CHEMICAL AND BIOLOGICAL FACTORS IN THE ACTIVITY OF ADRENOCORTICAL STEROIDS

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I. INTRODUCTION

This review is concerned principally with a discussion of the molecular structure of adrenocortical steroids and the possible nature of the chemical events that produce the biological activities of these substances. In order to give a proper account of the matter, it will be necessary first to review the recent work on the nature of the biological activities of adrenocortical steroids (pages 320–354). Because of the enormous amount of work that has been done over the last fifteen years or so, this review will be incomplete, but an attempt will be made to give sufficient references to reviews and certain crucial papers to enable those unfamiliar with the field to make acquaintance with the recent literature. On the other hand, the huge volume of work on synthetic analogues of the steroid hormones is quite essential to the main subject of this review and is contained largely in chemical journals which are not the habitual reading matter of many pharmacologists. I have therefore attempted to give as complete a review as possible of this work although many of the references are rather short and condensed communications. The excellent review of Fried and Borman (252) deals very fully with the synthetic analogues that had been studied up to 1958; I shall attempt to cover the more recent work and also to relate it as far as possible to earlier work. The search of the literature was finished in May, 1960, but a certain number of more recent papers have been considered which are of outstanding interest.

The main biological activities of the adrenocortical steroids are usually divided into two main classes, those affecting the intermediary metabolism of carbohy-

drate, protein, and fat, which are associated with the remarkable suppression of certain inflammatory processes, and those affecting the distribution and excretion of water and electrolytes. The adrenal cortex, however, is also capable of synthesizing, and probably of secreting, steroid sex hormones of the three main classes although the functional significance of these secretory products is not fully understood. Since our ideas of the molecular events underlying the biological actions of the principal adrenocortical steroids are greatly influenced by studies of the structure-activity relationships of steroid sex hormones and their synthetic analogues, a fairly complete review will be given of the recent and early work in the field of androgens and gestagens, and a limited review of similar studies on steroid estrogens. No attempt has been made to give a complete review of the estrogens because the very large number of synthetic and natural nonsteroidal estrogens suggests that the structural requirements for this type of activity are much less strict than for the other classes of steroid hormones, and at present poses an insoluble problem to would-be interpreters.

In interpreting the existing literature it should be emphasized that a great majority of the papers deal with routine biological assays carried out on large numbers of compounds supplied by the synthetic chemist. The assays are designed mainly to detect compounds of possible therapeutic interest. It is usually difficult or impossible to compare the results obtained by one group of workers with those of another. The majority of workers carrying out such assays are well aware of this. In criticizing this aspect of the literature, no slight is intended on the many industrious workers who have provided the enormous and valuable body of information that is presently available, but the criticism must be made lest those unfamiliar with this field should be misled into thinking that the relative activities of different steroids reported in the literature have more than a limited pragmatic value in the discussion of the structure-activity relationships of this class of substances. Despite this difficulty, certain arguments can safely be employed which take us a long way towards an understanding of the molecular events underlying the biological activities of the steroids.

Up to 1952 it was held that with the exception of the estrogens, the structural requirements for the biological activities of steroids were extremely strict. What were considered relatively slight modifications of their structures seemed invariably to produce the abolition or a very considerable reduction of their biological activity. It is now clear that these views were unnecessarily pessimistic, even on the evidence available at the time, but it is fair to observe that the profitability of exploring the effects of major structural alterations to steroid molecules was realized only after the demonstration by Fried and his group (252) that 9α-halogenated adrenocortical steroids possessed much greater biological activities than the natural substances to which they were related. This realization has led to the production of large numbers of synthetic steroids with greatly enhanced biological activities and with extremely interesting variations in the balance of their different effects. This is true for all classes of steroid hormones, although there is as yet only one type of steroid analogue of estradiol in which the enhancement of biological activity is of the same order as has been ob-

tained with many different types of analogues in the fields of the corticoids, androgens, and gestagens.

In order to give a full discussion of these structure-activity relationships, however, it is first necessary to consider the biology of the steroid hormones.

II. BIOLOGICAL CONSIDERATIONS

A. The nature of the adrenocortical secretions

1. The chemistry of the secretory products. The subject was last reviewed in detail in 1954 by Hechter and Pincus (310) and it is not necessary to add greatly to their account for the purposes of this review, except to point out that recent comparative work has shown that the products recognized as the principal hormones of the adrenal cortex in mammals have now been demonstrated to be present in a wide variety of vertebrate species. Fontaine (242a, 242b) and Phillips in particular have pioneered the study of these steroids in fish, birds, and reptiles and this work has been confirmed and extended by others. Cortisol and corticosterone were found in the blood plasma of three species of *Cyclostomata*, eight species of *Elasmobranchii*, and three species of *Teleosti* (353, 547, 548). In one large sample (8,868 ml) of pooled plasma from spawning Sockeye salmon, Phillips, using a number of chromatographic criteria, identified aldosterone in a concentration of 0.12 μg/100 ml (547). Less convincingly, he was able to show that the interrenal tissue and head-kidneys of killifish incorporated tritiated progesterone (16-^{3}H-progesterone) into cortisol and aldosterone on incubation of the isolated tissues (547, 549). The functional significance of these steroids in fish is at present unknown; it may well be similar to that in mammals, since their concentration in peripheral plasma undergoes a 4- to 5-fold increase during the spawning migration of the Sockeye salmon (353, 589). Ulick and Solomon (751) showed that adrenal tissue from *Rana catesbeiana* synthesized aldosterone from progesterone *in vitro*. Hisaw and his group (91) isolated very small amounts of material tentatively identified as estradiol and progesterone from 6.552 kg of ovaries from an invertebrate, the starfish.

Our present evidence suggests that the principal adrenocortical steroid hormones, *i.e.*, cortisol, corticosterone, and aldosterone, are found in the blood of a wide variety of vertebrate species and may well prove eventually to be present in all of them. There is no obvious evolutionary trend in the proportions of the two principal glucocorticoids, cortisol and corticosterone, that are found in these different vertebrate species, nor is there any obvious relationship between these proportions and the dietary or environmental habits of the species that have been examined. One remains with the concept that the normal functions of these two corticoids are probably essentially the same in the different species, and that their proportions in the adrenocortical secretion are a matter of biochemical accident (see also 479). Changes in these proportions may be produced by prolonged overdosage with adrenocorticotrophic hormone (ACTH) (397).

The possible significance of the androgens, estrogens, and progesterone and other steroids that are probably secreted by the adrenal cortex of most mammals

is still a matter of debate (*e.g.*, 32a, 768). The structure-activity relationships of these classes of steroids will be discussed from the point of view of throwing light on the problem of the intimate mode of action of steroids in general, and not because the biological importance of the secretion of these compounds by the adrenal cortex is at present known to be at all great.

2. The regulation of adrenocortical secretion. It is still agreed that the secretion of the glucocorticoids cortisol and corticosterone, is largely or entirely under the controlling influence of the adrenocorticotrophic hormone (ACTH) of the anterior pituitary gland. It is necessary to emphasize that the speed of the reactions of this regulating system is considerable (*e.g.*, 629, 712, 717). Thus the adrenal cortex shows a detectable response to the close injection of ACTH in animals within seconds (310), and its secretion rate falls to between a tenth and a twentieth of that usually found during an acute experiment within 20 to 30 minutes of carrying out hypophysectomy in the rabbit (310). Similarly, the ingestion of 9α-fluorocortisol or 9α-fluorocortisone produces a more than 50% fall in the concentration of cortisol in the peripheral plasma of young male volunteers within 1.5 hours. Since this is little, if any, greater than the normal half-life of cortisol in the plasma of men, it is clear that the inhibitory effect of this rapidly absorbed and extremely potent steroid on the secretion of ACTH must have been extremely rapid (*cf.* 77, 230, 762).

Another important development in recent years has been the steady growth of the idea that emotional factors of various kinds probably play the major part in causing fluctuations of adrenocortical secretion rate in man, monkeys, and possibly other species. It is probable that very severe burns, and large doses of certain agents such as bacterial pyrogens, histamine, and peptones, cause a brisk release of ACTH that is independent of any emotional concomitants (629, 649); but it is extremely doubtful whether any of the physical stimuli which are commonly supposed to be "stresses" are effective in causing the increased secretion of ACTH at all. Thus, severe exercise, cold, and fasting produce little or no effect on the secretion and metabolism of cortisol in man unless they are part of a situation that provokes emotion. On the other hand, strong emotion in the absence of any recognizable physical stimuli or "stresses" regularly causes maximal increases in the secretion rate of cortisol and in its concentration in peripheral blood. These emotional factors have been demonstrated most thoroughly and strikingly by Thorn (327) in man and by Mason (478) both in monkeys and in man. It seems most likely that the emotion that is most effective in producing these effects on the secretion of ACTH is best described as "competitive anxiety," that is to say, anxiety of the type and severity that calls forth the desire to overcome adverse circumstances or the challenge of competitors (82, 228, 244, 477, 478; *cf.* 491). It is difficult to overemphasize the importance of these findings for adrenocortical physiology. In the first place, all previous experiments on conscious animals purporting to demonstrate that physical "stress" affected the secretion rate of the adrenal cortex are thrown open to considerable doubt since they all involved the exposure of the animals to unpleasant and threatening experiences. In the second place, our whole concept of the adrenal cortex as a gland the secretions of which

regulate an as yet undiscovered metabolic process that affects the metabolism of carbohydrate, protein, and other substances is thrown into confusion by the suggestion that the most important natural stimulus to the activity of the gland is psychological in character. The magnitude of the increases in secretion rate that have been observed is far too large to be neglected. Thus, the increases in the concentrations of cortisol in plasma and in the excretion rate of metabolites of cortisol that are seen during such relatively common and mild periods of anxiety as being given an oral examination or interview for a senior position are such as are seen otherwise only in patients with severe Cushing's syndrome. Much further work is needed to elucidate the question of what part this phenomenon might play in psychosomatic disease.

The regulation of the secretion of aldosterone is still a matter of considerable controversy. It is probable that ACTH plays a minor and possibly negligible part but the nature of the hormone or hormones controlling the secretion of aldosterone is still in doubt (see 43, 44a, 457, 665). The work of Bartter and his colleagues has shown clearly that the plasma concentration of sodium is not the variable directly affecting the regulatory system and that some function of the extracellular fluid volume and possibly of plasma potassium concentration is probably the effective stimulus. The subject will not be considered further here since there are numerous reviews available (43, 231, 296, 457, 731, 736, 809) and the present state of this field throws little or no light on structure-activity relationships.

B. The nature of adrenocortical insufficiency

1. The general characteristics. The nature of adrenocortical insufficiency is very germane to any discussion of the actions of adrenocortical steroids. In the first place, the adrenalectomized animal is usually the test object of biological assays of such steroids, and the assays depend upon the reversal or amelioration of some feature of the syndrome of insufficiency. In the second place, the characteristics of this syndrome may give some clue to the nature of the biological functions of these steroids. In actual fact, the syndrome of adrenocortical insufficiency is extremely complicated and needs much further study. Those parts of the earlier literature that are not explicitly mentioned here are referred to in earlier reviews (see 629).

The removal or destruction of the adrenal glands results in a condition of weakness, inability to withstand fasting or other relatively mild adversities, and death after a relatively short interval. The concentration of sodium and chloride in the extracellular fluid falls; that of potassium in both extracellular and intracellular fluids rises; the volume of extracellular fluid falls; the concentration of glucose in the blood falls; and the concentration of liver glycogen falls. A feature of the syndrome that was emphasized by Thomas Addison in his famous monograph on Addison's disease and which has received closer attention in recent years is the weakness of the action and fall in weight of the heart, the low blood pressure, and the inability of the circulatory system to respond to situations calling for an increased cardiac output (see 78, 109, 285a, 561a). The cause of death in adrenal in-

sufficiency is usually attributed to the sum of the effects described above but remains somewhat mysterious. Thus, adrenalectomized dogs are maintained in seemingly good health by daily intramuscular injections of cortisone acetate (0.93 mg/kg per day) as judged by their behavior, appetite, and blood urea concentrations. On the other hand, the concentrations of sodium, potassium, and chloride in their blood plasma are those characteristic of adrenal insufficiency and the animals may collapse and die suddenly immediately after a meal or playful fights with their companions. Such animals show raised concentrations of potassium in their plasma and electrocardiographic changes characteristic of hyperkalemia. If the dose of cortisone acetate is raised to 1.86 mg/kg per day, the plasma electrolytes are maintained at normal concentrations and sudden collapse and death of this sort are avoided (704). On the other hand, it is well known that adrenalectomized animals, especially rats and dogs, can be maintained in apparently good health, and with normal concentrations of sodium, potassium, chloride, and urea in their blood plasma, by daily treatment with deoxycorticosterone or by simply substituting isotonic saline for water in their drinking bottles, but that they will collapse and die rapidly when fasted or subjected to cold or a mild infection (*cf.* 105, 106).

Before 1950 or thereabouts, it was thought by some that the adrenocortical secretions acted mainly or exclusively on the excretion of sodium and potassium by the kidney, and upon the metabolic conversion of protein to carbohydrate in the liver. In 1950, Sayers in his magnificent review (629) suggested that the actions of the adrenocortical steroids were more far-reaching than this and involved most or all tissues of the body. This "ubiquitous" action of these hormones was postulated to depend upon or be associated with their "utilization" by the tissues concerned. It was postulated that this "utilization" lowered the plasma concentration of the hormone or hormones which in turn released the anterior pituitary (or hypothalamus) from the inhibitory influence of the steroids on the secretion of ACTH. It soon became clear that situations which provoked an increased secretion rate of the adrenal cortex in man and experimental animals produced in fact an increased concentration of these hormones in the peripheral plasma and that there was little or no evidence of their increased "utilization" in such situations in the sense implied by Sayers. Recent work has, however, tended to emphasize the extrarenal actions of adrenocortical steroids on the distribution of fluid and electrolytes; their actions on the heart and blood vessels; their local actions on inflammatory reactions and on growing tissues in culture; and most important of all, the "permissive" or "supportive" function of these hormones (224, 355, 356). The last-mentioned topic is best considered here since it underlies all the later discussion of the biological activities of these steroids.

It is still commonly supposed that the adrenocortical hormones have the function of *regulating* certain processes in the same way that sympathetic nerves can be said to "control" the contraction of arteriolar smooth muscle, or the antidiuretic hormone to "regulate" the reabsorption of water by the kidney. Ingle showed that the experiments upon which such conclusions were based admitted another concept of the actions of these hormones, and in subsequent work he

carried out a new type of experiment which, in fact, demonstrated that his new concept was nearer the truth (356). He adrenalectomized his animals and then kept them on a *constant* replacement dose of adrenocortical extract or steroids. While on this regime, a number of the animals were then subjected to the natural stimulus and the usual response measured; the other animals continued on the regime to serve as controls. Every response that was examined which had previously been supposed to be subject to the *regulatory* action of the glucocorticoids of the adrenal cortex (cortisol and corticosterone) was shown by this type of experiment to depend upon the *presence* of this type of steroid but to be under the *regulation* of some other system. Thus, the animals on a constant daily dose of cortisone or adrenal extract showed a perfectly normal response to the stimulus or "stress" although they were deprived of their adrenal glands and hence were unable to increase their endogenous supplies of adrenocortical hormones.

One can hardly over-emphasize the importance of this concept of adrenocortical physiology. The only serious modification to the original hypothesis that has been found necessary up till the present is that it is now clear that the full response to a large stimulus or "stress" is often obtained only with *large* constant doses of hormone, while the full response to smaller stimuli can be obtained when the animals are kept on relatively low doses. It should be added that this concept applies at present only to the glucocorticoids and has not been demonstrated convincingly for the mineralocorticoids. It suggests that the glucocorticoids may not, in fact, have a "regulatory" function at all in the sense that is usually applied to hormones in general. They seem at present rather to play the part of an "endogenous vitamin," needed in differing amounts for the optimal response of the organism to different environmental conditions and different physiological states (356). At present, it seems that the normal metabolic machinery of the animal exists in the adrenalectomized animal and is sufficient for growth and reproduction as long as the animal is protected from extreme conditions or harmful agents. The close parallel between these hormones and the vitamins will be obvious, but an important difference exists. Unlike vitamin-deficient animals, adrenalectomized animals maintained on isotonic saline solution grow at a normal rate and tolerate moderate excesses of diet reasonably well. This suggests that the adrenocortical steroids are not essential for specific metabolic reactions in the way that the majority of the vitamins or their conversion products are known to be.

The second important general feature of adrenocortical insufficiency is that no therapy other than the glucocorticoids themselves has been found to protect the deficient animal from the lethal effects of extreme environmental conditions, or harmful agents (*e.g.*, 617, 629). There is at present no essential response or metabolic pathway that is common to all these situations which can surely be identified as being specifically dependent upon the presence of glucocorticoids.

2. The disturbance of fluid and electrolyte distribution in adrenal insufficiency. The electrolyte and fluid disturbances found in adrenal insufficiency are clearly due in part to a failure of the kidney to retain sodium in the normal way (306, 452). On the other hand, it was not quite so clear that a renal defect would

explain the rise in plasma potassium concentration. Conway and Hingerty (159) found that the known disturbance of potassium and magnesium concentrations (48) was largely or entirely due to a failure of the kidney to excrete these ions in a normal way. Thus, the concentrations of each ion were greatly elevated in both the plasma and muscles of adrenalectomized rats.

A prolonged controversy has occurred over the exact nature and magnitude of the disturbance of extrarenal factors affecting the distribution of fluid and electrolyte that have been postulated to occur in adrenal insufficiency. This subject has been studied very fully by Woodbury (810, 810a, 811). It seems likely that adrenal insufficiency in rats is characterized by a decreased intracellular concentration both of sodium and of nondiffusible solutes (154, 155, 159), but many workers have tried to establish that insufficiency is accompanied by a shift of sodium from the extracellular to the intracellular compartment of body water (242, 269, 328, 458, 686, 788) together with a shift of water in the same direction (306, 328). Rolf and his colleagues (596), however, were unable to confirm these findings and pointed out that comparisons of the loss of sodium to the exterior with the apparent loss from the extracellular compartment were suspect due to the methods used for estimating the volume of the extracellular compartment. They found that when this volume was estimated by using continuous infusions of sucrose to obtain a steady-state, the calculated loss of sodium from the extracellular fluid was not significantly different from the loss of sodium in the urine and feces measured directly.

Flanagan *et al.* (242), however, found that the excretory loss of sodium was too small to account for the calculated loss of sodium from the extracellular fluid, and found that there was no observable increase in the intracellular concentration of sodium in the soft tissues, in effect a confirmation of Conway and Hingerty's findings (159). Since bone contains a very large fraction of the total sodium in the mammalian body [up to 40% (520)], they suggested that the missing sodium might be taken up by bone in adrenocortical insufficiency. White and Rolf (788) were unable to show that bone was in fact responsible; as with muscle, there was a fall in the sodium content of bone in adrenocortical insufficiency. While accepting the criticisms of Rolf and his group of the conclusions based on measurements of extracellular volume, it is fair to note, nevertheless, that certain studies which are not open to this criticism suggest that adrenocortical insufficiency leads to large shifts of electrolytes and water between the extracellular and intracellular fluids, and that these shifts are reversed by the administration of adrenocortical steroids.

Woodbury (810) and Friedman (260, 261) found that the concentrations of electrolytes in the plasma of nephrectomized rats were altered by adrenalectomy and that the alterations were reversed by deoxycorticosterone. These effects were rapid, and were not due to excretion or absorption of sodium from the gut (810). Both groups found that adrenalectomy caused a fall and deoxycorticosterone caused a rise in the concentration of sodium in the plasma of nephrectomized rats, but only Friedman and his group found that the rise in the plasma concentration of potassium that normally follows nephrectomy was increased by adren-

alectomy (*cf.* 361). Much more convincing, however, are the careful studies of Swingle and his group on adrenalectomized dogs (706, 707, 709, 710, 712, 713, 714, 715). These authors showed that adrenalectomized dogs which were allowed to go into crisis after the withdrawal of maintenance therapy could be restored to normal health and vigor within 12 to 48 hours by the administration of potent sodium-retaining steroids such as 2α-methyl-9α-fluorocortisol *while fasting*. During recovery they continued to excrete variable but often large amounts of sodium in the urine. Nevertheless, the concentrations of sodium, potassium, and urea in their plasma were restored to normal concurrently with their return to vigor and strength. It is clear that the restoration of normal extracellular concentrations of these ions must have been due largely to internal redistribution of water and electrolytes.

Mendelsohn and Pearson (485) studied the withdrawal of cortisone from seven adrenalectomized human patients. They found that retention of water in the *extracellular* compartment was the principal consequence and that the fall in the plasma concentration of sodium was not significantly different from that expected from the dilution of this compartment by the retained water. There was no significant increase in the rate of sodium loss in the urine, and the urine flow fell to low values.

The weight of evidence suggests at present that important internal shifts of water and electrolyte occur in adrenocortical insufficiency and that they can be reversed by sodium-retaining steroids.

The interpretation of the effects of adrenal insufficiency is made extremely difficult in all spheres of metabolism by the protean nature of the systemic disturbance, particularly of the circulatory system. Nowhere is this more pronounced than in the field of electrolyte and water movements. For instance, it is clear that the predominant phenomena are a failure to conserve sodium by the kidney and an inability to excrete water loads (*e.g.*, 265) but it was pointed out by Harrop long ago that the adrenalectomized animal was killed as rapidly by an excessive intake of sodium chloride as by an inadequate intake (see 629). Further work will be required to explain the complexities of certain observations (*e.g.*, 174) and the interaction of the effects of steroids and of antidiuretic hormone (176, 594, 807).

3. The effects of adrenal insufficiency on intermediary metabolism. The period following Sayers's review (629) has been dominated by a large number of studies attempting to demonstrate an action of glucocorticoids on isolated systems, usually specific enzymes. Most of these have been reviewed by Hechter (308a) who came to the conclusion that none of these findings satisfied the criteria that would be required to establish that the physiological functions of these hormones were due to a simple and definite interaction with one or several enzyme reactions. In the present review, our attention will be confined to a few seemingly crucial observations in this field and some references to other reviews of it.

It is valuable first to recall Umbreit's paper (754) in which he pointed out that homogenates of kidneys from adrenalectomized animals showed a reduced ability to oxidize a wide variety of substrates with the striking exception of succinate.

Since this was the only oxidation which did not depend upon the presence of adenosine nucleotides, he examined the oxidation of the other substrates after fortification of the homogenates with adenosine triphosphate (ATP). The rates of oxidation of all tested substrates, except proline, by homogenates of kidneys from adrenalectomized rats were not different after fortification from those obtained with tissue from intact rats (754, 755). In other words, the kidneys showed a deficiency of adenylic acid derivatives but no real deficiency of the enzyme systems that had previously been thought to be affected by adrenalectomy.

The situation is much more complicated when chronic effects are studied or when the whole animal or intact but isolated tissues are used. Thus, one suspects strongly that most of the many effects on enzyme systems of adrenalectomy are secondary to the systemic disturbance, even though many of them fit attractively into our present knowledge of the metabolic pattern of adrenocortical insufficiency (18, 263, 483, 597, 753, 757, 764).

An extremely important modification of previous ideas on the nature of adrenocortical insufficiency has been suggested, however, by the work of Winternitz *et al.* (808). The classical studies of Long and his colleagues (see 453, 808) had suggested that the depletion of liver glycogen in fasted adrenalectomized animals was due to a deficiency in the conversion of noncarbohydrate material into carbohydrate, *i.e.*, in gluconeogenesis, and that this material was in fact largely or wholly protein. Long and his group have now shown that the conversion *in vivo* of a large number of nonnitrogenous precursors to glycogen is greatly diminished in adrenalectomized rats. These included glucose, pyruvate, lactate, malate, fructose, and glycerol. Since the rate of removal of these precursors from the blood after intravenous infusion was not diminished in adrenalectomized animals, they have suggested that the adrenalectomized animals metabolized a larger proportion of the precursors by other metabolic pathways which are inhibited by glucocorticoids in intact animals. They suggested that the principal pathway in these experiments might have been the pentose phosphate cycle, and that the main action of cortisol might be to inhibit some reaction in this cycle. Long *et al.* (453a) have recently reviewed this field very succinctly and laid great emphasis on the fact that cortisol produces effects on carbohydrate metabolism which are dissociated from effects on protein catabolism when the animals are fed; these effects are absent in the eviscerated animal, suggesting that the primary effect of the glucocorticoids is on the liver and not on the peripheral tissues (see below).

4. The cardiovascular system in adrenocortical insufficiency. The weakness of the pulse and the smallness of the heart in patients with Addison's disease were emphasized strongly by Thomas Addison in his famous monograph. The earlier work was reviewed by Sayers (629) and by Brown and Remington (109). The latter authors have reinvestigated the popular theory that the adrenalectomized dog suffers from cardiovascular collapse which is due mainly to a diminished response of the arterioles to noradrenaline and sympathetic constrictor fibers. Previous work suggested that animals in or near to crisis showed: (a) a raised or normal venous pressure; (b) a lowered mean systemic and pulse pressure; (c)

a lowered cardiac output; (d) a pooling of blood in the splanchnic area (cats); (e) a failure of both the systemic arterial pressure and of small vessels observed under the microscope in the meso-appendix to respond in the normal way to injections or infusions of noradrenaline and adrenaline; (f) an increased hematocrit due to a decreased plasma volume (*cf.* 272); (g) raised concentrations of potassium, magnesium, and urea and lowered concentrations of sodium, chloride, and bicarbonate in the blood plasma.

Levine and his colleagues showed that the decreased ability of adrenalectomized animals to do muscular work is largely or entirely secondary to circulatory failure (285a, 561a). It is still widely believed, nevertheless, despite Ingle's analysis of this phenomenon (see 285a, 358, 359, 360), that it is one of the consequences of the *disorder of carbohydrate metabolism* found in such animals. Levine's group showed that the maximum rate of work that could be obtained by repetitive stimulation of the gastrocnemius of the dog bore exactly the same relationship to the blood pressure in both intact and adrenalectomized dogs. When the muscle failed in the adrenalectomized dog, it was resuscitated immediately by raising the blood pressure with infusions of saline and noradrenaline. Similarly, a slip of the diaphragm, that had failed in an adrenalectomized rat during repetitive stimulation, became responsive immediately after it had been excised and put into a bath of oxygenated Locke-Ringer solution.

Brown and Remington (109) emphasized, however, that previous authors investigating responses to noradrenaline and adrenaline had assumed that it was reasonable to make comparisons of these responses at different mean arterial blood pressures, and had neglected the part played by the heart in such responses. In order to reassess these studies, they gave intravenous injections of noradrenaline, adrenaline, and nicotine to intact dogs and to dogs which had been adrenalectomized 10 to 12 hours before the experiments, and in which the blood pressures at the start of the experiment were brought to the same value by reducing the dose of morphine used in the anaesthesia of the adrenalectomized dogs and by bleeding the intact dogs. During the course of circulatory failure, the peripheral resistance in the hind leg was much the same in both groups of dogs. Furthermore, the injection of noradrenaline produced a *larger* increase in this peripheral resistance in the adrenalectomized dogs than in the controls, even though the average increase in mean systemic *arterial pressure* was less than in the controls.

The earlier simple interpretations of Levine and others of the circulatory collapse of adrenocortical insufficiency are rendered somewhat doubtful by this work (see 109, 826), and previous results could be explained largely in terms of the consequences of a low mean systemic arterial pressure from whatever cause. Nevertheless, it remains true, as Levine emphasized, that the circulatory inadequacy of the animal suffering from adrenocortical insufficiency is a major and possibly dominating feature of the syndrome and that further work on it is badly needed.

5. Summary of recent views on adrenocortical insufficiency. The most striking result of the work of the last ten years has been the extent to which further analy-

sis of this complicated deficiency syndrome has failed to pin-point any single tissue or metabolic pathway as the principal or sole site of action of the adrenocortical hormones, while many phenomena previously attributed to the "ubiquitous" role of these hormones have been shown to be secondary to circulatory or other disturbances. The three outstanding changes in current thought on the matter would seem to be (a) the demonstration of the importance of the circulatory inadequacy in many phenomena previously attributed to metabolic abnormalities; (b) the demonstration of the supportive or "permissive" role of the glucocorticoids in all the responses investigated so far that had previously been thought to be dependent upon a regulatory function of the glucocorticoids; and (c) the demonstration that the diminution of liver glycogen is not due simply to a failure of gluconeogenesis from nitrogenous substances.

C. The classification of the biological actions of adrenocortical steroids

Before proceeding further it is necessary to give a brief consideration of the usual classification of the biological activities of steroid hormones and their synthetic analogues. It is probably impossible to please everyone in this so that at the outset I must emphasize that I am concerned with the complexities of the biological actions of these substances only insofar as they will help in the discussion of the structure-activity relationships of steroids. The terms to be employed in the subsequent argument are used purely for their convenience and do not imply any suppositions about the nature of the biological activities that are under discussion.

Steroids which cause a fall in the ratio of the concentrations of sodium and potassium in the urine of adrenalectomized animals, or which produce a definite positive sodium balance in such animals will be referred to as sodium-retaining steroids (see 190). This type of action will be called sodium-retaining activity. It is usually associated with a definite action increasing the urinary excretion of potassium but this action is probably at least partly due to a different mechanism from that leading to the similar overall effect of glucocorticoids (499). It will be seen later that the overlap of the actions of glucocorticoids and sodium-retaining steroids on electrolyte metabolism is considerable.

Steroids which have a definite activity in the liver glycogen assay (534a) will be called glucocorticoids. It may reasonably be assumed that all or most of them will also possess the other types of biological activities that are possessed by cortisol or cortisone on inflammatory processes, cytological phenomena, and intermediary metabolism. They will probably all tend to produce a negative nitrogen balance and an associated negative balance of potassium, phosphate, calcium, and water (148, 224, 356, 735). But they will not necessarily show the weak sodium retaining activity of cortisol; it may be much greater than that of cortisol, much less, or actually reversed so that a negative sodium balance is caused. "Glucocorticoid" or "glycogenic" activity will refer solely to an action in the liver glycogen assay as customarily performed, and "anti-inflammatory activity" will refer to an action in the type of assay that is based on the inhibition of the formation of granulomata in response to a foreign or irritant material

such as cotton wool, asbestos, turpentine, or croton oil unless otherwise specified (see 316, 583). Such anti-inflammatory activity is nearly always associated with similar activity in the suppression of the inflammatory phenomena of the so-called collagen diseases, and the inflammatory response of certain hypersensitivity reactions, but the activity in the granulomatous reactions is often much greater than that in the latter type of inflammation.

Steroids with definite activity in the usual tests for progestational activity which employ a direct endometrial response in an estrogen-treated animal will be referred to as gestagens or as having progestational activity. Other types of activity, such as the inhibition of gonadotrophin secretion or the antagonism of the endometrial or vaginal actions of estrogens, will not be included in these terms unless mentioned specifically. Steroids which cause an increase in the uterine weight of ovariectomized, hypophysectomized or immature animals will be called estrogens or steroids having estrogenic activity.

Steroids capable of increasing the weight of the prostate and seminal vesicles of immature or castrate animals will be called androgens or said to have androgenic activity. The same terms will be used for steroids capable of increasing the weight or size of the combs and wattles of immature or castrate birds.

The term "anabolic steroid" or "anabolic activity" is now almost universally used for compounds that increase the weight of the *levator ani* of the castrate rat. Despite the convenience and wide use of this term, its connotations are so misleading that it is almost a public duty to avoid using it until such time as these connotations have been justified by sound experimental work. Those unfamiliar with this field should realize that the term arose from the desire to find substances which retained the truly anabolic activity of typical androgens such as testosterone while lacking their virilizing effects. Such substances might be of great use in promoting the healing of wounds and muscular regeneration after surgery or wasting diseases. A proper assay for anabolic activity has not yet been achieved in a form which would provide an economical screening test for such substances on a large scale. Following the demonstration by Eisenberg and Gordan (see 198) that the *levator ani* of the rat responded to testosterone by an increase in its weight, it has become customary to regard such activity as "anabolic" in the general sense. This supposition has never been firmly stated or proved to be true, but has been tacitly implied for so long and has received so little public challenge that the term has become firmly entrenched. The only justification for allowing the term at all is that activity in this assay can be dissociated from androgenic activity itself by certain chemical modifications of the steroid molecule. From the purely pragmatic point of view, it is an action which is different from and not dependent upon androgenic activity as defined here. In order to emphasize this point, such activity will be referred to as "levator activity." This is not to deny that there is good biological and biochemical evidence that many typical androgens do in fact have a general anabolic effect in certain species under certain conditions and that this is known to extend both to kidney tissue (423, 424) and the general skeletal musculature (see 437).

D. The biological actions of sodium-retaining steroids

1. Actions on the kidney. The early work mentioned above in the discussion of the electrolyte disturbance of adrenocortical insufficiency established clearly that at least part of the actions of adrenocortical steroids was upon the renal excretion of electrolytes. Neher (515) has written a useful review of the work before 1957.

Following the demonstration that aldosterone was a genuine secretory product of the adrenals of dog, monkey, and rat (310, 664, 665), much attention has been devoted to clarifying the nature and magnitude of its action upon the kidney. Numerous studies have shown that aldosterone produces an increased excretion of potassium and a decreased excretion of sodium by the kidney, which is due to an action on tubular reabsorption and not upon glomerular filtration rate or other extratubular effects (43, 174, 457, 499, 517, 602a, 654). The effect is similar to that of deoxycorticosterone (176, 270, 293, 294) and the potency of this steroid in man relative to deoxycorticosterone is comparable to that in animals, although not as great as in the rat (480, 679, 736). Swingle and his group (710) came to the conclusion that aldosterone exerted all its effect upon the renal handling of electrolytes and that even in large doses it did not produce the internal redistribution of water and electrolytes by extrarenal actions which was so pronounced with steroids such as the 9α-fluorosteroid analogues. In most of these studies, the effects of aldosterone in dogs or in man began to be observable within 1 to 2 hours of starting an intravenous infusion.

Ganong and Mulrow (268) found that intraaortic injections of aldosterone in the dog in doses of 2 to 10 μg produced an observable change in sodium and potassium excretion within 5 to 30 minutes. Barger *et al.* (39) used one renal artery as the route of injection and obtained unilateral changes in the renal excretion of sodium and potassium with low doses of aldosterone or 9α-fluorocortisol. These began within 40 to 60 minutes and reached their peak after a further 100 minutes. Both steroids produced an effect on potassium excretion in normal and adrenalectomized dogs, but a fall in sodium excretion was obtained only in the adrenalectomized animals. Furthermore, the increase in potassium excretion in the adrenalectomized dogs occurred only 40 minutes after the decrease in sodium excretion had become significant. They concluded, in agreement with Mills *et al.* (499), that aldosterone and 9α-fluorocortisol were not influencing the excretion of these two ions by acting on a simple Na/K exchange mechanism. Two critical attempts to locate the tubular site of action of aldosterone and deoxycorticosterone have been made, but these have given opposed results. In one, the stop-flow method suggested that aldosterone acted on the distal tubule in the dog (758). Nicholson, however (521), found that renal arterial infusions of racemic sodium tartrate, which damage the proximal tubule, completely abolished the renal response to aldosterone and deoxycorticosterone on the side of the infusion, while damaging the distal tubule, by retrograde infusion of mercuric chloride *via* the ureter, left the response to these steroids unchanged.

Despite the uncertainties of the stop-flow method, Nicholson's conclusion is not unassailable. Thus, for instance, the effect of different sodium loads would be expected to make a large difference to the response (104, 170) and to the relative importance of the proximal and distal tubules.

An important source of complication in the renal response to salt-retaining steroids has been demonstrated by Nelson and August (517) who found that patients with edema from various causes showed a much larger salt retention than normally found with aldosterone and deoxycorticosterone, and yet failed to show the normal increase in potassium excretion. Several workers have emphasized that deoxycorticosterone, both in man and in the dog, produces a considerable increase in the rate of water excretion by the kidney (112, 236, 293, 294), an effect which is partly, and probably largely, due to diminished tubular reabsorption (293, 294). Water diuresis has previously been considered more characteristic of typical glucocorticoids than of salt-retaining steroids.

An extremely interesting analysis of the effects of cortisol and of aldosterone in man has been made by Mills *et al.* (499). They found that in the afternoon cortisol caused a retention of sodium that was approximately balanced by an equivalent increased excretion of potassium and was associated with no change in the excretion of hydrogen ion. In contrast, both aldosterone and deoxycorticosterone caused a moderate increase in the excretion of hydrogen ion (see 616a), and the sum of the increases in hydrogen ion and potassium was equivalent to the decrease in sodium excretion. At night cortisol caused a fall in hydrogen ion excretion which was balanced by a further increase in potassium excretion. When hydrogen ion secretion was blocked by administration of acetazoleamide, all three steroids caused an increase in potassium excretion and a retention of sodium that were approximately equivalent.

Attempts to obtain effects of adrenalectomy and of steroids on isolated particles from homogenates of rat liver and kidney gave rather inconclusive results (652). It should be remembered, however, that the renal handling of sodium and water is a highly complicated process involving all parts of the renal tubule and the glomerulus and renal blood vessels.

When we consider the sodium-retaining activity of steroids which are mainly notable for their potent glycogenic activity, a number of complications arises (see, *e.g.*, 443, 506). It was shown early by Thorn *et al.* (733) that deoxycorticosterone and corticosterone produced definite sodium retention in dogs, while cortisone and cortisol produced a moderate increase in sodium excretion. It is now clear that the weak sodium-retaining action of steroids like cortisol is easily obscured by their effects on other factors which tend to cause an increase in sodium excretion. The clearest demonstration of this has been given by Johnson (375) who showed first that the sodium excreting effect of cortisol could be reduced considerably by reducing the water load given during an assay; and second, that the effect of corticosterone, which has considerable activity both in sodium retaining and liver glycogen assays, varies with the dose. Kagawa and Van Arman (383) found that cortisol caused sodium retention over the first 300 minutes and increased sodium excretion in the second 300 minutes in adrenal-

ectomized rats. In order to overcome these interfering effects, Simpson and Tait devised the assay based upon alterations in the Na/K concentration ratio in urine of adrenalectomized rats (663). Even with deoxycorticosterone, some workers have found that very low doses cause an *increased* rate of sodium excretion in the urine (243) or that animals given a hypertonic load of sodium chloride respond to deoxycorticosterone with an increase in sodium excretion (*e.g.*, 293).

Clear-cut evidence that different types of pharmacological activity are being observed in the sodium-retaining actions of glucocorticoids has been provided by Borman *et al.* (89), who found that the log-dose plots of the sodium-retaining activity of 9α-chlorocortisol acetate and of deoxycorticosterone were parallel, while the plots for 9α-fluoro- and 9α-bromocortisol acetates were not (see also 418). This finding and those of Mills *et al.* (499) make it difficult to believe that the sodium-retaining action of some glucocorticoids, even when pronounced, is pharmacologically similar to the same action of "pure" sodium-retaining steroids like deoxycorticosterone. This is not to say that even weakly sodium-retaining steroids such as cortisone cannot be shown to have clear-cut actions on renal tubular reabsorption of sodium under certain conditions (*e.g.*, 585). On the other hand, even such a potent salt-retaining steroid as 9α-fluorocortisol has been shown to cause *increased* sodium excretion in adrenalectomized dogs when given in large doses (444).

2. Extrarenal and other actions of sodium-retaining steroids. Two features of the sodium-retaining steroids need emphasis. First, Borman *et al.* (89) showed that in the growth-survival assay in adrenalectomized rats 9α-bromocortisol acetate was a good deal more active than 9α-fluorocortisol acetate, although the latter was much more active in the sodium-retaining assay. This is in contrast with earlier work which tended to show a strong correlation between these two types of activity (see 122b, 629). The second is that the extrarenal effects of aldosterone and deoxycorticosterone appear to be much weaker relative to their renal effects than those of the sodium-retaining steroids which also possess strong glucocorticoid activity (706, 710). Another important difference is that the blood pressure and vigor of adrenalectomized dogs are maintained even in the presence of severe reductions of the plasma concentrations of sodium and chloride by glucocorticoids with weak sodium-retaining activity such as prednisone, while sodium-retaining steroids, such as aldosterone, fail to restore either the blood pressure, vigor, or the electrolyte concentrations in the plasma of fasting adrenalectomized dogs which have been allowed to go into crisis (710). On the other hand, aldosterone and deoxycorticosterone appear to be far more active than cortisone in maintaining a normal Na/K concentration ratio in the saliva of sheep (282), showing that they are not devoid of extrarenal activities of certain kinds.

Extrarenal actions of deoxycorticosterone on other tissue are far more difficult to assess. Woodbury and Koch (811) pointed out that both aldosterone and deoxycorticosterone cause a rise in the ratio of intracellular/extracellular concentrations of sodium in muscle even though the concentration of sodium in plasma is increased by the steroids, but that this may well be secondary to the con-

siderable loss of potassium that is also caused (236, 417). Ferrebee *et al.* (236) were, in fact, able to prevent the increase of intracellular sodium concentration caused by the chronic administration of deoxycorticosterone to rats by administering large supplements of potassium with the diet.

Berger *et al.* (51, 151, *cf.* 167) showed that in the dog deoxycorticosterone had no effect on the movement of potassium or sodium across the wall of the ileum or jejunum. On the other hand, it caused a definite increase in the sodium efflux from the colon, no effect on the sodium influx, and an increase in both the influx and efflux of potassium. The sodium efflux bore no relation to the potassium influx, which suggested that the steroid was not affecting a simple Na-K ion-exchange mechanism.

Various attempts have been made to demonstrate a convincing action of salt-retaining steroids upon isolated cells or tissues. Most of the effects appear to be either small or inconsistent with the changes that would be expected *in vivo*. There seems to have been little advance beyond the earlier literature (see, however, 651, 730) and most of these experiments have been carried out with concentrations of steroid that are from 10^2 to 10^4 times those found *in vivo* (*e.g.*, 107, 298, 299, 377, 630, 645, 803). A rather more convincing effect was obtained by Tanz *et al.* (726), who produced a strong positive inotropic effect on the isolated papillary muscle of the cat heart with only 0.5 μg of 9α-fluorocortisol/ml in the bathing fluid. Even if we make the unlikely assumption that this potent synthetic steroid has no greater intrinsic activity than cortisol, the effective concentration used is probably equivalent to 100 times that of cortisol in tissue fluid *in vivo* (see below). Glynn (280, 281), in a detailed and valuable study of the potassium and sodium exchanges of erythrocytes *in vitro*, found no significant effects with aldosterone although the cardiac glycosides had a pronounced effect upon potassium movement (see also 430, 670). On the other hand, Kessler *et al.* (403) obtained definite increases in the internal sodium concentration of human erythrocytes *in vivo* 20 to 40 minutes after an intravenous injection of hydrocortisone, and a rise in the pH of the plasma within 5 to 10 minutes of the injection (*cf.* 499).

Further work on the action of sodium-retaining steroids lacking glucocorticoid activity has continued to confirm the earlier conclusion that they fail to show the protective action of glucocorticoids against a wide variety of harmful stimuli in adrenalectomized animals (*e.g.*, 617) and have few cytological effects *in vivo* (276, 522). The concept of "stress" has been reviewed and modified yet again by Selye (648) who has devised an ingenious technique using a double granuloma pouch (647) in which it is possible to show "hypercorticoidism" and "hypocorticoidism" simultaneously in different regions of the same animal. The dependence of the effect of cortisol on the response to a large dose of croton oil upon whether or not the pouch has been pretreated with small doses of croton oil is of considerable interest, but it is difficult to accept some of the interpretations that have been offered. The most important findings in this field have been those of Ingle and his colleagues who have found that the full-blown syndrome produced by excessive doses of deoxycorticosterone in rats can be obtained in

adrenalectomized rats by sufficiently intense and prolonged treatment with a high-salt intake alone (356a; see also 629, 694, 700).

3. Antagonists to sodium-retaining steroids. The discovery of the action of the spiropropionyl lactones of testosterone and related steroids has led to an intensified interest in steroid antagonists (140, 141, 142, 384, 386, 402, 441, 698, 699, 790; *cf.* 287, 513). The evidence that they act by antagonizing the actions of aldosterone, and indeed of other sodium-retaining steroids, on the renal tubule is good (442).

The work with the spirolactones has led to a re-examination of the antagonism of salt-retaining steroids by naturally occurring steroids. Kagawa (381, 385) reinvestigated the antagonism of deoxycorticosterone by progesterone and testosterone. Both the latter steroids reduced or blocked the sodium retaining action of 12 μg of deoxycorticosterone when given to adrenalectomized rats of 150 to 200 g body weight in doses of 2 to 20 mg. It is of note that, as with the spirolactones, a very large dose of antagonist is required to produce an effect on the action of small doses (by weight) of the sodium-retaining steroid. Testosterone had no effect at all on the urinary Na/K concentration ratio in the absence of deoxycorticosterone, while progesterone administered alone had a weak sodium-retaining action, 20 mg of progesterone being equivalent to 10 μg of deoxycorticosterone acetate. The log-dose plot of the antagonistic effect of testosterone on the Na/K concentration ratio was linear over the dose-range that was studied while that of progesterone reached a peak at 4 mg. The interpretation of these effects of very large doses of antagonists is very difficult because of the real possibility in such cases that the active material is a metabolite of the steroid that has been administered (see below).

The considerably increased sodium excretion caused by some of the newer steroid analogues, especially the 16-substituted steroids (*e.g.*, 443, 495) may in part be due to a similar type of antagonism, but occurs in adrenalectomized animals and must be due largely to direct renal and extrarenal actions (443).

Another type of antagonism of electrolyte effects which has been given considerable prominence recently is that between sodium-retaining steroids and cardiac-active glycosides and aglycones. Wilbrandt has published a number of reviews (793, 795, 796) and papers (702, 791, 792, 794) on this topic (see also 630). His main findings are that strophanthoside K, a typical cardiac glycoside, antagonizes the sodium retaining action of deoxycorticosterone in adrenalectomized rats while having no effect on the potassium excretion other than that found with the glycoside itself. A similar antagonism was found between these two types of compound in their effects on the potential difference across the frog skin preparation (see review of Ussing, 756). This will be considered later in this review, but it is only fair to point out that strophanthoside K is very active in increasing the excretion of sodium and potassium (702) which is well known to be a complicated effect due at least in part to its extrarenal effects, particularly on the heart and vascular system.

4. The physiological function of sodium-retaining steroids. If we consider the "pure" sodium-retaining steroids, that is those in which glucocorticoid activity

is absent or weak relative to their sodium-retaining activity, such as deoxycorticosterone and aldosterone, the weight of present evidence suggests that most, if not all, of their activity is due to an action on the renal tubular handling of sodium and hydrogen ions. The effects of these steroids on extrarenal factors controlling the movement of electrolytes is far less prominent (710) and in many cases has been shown to be due to the effects of potassium depletion and, with large doses, of water depletion. It might not be too extreme a view to suggest that a *direct* extrarenal action of these steroids on normal mechanisms of electrolyte transport be considered unproven until further work has been done to clarify the position (*cf.*, however, 51, 810, 810a).

When we consider the sodium-retaining steroids which also possess considerable glucocorticoid activity, there is a strong indication that most authors tend to correlate the strength of their extrarenal actions on the distribution of electrolytes and water with their sodium-retaining activity in standard assays. This might suggest that the extrarenal receptors responsible for these actions were in some way similar to the renal receptors for sodium-retaining steroids. Before accepting this, however, it is important to remember that the glucocorticoid activity of such steroids is also much greater than that of the natural steroid hormones, such as cortisol, or even of analogues such as prednisolone (1,2-dehydrocortisol). There is also the possibility that the receptors for such synthetic analogues, while being responsible for extrarenal actions which resemble those of the natural steroid hormones, are in fact quite unrelated to the receptors for the natural steroid hormones.

In considering the structure-activity relationships of sodium-retaining steroids, therefore, we shall pay most attention to their renal actions which resemble, and may actually be due to the receptors for, aldosterone and similar "pure" sodium-retaining steroids. The only extrarenal structures on which a clear and definite action of "pure" sodium-retaining steroids has been demonstrated are those such as the amphibian skin, the goldfish gill (651), the mammalian sweat glands, the mammalian colon, and the sheep salivary gland (282), all of which are specialized for excretory or conservatory functions similar to those of the renal tubule.

E. The biological actions of glucocorticoids

1. The actions of glucocorticoids on the whole animal and on organs and tissues. This field has been the subject of numerous reviews in recent years and once again papers which are of relevance to the question of understanding the structure-activity relationships of the glucocorticoids have been selected for the account which follows. The influence of such steroids on carbohydrate metabolism (105) has been discussed by Thorn (734a, 735), by Frawley (247) and by Renold *et al.* (568). Good general reviews emphasizing the organic chemistry and synthesis of these steroids have been given by Wettstein (786, 787). A very valuable review of the effects of various agents, including steroid hormones, on enzyme systems *in vivo* and *in vitro* is that by Knox *et al.* (422). Roberts and Szego (587) have discussed in detail the interactions of different types of steroids, including the glucocorticoids, in their effects on the reproductive organs and

tissues. When considering these articles, however, one would re-emphasize the complications of many current concepts that result from the theory of the permissive action of adrenocortical steroids (224, 356).

Considerable progress has been made in the analysis of the eosinopenic action of glucocorticoids although the phenomenon is still not completely understood. Since this type of action is still occasionally used for assaying glucocorticoids (*e.g.*, 737) it is worth remarking that the effect of cortisone on the blood eosinophil count is less in adrenalectomized than in normal animals (17), and is followed by a drop in the eosinophil count in the spleen (650; *cf.* 776). It is now well known that eosinopenia is caused in adrenalectomized animals by many forms of "stress" and by adrenaline, histamine, and other drugs (227, 746). These eosinopenic effects are prevented by antihistamines (*cf.* 13) while the eosinopenic action of cortisone is not prevented in this way (711). It might be valuable to find out whether this type of assay for glucocorticoids could be made more specific and reliable by using adrenalectomized animals treated with antihistamines, since it is an extremely sensitive and simple assay.

The effects of steroids and adrenalectomy on the mitotic activity of skin have been made by Ghadially and Green (274, 275). As with other types of action of glucocorticoids, the effects of steroids can be produced with other agents in adrenalectomized animals, and the effect of adrenalectomy disappears after a time (274). Roberts *et al.* (584) found that cortisone reduced mitosis in the liver of the mouse, but had no effect on mitosis in the intestinal epithelium.

Despite the emphasis by Long and his colleagues that glucocorticoids can produce effects on glycogen production independently of actions on protein metabolism, and that actions on protein metabolism probably occur later than those on the concentrations of blood glucose and of liver glycogen (453a), the actions of these steroids on protein and nitrogenous metabolism remain striking and important (18, 45, 87, 88, 150, 201, 224, 262, 285, 301, 455, 456, 524, 540, 567, 586, 640, 653, 812).

Whether or not these effects are secondary to an effect on a crucial reaction in carbohydrate metabolism, one of the most important facts is the finding of Engel (224) that none of the steps in the conversion of protein in the plasma to nonprotein nitrogen in the plasma of nephrectomized rats was affected by the administration of adrenal extract, although this extract or cortisone increased the rate of accumulation of nonprotein nitrogen in such animals. He therefore concluded that the effect of the steroid was on some step leading to the mobilization of protein from the peripheral tissues, a conclusion in agreement with the findings of Roberts (586) and of Fritz (262; also 773). All the studies on other phases of protein catabolism have time-courses which would fit this interpretation, *i.e.*, they could well be secondary to such an effect. Both Schwartz *et al.* who studied the effects of adrenal steroids on dipeptidase activity in diaphragm (640), and Rosen *et al.* who observed enormous increases in the activity of hepatic glutamic-pyruvic transaminase, concluded that these phenomena came on too slowly to be considered a direct effect of cortisol on the enzymic reactions themselves (598, 599, 600, 640).

The nature of these effects is not clear. In the case of tryptophan peroxidase, it has been found by Knox and his group that the injection of substrate into rats induces the formation or activation of the hepatic enzyme (420, 421; *cf.* 732). Knox has also shown that the induction of tyrosine-α-ketoglutarate transaminase by the administration of L-tyrosine is absent in adrenalectomized rats but is restored by treatment with cortisone, that is to say, it represents another permissive action of glucocorticoids (449).

The three major controversies over the actions of glucocorticoids in the whole animal are concerned with (a) the nature of the relationship between the effects on nitrogenous and nonnitrogenous metabolism; (b) whether the effect on nitrogenous metabolism is mainly one of protein catabolism or of the inhibition of protein anabolism, or a mixture of both; (c) whether the action of the steroids is exerted on the peripheral tissues or on the liver, a problem with many resemblances to the similar dispute over the renal and extrarenal actions of sodium-retaining steroids.

Many of the gross effects of these steroids suggest that they inhibit protein synthesis, particularly the inhibition of mitosis and cellular growth in many types of tissues (33, 45, 114, 117a, 201, 371a, 395, 440, 554, 658; *cf.* 584). However, the effects of these steroids on lymphocytolysis and involution of the thymus would at first sight suggest the opposite interpretation (34, 113, 747a, 774). The same is true of some studies on the incorporation of isotopically labelled amino acids into tissue protein (262, 540, 812). The experiments of Fritz (262), however, show that there is a movement of protein from peripheral tissues to the liver under the influence of cortisone, and also suggest that the more recently synthesized protein in the muscles is mobilized earliest. However, the majority of experiments in this field do deal, as Long suggested (453a), with rather late and extremely complicated metabolic changes which may well be entirely secondary to a direct effect of these steroids on nonnitrogenous metabolic pathways.

One cannot, however, be quite so sure about Long's proposal (453a) that the *direct* metabolic action of these steroids is upon the liver, and that peripheral actions are secondary to this hepatic effect. Thus, for instance, effects can be produced on both carbohydrate and protein metabolism in the eviscerated animal (*e.g.*, 88) and the fact that these effects are not as large as in the intact animal is not a serious criticism of these findings. Similarly, there are definite cytological and anti-inflammatory effects (*e.g.*, 31; see below) which occur *in vivo* with local application of steroids in doses which are too small to produce systemic effects. The same doubts exist over the effects of these steroids on lipid metabolism (19, 102, 146, 311, 362, 755).

On the other hand, numerous studies on the carbohydrate metabolism of patients with Cushing's syndrome, and of the effects *in vivo* of cortisone and similar steroids in other mammalian species, tend to confirm Long's picture of the primary importance of the effects of glucocorticoids on nonnitrogenous metabolism. Thus, many workers have found that glucocorticoids raise the plasma concentration of pyruvate and decrease pyruvate tolerance (247, 248, 277, 312, 404, 454), and others have demonstrated effects of cortisone and similar

steroids on glucose metabolism which cannot easily be explained as secondary to, or even associated with, major changes in the metabolism of nitrogenous substances. Numerous workers have also found that the synthesis *in vitro* of glucose and glycogen from lactate or pyruvate by liver slices which have been taken from animals treated with glucocorticoids is much greater than by slices of livers from untreated animals (19, 425, 569).

The exact nature of the "steroid diabetes" that can be produced with glucocorticoids is still not clear. In the first place, the subject is complicated by species differences, the guinea pig being very sensitive, and the cat and the rat being rather insensitive to this type of effect of glucocorticoids (363; *cf.* 695). In the second, the evaluation of experiments on the whole animal is made difficult by the number of pathways that may be involved, and by temporal changes in the nature of the response (*e.g.*, 46, 357, 559, 644). On the other hand, the older idea that the glycosuria caused by glucocorticoids in many species, including man, was due largely to a diminished tubular reabsorption of glucose in the kidney seems to be invalidated by most of the more recent work which has shown that such steroids definitely diminish glucose tolerance (264, 341, 593, 777, 779; *cf.* 569, 695); Froesch *et al.* believe that the resultant rise in blood sugar coupled with increased glomerular filtration rate is enough to explain the magnitude of the glycosuria in man (264). The diabetogenic action of glucocorticoids is one of the most important effects of these steroids that has been shown to be permissive in type (355).

The relationship of the adrenal cortex to diabetes mellitus has been the subject of several papers (229, 355, 492, 734a, 735, 771, 804). One of the most succinct and useful reviews of the metabolic actions of adrenocortical steroids which also gives an excellent account of this particular topic is the paper by Thorn and his group (735). The most distinctive difference between diabetes mellitus and steroid diabetes is the considerable increase in blood glucose that follows an infusion of fructose into subjects with the latter condition (*e.g.*, in Cushing's syndrome). Similarly, the rise in the blood concentration of pyruvate following an infusion of glucose is normal or abnormally large in patients with steroid diabetes, while it is reduced in diabetes mellitus (247, 311, 312).

The most important line of work on anti-inflammatory steroids has been the firm confirmation of earlier work (see 273) that suggested that the anti-inflammatory effect of the glucocorticoids was part of a direct action on the inflamed tissues and not simply a secondary consequence of any "general" metabolic action of these steroids (*e.g.*, 178). A large number of papers have confirmed this in various species for skin (68, 633, 815), the nasal mucosae (502), and for the eye (9, 86, 292, 438). In some cases the lowered resistance to infection that is a well-recognized hazard in the use of these steroids as systemic drugs (see 47, 273) is also seen with local applications. However, glucocorticoids are not effective in inhibiting all types of inflammation, and some types of inflammation (*e.g.*, turpentine abscesses) respond to steroids in one site but not another (677, 678). On the other hand, one must not assume that a local anti-inflammatory action of a steroid is due to *one* type of direct effect. It is now well known

that inflammatory processes are extremely complicated, vary from species to species and probably from tissue to tissue, and that many anti-inflammatory agents act on different steps in the inflammatory process (273, 352, 677, 678). It is by no means certain, therefore, that the local anti-inflammatory action of the 11-deoxy-9α,11β-dichloroprednisolones, for instance, which are inactive by the oral or systemic route, is due to the same type of direct effect as that of cortisol (95a, 591). It is surprising how often it seems to be forgotten that respectable nonsteroids, such as the antihistamines, salicylates, and time-honored preparations of lead, are spectacularly effective local anti-inflammatory agents.

Earlier work on the clinical uses of cortisone and related steroids (*e.g.*, 84, 117, 400, 734, 772, 780) has been confirmed and extended by several clinical trials (10, 307, 482, 748), of which the most interesting is that of the British Medical Research Council's panel (10, 482). This group has shown that patients with rheumatoid arthritis who have been treated for two years with prednisolone are in better health than their controls by all clinical criteria except one. This one exception, however, is both worrying and of great potential significance. It is the titer of the sheep erythrocyte agglutinins that occur in the serum of patients with this disease, which was higher in the treated patients than in the controls. Another interesting feature of this report is the conclusion that 10 mg/day is the highest acceptable dose for the chronic treatment of this condition with prednisolone, and that doses of 20 mg/day led to unacceptably severe side-effects. The recommended dose should be equivalent to about 45 mg of cortisol per day, or just under twice the probable average secretion rate of cortisol in man, in agreement with Savage's finding that treatment with corticotrophin (ACTH) is nearly always successful with a dosage that secures a 1.5- to 2-fold increase in the excretion of 17-ketogenic steroids in each patient (627, 628). Savage's results are also interesting in confirming the longstanding suspicion that the dyspepsia and peptic ulcers often seen in patients treated with oral cortisone and other steroids are due to a local effect of the steroid on the alimentary mucosa or due to its absorption by the portal route, rather than to their systemic effects.

Further work on the effects of glucocorticoids on the healing of wounds and on small blood vessels generally confirms earlier work (273, 427, 428, 429, 629) showing that definite local effects can be obtained with doses that do not produce systemic effects, and that there is a definite difference between mesenchymal cells and epithelial cells, the latter showing no inhibition of growth or division (202, 336, 344, 371a, 372, 429, 440, 535, 800). On the other hand, numerous other agents exert similar effects, particularly in the case of small blood vessels (136, 399, 426, 486). These effects are as complicated as any other feature of the biological activity of the glucocorticoids. Thus, prednisolone administered to rabbits immediately after local irradiation of the stomach inhibits the appearance of gastric ulcers, whilst it increases the incidence of perforation of the ulcers if administered after the ulcers have developed (303). Similarly, the slight protective action of cortisone against anaphylactic shock (*e.g.*, 134, 273) is usually measured by observing the proportion of deaths in the population of mice or guinea pigs being used. In both these species, however, death in anaphylaxis is

due largely or entirely to asphyxia, and the major effect of cortisone is exerted not by inhibiting the inflammatory process of anaphylaxis but by increasing the animals' tolerance of anoxia (273; *cf.* 317).

A most important consideration in reviewing the anti-inflammatory activities of different glucocorticoids is that nearly all the activities reported are based upon assays that employ some form of artificial granuloma as the test object (*e.g.*, 314, 583, 591; *cf.* 646). Although part of the inflammatory process in this type of assay may be similar in part to those of the so-called collagen diseases (273, 730b), it is very unlikely that the resemblance is at all close. It is possibly this which results in the activity of cortisone analogues in therapeutic applications in man being much lower than expected on the basis of their anti-inflammatory activities in animal assays (*e.g.*, 85a, 248). Silber (660) concluded that the closest parallel to the therapeutic activities of cortisone analogues is usually given by their activities in the liver glycogen assay and not by their anti-inflammatory activities in the usual assays. It would be interesting to reinvestigate the anti-inflammatory activities of cortisone-like steroids, using an animal assay based on a delayed allergy. A few steroids have been assayed using D. A. Long's tuberculin sensitivity test (133), and the proliferative arthritis caused by injections of the exudate from Murphy's lymphosarcoma might form the basis of a useful assay (315).

One of the most interesting metabolic actions of glucocorticoids which may well be related to their action on the collagen diseases is on the synthesis of polysaccharides of the type found in cartilage and in the ground substance of connective tissue (see below). A useful review of this field has been given by Bazin and Delaunay (49), and of the more physiological aspects by Baker and Adams (32).

2. Actions of glucocorticoids on isolated tissues and on enzyme systems. It is convenient to group together these two fields of study. It is in these two fields that we expect to find the clearest demonstrations of the absence or presence of specific and direct actions of humoral agents. The first clear demonstration of an action *in vitro* of adrenocortical steroids on well-defined enzyme systems is probably that of Hayano and Dorfman (308), who showed that deoxycorticosterone, cortisone, and other steroids inhibited the action of D-amino acid oxidase on a number of substrates and, in less detail, that the same was true of tyrosinase, urease, ascorbic acid oxidase, and transaminase. Since then, many other enzymes have been studied in this way (see companion papers to 308). In all cases it appears that the effects that have been obtained either require very large concentrations of steroid for their appearance, or else bear little resemblance to *in vivo* phenomena in the relative effectiveness of different steroids in producing them (*e.g.*, 166, 373, 512). Engel and Scott (225a) have drawn the important conclusion that the relative activity of different steroids in many of these effects *in vitro* is governed mainly by their free energies of adsorption to a hexane/water interface (504), in other words, by their unspecific properties as surface-active agents. That the same type of unspecific action may be at the bottom of many studies *in vitro* is suggested by the fact that it is usually found in such studies

that a wide range of steroids is active in producing the phenomenon under observation (*e.g.*, 81; see also 273, 629) and that the steroids which are active come from many or all types of steroid hormone; the findings in fact are interesting from the pharmacological point of view but make little or no physiological sense. Much of the earlier and even recent work is subject to the criticisms of Umbreit (754, 755) (see above).

A few studies, however, are not subject to these criticisms. Among these the most interesting is probably the demonstration by Overell *et al.* (533) that cortisol and prednisolone in concentrations of 0.01 μg/ml or above inhibit strongly the uptake of glucose by isolated strips of mouse skin *in vitro*. Deoxycorticosterone and a number of other steroids, including estradiol, testosterone and progesterone, were less than $\frac{1}{200}$ times as active as cortisol.

It is not easy to assess the physiological importance of the action of glucocorticoids on the uptake of sulphate by connective tissues that has been reported by several groups of workers (149, 432, 789). In both rat and chick tissues cultured *in vitro*, the uptake of radioactive sulphate is inhibited by various glucocorticoids in concentrations around 1 to 2 $\times$ 10^{-4} M. Whitehouse and Lash (789) have made the interesting observation that the synthesis of polysaccharide itself is not inhibited by the steroids, but simply the sulphation process that leads to the formation of chondroitin sulphate and similar substances. The phenomenon shows, however, several peculiar divergences from what would be expected if this action were the basis of the biological actions of glucocorticoids *in vivo*, or even the basis of their anti-inflammatory activity. Thus, a variety of cortisone-like steroids, including 6α-methylcortisol and prednisolone, were found to be active by Whitehouse and Lash (789), but 6α-methylprednisolone was inactive. Similarly, Clark and Umbreit (149) found that cortisone and deoxycorticosterone inhibited, while cortisol stimulated the uptake of radioactive sulphate by rat cartilage *in vitro*. Cortisone was, however, effective only at a concentration of 0.25 $\times$ 10^{-3} M. Despite the very attractive possible connection between this work and the effect of glucocorticoids on the ground-substance of connective tissues in the "collagen diseases," one must regretfully conclude that these actions *in vitro* of such steroids cannot be regarded yet as of proven physiological significance.

A very large number of papers has appeared describing changes in the activity of enzymes that are demonstrated by assays *in vitro*, but in which the steroid is effective only *in vivo*. The majority of such work has been on enzymes concerned with steps in the Embden-Meyerhof pathway of carbohydrate metabolism, and while interesting provides little or no evidence as to whether these changes are direct or secondary effects of glucocorticoids (20, 263, 401, 437, 483, 501, 775). However, Civen and Knox (146) in an important paper showed that the activity of tryptophan pyrrolase in the liver of rats can be increased either by induction with tryptophan in the diet or by giving cortisol. By using pyridoxine-deficient rats, they were able to show that the effect of cortisol on the enzyme was not a simple induction of the enzyme by an action of the steroid leading to an increased supply of substrate to the liver.

Perhaps the best example of the use of ludicrously large concentrations of steroid hormone in an experiment *in vitro* is the reported antagonism of histamine-induced contractions of isolated guinea pig tracheal muscle by cortisol hemisuccinate in a concentration of 1 to 2 mg/ml (435).

F. The biological actions of steroid sex hormones

1. The biological actions of estrogens. The interpretation of the structure-activity relationships in this field is extremely difficult because of the large number of nonsteroid substances which are extremely active (*e.g.*, 716). This may well be correlated with the fact that the vast majority of substances with this type of activity are phenols. A brief account of some of the recent work is, however, necessary.

The activity of estrogens is nearly always assayed by means of their action on the uterus, usually taking the increase in the weight of this organ as the measure of the response. In a number of detailed studies, Huggins and Jensen have shown how complicated this response is (347, 348, 349, 350). First, the log dose-response curve of steroid estrogens with oxygen-containing substituents at positions 6 or 16 is quite different from those of other types of steroid estrogens in that it has a much smaller slope and reaches a maximum or plateau only at very high doses. More typical estrogens give curves with a sharp rise from a definite threshold dose, reaching a plateau at low doses. The atypical estrogens they called "impeded estrogens"; these steroids are also peculiar in that they *antagonize* the increase in uterine weight caused by typical estrogens. They also pointed out that many typical androgens cause an increase in uterine weight; this, however, is histologically quite distinct from that of estrogens and is further distinguished by the fact that it is not antagonized by progesterone. They have also shown that many steroids produce estrogen-like effects upon the vagina of rats (347), but that very few of these compounds produce the full-blown histological changes with keratinization of the vaginal epithelium that is caused by administering a typical estrogen.

The activity of estrogens in changing the metabolism of lipids, particularly as shown by a rise in the plasma concentration ratio phospholipid/cholesterol, has recently been the subject of many investigations (161, 196, 197, 503, 561, 564, 590). Because of their possible use in the study or treatment of atheroma, efforts have been directed towards the synthesis of substances possessing this action but devoid of the actions of estrogens on primary and secondary sexual tissues. It is not known how this effect is produced nor what relationship it bears to the other extrasexual actions of estrogens, such as on the metabolism of other types of steroid by the liver (747), on the histology of skeletal muscle (422a), on the blood pressure of the rat (692), on the biochemistry of the adrenal cortex (*e.g.*, 342, 389, 767), and on the effects of thyroid hormones (189).

Although estrogens affect most tissues of the body, many recent studies have attempted to discover the earliest recognizable changes that are produced in the uterus by estrogens. These have been remarkable for their emphasis on the uptake of water and certain solutes by the uterus and the vascular and cytological

changes that might be responsible for this. Holden in 1939 (377) observed that the uterus became hyperemic within 1 to 2 hours of giving estrogen to the immature rat, and Williams in 1948 (801) confirmed this and showed that while progesterone enhanced this effect of estrogen it had no effect on its own. Kalman and his colleagues found that the uptake of albumin labelled with I^{131} by the rat uterus *in vivo* was noticeably increased within 1 hour of injecting ethinyl estradiol, and that the uptake of water and sodium had reached their maximum within 4 hours (391, 392). They found that the uptake of chloride and potassium was both smaller and later than that of sodium, and concluded that the phenomenon was not due simply to an increased rate of ultrafiltration of plasma from the uterine capillaries. Hechter and his colleagues, however, found that estrogen caused an increase in the extravasation of dyes from the uterine capillaries and that estradiol produced no effect on the penetration of labelled D-xylose or α-aminoisobutyrate into the uterine cells (300, 309).

Following up earlier work on the rapid changes in water content and β-glucuronidase activity of the uterus that were caused by estrogens (see 587, 675), Spaziani and Szego have obtained strong evidence that one of the earliest effects of estrogens is to cause a release of histamine from the uterus (673, 674, 675). They were able to produce the maximum uptake of water by the uterus that could be obtained with estrogen by intrauterine injections of histamine or of the histamine-releasing agent, compound 48/80. Cortisol did not prevent the fall in the uterine concentration of histamine due to estrogen but did prevent the usual uptake of water; similarly it prevented the uptake of water caused by histamine or 48/80. Two out of four antihistamines that were tried prevented the effect of estradiol on the uptake of water by the uterus. The time-course of these effects and the magnitude of the histamine release caused by estradiol (17% of the content of the control uterus) are sufficient to account for this effect of estradiol entirely in terms of its histamine-releasing action, and the authors suggested that the early effect of estrogens upon the uterus may in fact be mediated by histamine. However, Spector and Storey (676) have been able to extract from the uteri of estrous rats a substance which causes the emigration of leucocytes from the blood vessels of rat skin and which might be a mediator of the action of estrogen on the uterus itself; this substance, however, is unlike histamine in other respects.

Other works on the actions of estrogens cannot be considered here in detail. Attempts to demonstrate early actions of estrogens on uterine enzyme systems have led to many interesting results (see, *e.g.*, 503a) but they are mostly subject to the same considerations as apply to similar experiments with glucocorticoids (*e.g.*, 781) with the exception of the work of Talalay and Williams-Ashman on the catalysis of the transhydrogenation of pyridine nucleotides by estrogens (724, 802; see below).

2. The biological actions of gestagens. Earlier work has been reviewed by Roberts and Szego (587) and there is little to report that affects seriously the consideration of structure-activity relations in this class of sex hormones. Many new progesterone analogues with enhanced activity, particularly activity by the oral route (*e.g.*, 199, 234, 235, 413, 626), have been synthesized. The effectiveness of

progesterone and deoxycorticosterone in maintaining pregnancy in the spayed rat has been analyzed by Alexander *et al.* (4). They made the very interesting observation that survival of fetuses was greatly increased in the spayed rat by incising the uterus so that the amniotic sacs were not subjected to intrauterine pressure. This work suggests that the principal action of progesterone in maintaining pregnancy is upon the factors determining intrauterine pressure in the later stages of pregnancy (*e.g.*, 636), and not upon those determining the efficiency of the placenta in its metabolic and nutritive functions.

While the assay of gestagens is still largely carried out using the Clauberg method as modified by McPhail (463), a number of other types of biological activities has been investigated in recent years, particularly those connected with fertility (571, 620, 621). However, in an assay using the occurrence of pregnancy in a mixed population of rats as the "response," Saunders (620) found that diminution of the pregnancy rate by synthetic steroids was correlated much more closely with their estrogenic activity than with their progestational activity.

Amphenone, a compound known to reduce the secretion rate (per weight of tissue) of corticosterone and cortisol by the adrenal cortex (749, 767), has a progestational action *in vivo* as judged by a secretory reaction of the endometrium. This quasi-progestational action has been investigated by Tullner (749), who has shown that it does not occur in adrenalectomized rabbits maintained on a constant dose of cortisone; this suggests that it is due to the progestational effect of one or more adrenocortical precursors that are secreted during treatment with amphenone.

As with several other types of steroid, progesterone and other gestagens in very large concentrations (100 to 400 μg/100 ml) have been shown to have effects upon the contractions of the isolated papillary muscle of the cat heart (603) and to affect the beating of the isolated perfused heart (15, 603). It causes a reduction in the amplitude of contraction; this may be masked in the perfused heart by a more slowly appearing bradycardia, and the effect is greatly reduced by adding serum to the perfusion fluid, probably because progesterone is strongly bound by albumin and other serum proteins (167b, 612, 632).

3. The biological actions of androgens. The field has been dominated by the search for compounds with a high so-called "myotrophic/androgenic" activity ratio, that is, for the ideal "anabolic" steroid (365, 592, 623, 624). The earlier work of Kochakian on the renal effects of androgens (423, 424) has been followed up by Hewitt (326), who has found that very low doses of testosterone propionate will protect the kidneys of castrate mice against the tubular necrosis that is caused by small concentrations of chloroform. Many androgens prevent the full degree of atrophy of the adrenal cortex that is caused by hypophysectomy or by large doses of cortisone (see 108). On the other hand, the secretion of ACTH in man and in rats appears to be inhibited by androgens (103, 108, 825). The evidence in man (108) may, however, be complicated by the effects of androgens on the metabolism of thyroid and adrenocortical steroids (232, 747); the evidence in the rat is less affected by this possible criticism.

The effects of androgens on metabolic reactions have been studied in a number

of papers (160, 165, 437, 526, 563, 718). Contrary to expectations based on clinical findings, testosterone was found to reduce the blood concentration of cholesterol and the incidence and severity of atheromatous lesions in rabbits on a high cholesterol diet (160). Ranney and Drill (563) found that a wide variety of androgens reduced or prevented the fatty infiltration of the liver caused by giving ethionine to rats. This was correlated with their levator activity and quite independent of their androgenic activity. Androgens also potentiate the hypoglycemic action of insulin and protect animals against diabetogenic agents. This work was developed by Houssay and has now been extended to man (719).

Lindner (450) has shown that in many species the testis of the immature animal secretes mainly the weak androgen, androst-4-ene-3,17-dione; at puberty the secretion of testosterone begins to increase so that it becomes the main secretory product. The concentration of testosterone in the peripheral blood of man is low, but it is present in very low concentration in the blood of women as well (527). The wide variety of actions possessed by androgens is most strikingly demonstrated by the interesting work of Talalay and his colleagues (see 472), who have shown that a number of androgens and similar inactive steroids will induce the formation of enzymes capable of utilizing such steroids as the sole source of carbon in *Protozoa* such as *Pseudomonas* spp. An important feature of the actions of androgens is that the truly anabolic effect of testosterone (*i.e.*, a definite positive change in nitrogen balance) begins only at a time when all but traces of the effective dose of the steroid have been metabolized and excreted by the body (779a).

G. Specificity and overlap in the biological actions of steroids

1. Glucocorticoid activity. There is very little evidence that the majority of steroids with pronounced estrogenic, androgenic, or gestational activity have any activity at all in the liver glycogen assay. It is clear, however, that a large number of steroids exist which possess both glucocorticoid and sodium-retaining activity. However, the most striking fact is that the very potent and chemically simple sodium-retaining steroid, deoxycorticosterone, is inactive in the standard liver glycogen assay. Similarly, glucocorticoid activity is possessed by a large number of synthetic steroids which have either no sodium-retaining action or the effect of increasing the excretion rate of sodium.

On the other hand, Fried (250) has pointed out that the synthetic steroid, 9α-fluoro-11β-hydroxyprogesterone, is like a "skeleton key" to many of the receptors for steroid hormones. It thus possesses considerable glucocorticoid, sodium-retaining, and progestational activities.

2. Sodium-retaining activity. No serious claim has been made that typical androgens possess sodium-retaining activity, and the same is true for estrogens, although the latter of course have profound effects on the movement of water and salts in the primary and secondary sexual tissues. Certain gestagens, including progesterone itself, have a weak but definite sodium-retaining activity (23, 250). On the other hand, this is complicated in some compounds by an action which antagonizes the sodium-retaining activity of deoxycorticosterone in

certain dose ranges, and which is almost certainly exerted on the renal tubule (381, 431). The sodium-retaining activity of glucocorticoids has already been discussed. One would emphasize again that these two types of activity can be dissociated completely in many synthetic steroids, and that the two substituents which are responsible for the appearance of the maximum glucocorticoid activity in the *natural* steroid hormones, namely the 11β- and 17α-hydroxyl groups, reduce the sodium-retaining activity of the root steroid, deoxycorticosterone, 20- to 50-fold.

3. Estrogenic activity. The vast majority of glucocorticoids, sodium-retaining steroids, androgens, and gestagens show no estrogenic activity in terms of their effect on uterine weight and histology. Many steroids produce effects on the histology of the vagina and its desquamated products, but close analysis of these actions shows that they are nearly all different from the full picture produced by estrogens (349). On the other hand, certain androgens, especially the $\Delta^{5,6}$-3β-hydroxysteroids, come very close to producing a typically estrogenic effect on the vagina of the rat and the mouse (*e.g.*, 171).

Some recent synthetic steroids based on estr-5,10-ene-3-one undoubtedly possess significant estrogenic activity at the same time as possessing very weak androgenic or levator activity, and quite considerable progestational activity. These findings are, however, subject to some of the reservations given later, which make it impossible to state definitely that such steroids themselves interact with the receptors for estrogenic activity. The number of steroids, often with enormous potency as gestagens, androgens, or corticoids, which possess no estrogenic activity is much more impressive than the small number of synthetic steroids which possess estrogenic actions in addition to other types of action. This is all the more striking when one considers the very large number of non-steroids which are potent estrogens.

4. Androgenic activity. The androgenic activity possessed by typical glucocorticoids is certainly small. Despite the numerous statements that therapeutic doses of anti-inflammatory glucocorticoids are often virilizing in women (see, *e.g.*, 84, 85; see 482, 627, 628), it is probably more correct to conclude that their androgenic activity is either negligible or indirect. Up to 5% of a dose of cortisol is metabolized to 17-ketosteroids which are themselves weakly androgenic and many of the virilizing effects of cortisone could also be due in part to secondary effects, such as alterations in the metabolism of androgens or synergy with them. There is certainly no convincing case showing that glucocorticoids themselves interact with the receptors for androgens.

On the other hand, there are several progestational steroids, including progesterone itself, which have some weak androgenic effects, although progesterone has a general catabolic action in man (*e.g.*, 194, 431). It is probable but not certain that these steroids are interacting with the receptors for natural androgens. This field needs further study because of the existence of a long-continuing controversy over the clinical use of synthetic gestagens. Thus, numerous derivatives of the 17α-alkyl-19-nortestosterone type have been produced with very low androgenic activity in the usual assays. However, the majority of clinicians

still hold that definite virilizing effects are found in a large proportion of women who have been treated with these agents. The similar controversy over so-called "anabolic" activity has been discussed above.

5. Progestational activity. While there are many weak androgens with pronounced progestational activity (see Tables 5, 6) there are few, if any, very active androgens with more than small progestational activities. Certain steroids which have structural features of the glucocorticoids have low progestational activity. Apart from certain 9α-fluorosteroids, however (250, 467), these compounds either have no glucocorticoid activity at all or else are subject to the reservations given below. They possess usually a 17α-acetoxyl group (see also 198, 216).

6. Antagonistic interactions between steroids and other agents. A large number of antagonistic interactions of steroids has been described. Roberts and Szego (587) treated fully the very complicated effects that have been observed on the primary and secondary sexual tissues.

Since then, a number of simple antagonisms between androgens and estrogens and between gestagens and estrogens have been studied, and a number of new types of compounds with inhibitory or antagonistic actions have been discovered. A number of these investigations (*e.g.*, 204, 349) suffer from the defect that the two agents have been administered as a mixed solution or suspension at one site; because of the solubility properties of steroids, this procedure must be avoided at all costs when looking for truly antagonistic actions. Edgren (204) found that large doses of androgen inhibited the vaginal cornification caused by estrogens but that gestagens and corticoids had no effect on this response; the negative findings in this case can be accepted despite the above criticism. It seems clear that the uterine growth response to estrogens is inhibited by the majority of gestagens (205, 206, 207, 349) but the effect of androgens on this response depends upon the conditions of the experiments and on whether the histological characteristics of the response are taken into account (207, 348, 350, 587). Jackson and Robson (369) found that local application of methylcholanthrene prevented the cornification of the mouse vagina by small doses of estradiol, the response of the seminal vesicle of the castrate rat to androgen, and the progestational response of the rabbit endometrium to deoxycorticosterone. It also prevented the mucification of the vagina of the mouse during pregnancy or following the administration of progesterone.

Saunders (619) observed that estrogens have no antagonistic action on the response of the seminal vesicles, ventral prostate, or levator ani of the castrate rat to androgens. On the other hand, the reduction in growth-rate that is caused by estrogens is reduced or prevented by androgens.

Potent antiandrogen actions have been found by Dorfman and others with 1- and 2-methyloestrone, 1,2-dimethyloestrone (366), a dodecahydrophenanthrene derivative (191), and with two 10β-fluoro-19-nortestosterone derivatives (494). Studies of the possible antagonism by glucocorticoids of the endometrial effects of progesterone in the rat (608) and in the mouse (760) gave conflicting results.

There is little convincing evidence at present that any compound has been found with a definite antagonistic action towards glucocorticoids, in the sense that is usually understood by pharmacologists. Thus, Wilson and Tishler (805) mentioned that allodihydrocortisone (17,21-dihydroxy*allo*pregnane-3,11,20-trione) antagonized the anti-inflammatory effect of cortisone in rats, but there is no supporting evidence and no indication of whether single or separate sites of injection were employed. Similarly, Adams *et al.* (2) found that 21-acetoxy-12α,17α-dihydroxypregn-4-ene-3,20-dione prevented the effect of cortisone acetate on liver glycogen in a dose about sixteen times that of cortisone itself. Again, there is no mention of the use of separate or single sites of injection, and their finding remains unconfirmed. McGuckin and Mason (460) looked for an antagonistic action of 16α,17α-epoxy-21-hydroxypregn-4-ene-3,11,20-trione towards cortisone in the liver glycogen assay but found none. There are, however, suggestions that androgens do diminish or prevent the weight loss that is commonly seen during treatment with large doses of cortisone-like steroids while having no effect on their anti-inflammatory activity (*e.g.*, 371a). Marshall *et al.* (474) found that various 7-oxo- derivatives of $\Delta^{5(6)}$-3β-hydroxysteroids had antagonistic actions towards cortisone. In view of the instability of these compounds, and doubt about their method of administration, these findings require confirmation. It is highly unlikely that the discovery of a definite cortisone-antagonist would remain unknown or unconfirmed for very long in view of its potential importance in research and therapeutics.

Another interesting antagonism is that between heparin and glucocorticoids. Quite apart from the earlier interesting findings of Thomas (730b) that heparin inhibited the effect of cortisone on the Shwartzman reaction, it has been found that the eosinopenic and lymphocytopenic effects of cortisone are antagonized by heparin (302). That this antagonistic action is exerted at some stage fairly remote from the initial step in the action of cortisone is suggested by the finding that heparin is effective if injected up to 2.5 hours after the cortisone.

The only class of corticoid hormones to which steroid antagonists appear to be have been demonstrated at all convincingly is that of the sodium-retaining steroids. These antagonists are the spirolactone derivatives of testosterone and 19-nortestosterone which were discussed earlier in this article (384, 386, 402, 441, 442). These steroids have no effect upon sodium-excretion in adrenalectomized animals or in human patients with Addison's disease (441, 442) and as far as the crude response of the whole organism is concerned they appear to be true antagonists. The only cause for doubting this is the very large dose that is needed for effective action. This is of the order of 1 to 10 $\times$ 10^3 times the effective dose of the compound antagonized (*e.g.*, 386). Much less easy to evaluate are the findings of Sturtevant that a number of steroids antagonize the hypertensive action of deoxycorticosterone and lower the blood pressure of rats rendered hypertensive by previous treatment with deoxycorticosterone and a high-salt diet (689, 690, 692, 693, 696, 697, 701). These effects are of relatively small magnitude and were obtained with estrogens, androgens, and "anabolic" steroids or their closely related analogues. Similar findings with two nonsteroid substances (dihydroxy-

stilbenenitriles) seem even more questionable in view of the admitted toxicity of such substances (691).

A large number of nonsteroid estrogens and antagonists to estrogens are now known. These are of little help in understanding structure-activity relations among the steroids themselves and will not be discussed here.

H. The significance of specificity and overlap of the biological activities of steroids

At first sight this subject appears to be extremely confusing. Many pharmacologists are, in fact, tempted to throw up their hands in horror and assume that the field of steroids is too difficult to be analyzed with profit at present. The situation is not actually quite as bad as all that, as long as one takes into account the semantic difficulties which arise in a field in which assays are carried out in the whole animal, and in which the responses that are measured take anything from 6 hours to 16 days for their completion. It is impossible in such a situation to avoid calling the responses by names which convey far more precision than they really possess. Those familiar with drugs giving responses which are rapid and which can be obtained with isolated tissues are aware of the difficulty of making firm statements about structure-activity relations, or of identifying particular types of receptors for such drugs. It is obviously much more difficult when the whole animal is involved, but practical requirements force us to call such responses by names which have the same implication as those used to describe the faster and more clear-cut effects of drugs with simpler actions.

In defining the types of activity under consideration, the reviewer has therefore based most of the discussion which follows on one type of assay for each type of biological activity, in each case the one which comes nearest to being the *necessary* type of activity for that class of biological activities. At least by doing this one finds that the commonest assays are being considered, and hence the ones for which there is the largest amount of information available. It is now a commonplace that the known "effects" of steroids, and of many other types of hormone, probably all represent the results of long and complicated chains of events. The direct action of the steroid at its target organ, tissue, or molecular structure is what really interests us in most discussions of structure-activity relations; we want to know the first link of this chain of events and we want to know about it in molecular or chemical terms. Despite the existence of many interesting and suggestive leads, there is as yet no steroid for which it is certain that any particular type of cell or structure is its first target of action; nor is there any certainty whether one or several types of receptor are involved.

Nevertheless, much can be inferred about the structure-activity relations of steroid hormones and their analogues if certain facts are taken into account. First of all, the overlapping actions of steroids are not much more extraordinary than those found in other classes of pharmacologically active substances when the magnitude of the overlap is examined. Thus, aldosterone has a glucocorticoid activity approximately equal to that of corticosterone, but its sodium-retaining activity is approximately 400 times that of corticosterone (23, 663, 679). Similarly, many compounds described as having weak estrogenic activity are in fact

only $\frac{1}{200}$ or $\frac{1}{1000}$ as active as estradiol (*e.g.*, 69, 171, 347, 366, 367, 407, 465, 560, 759). When in addition one remembers that steroids are notoriously difficult to purify and that the synthesis of steroid analogues nearly always involves a large number of chemical reactions, in some cases microbiological, it becomes less surprising that certain steroids are reported to have biological activities in the range of 0.1 to 1.0% of that of a natural steroid with a different type of activity. Finally, it will be seen below that the metabolic alterations that are undergone by steroids play a significant and often considerable part in determining the magnitude and even the range of their biological activities. Although this argument can be overdone, it is by no means negligible in certain cases. Thus, for instance, it is now clearly established that testosterone is converted to estradiol to a small but significant extent (30, 607a). Chemically, this conversion is of marginal interest, but because of the very high molar biological activity of estradiol compared with that of testosterone, it is undoubtedly of significance. It is important, however, to assess the weight of this argument quantitatively in each case (see below).

Finally, we must consider the inadequacy of statements which are based entirely upon one type of assay. While the comparison of structure-activity relations among steroids of moderate or large potencies *within one class* of biological activities is best made on the basis of one well-established type of bioassay, it does not follow that the discussion of overlapping activities should be conducted similarly, especially when low potencies are being considered. Thus, for instance, a weak activity in depressing the blood pressure of rats treated with deoxycorticosterone would be expected of a wide variety of agents other than steroids [and indeed is found (689)]. Similarly, many steroids and some nonsteroids may cause an increase in the weight of the uterus of a spayed or immature mouse or rat or have "anti-inflammatory" actions; before such effects are classifiable certainly as "estrogenic," or "anti-inflammatory," it is necessary to examine their histological and functional characteristics.

I. Dissociation of the biological effects of steroids

The question of the dissociation of the different types of activities of steroid hormones is closely related to that of the specificity and overlap of their actions. All three topics bear on a discussion of the chemical nature of the receptors for these agents, and of whether or not their receptors are the same or different in the various tissues which are affected by them. It is impossible to answer questions such as these until unequivocal evidence has been obtained that the "effects" seen in different tissues and organs are indeed due to a "direct action" of the agent on the tissue concerned. Thus, for instance, it is known that insulin in concentrations close to or identical with those found to be effective *in vivo* produces effects on many different isolated mammalian tissues which are similar to, or accounted for by, those it produces on these organs *in vivo* (see 602). It is justifiable to conclude that receptors for insulin probably exist in all these tissues. No such clear evidence is available for steroid hormones and their analogues because of the lack of effects *in vitro* which would explain convincingly their actions

in vivo. Nevertheless, some evidence does exist which suggests that certain effects of steroids are most probably due to receptors in the tissues in which these effects are observed.

The only way of showing by means of tests *in vivo* that local receptors exist is by demonstrating a clear-cut local action of the agent with doses which produce little or no effect on other tissues. Dissociation of the effects obtained by systemic administration of the agent, while often seeming to be very suggestive, is always open to doubt because of the numerous factors which determine the distribution of the agent in the body. It has been emphasized elsewhere that the steroids are especially prone to be affected by such factors (124a) because of their markedly varying solubility properties, their strong tendency to associate with specific or nonspecific substances of high molecular weight (504, 505, 612, 632), and the importance of metabolic reactions in determining their elimination from the organism. The following pieces of evidence seem to be strong enough to suggest that certain steroids do in fact interact with receptors in more than one tissue or type of cell.

Sufficiently low doses of aldosterone and of 9α-fluorocortisol when injected into one renal artery produce their usual effects on sodium excretion by the kidney supplied by that artery while having no effect on the opposite kidney (39). On the other hand, aldosterone quite definitely has a moderate potency in causing an eosinopenia and in causing a deposition of liver glycogen (679). There is no evidence that the latter two actions are dependent upon the first, and, apart from its improbability, the demonstration of a local renal effect makes it unnecessary to consider that the first effect might be dependent upon either of the latter effects. Again, 9α-fluorocortisol and other 9α-fluorocorticoids produce changes in the electrolyte and fluid distribution of the adrenalectomized dog in crises that occur in the absence of, in fact in spite of, any change in renal excretion (707, 709). However, the best evidence for the existence of both renal and extrarenal receptors for sodium-retaining steroids has been obtained with synthetic steroid analogues which are not secreted *in vivo* (709). Aldosterone is at present notable for its lack of extrarenal effects on electrolyte and fluid distribution (710).

Similarly, many local anti-inflammatory and cytological effects of cortisol and similar steroids can be obtained in the absence of any of the systemic effects seen with oral or parenteral administration. The majority of such local actions have the same histological and histochemical characteristics as those produced by systemic administration (33, 193, 273, 427, 428, 429, 629, 747a). Although it is not certain whether the liver glycogen response is due to a direct action of glucocorticoids on the liver itself, it can be obtained with doses that cause little or no anti-inflammatory effect. However, early claims of the demonstration of a direct glycogenic action of adrenocortical extract on liver slices *in vitro* (143a) were not confirmed by subsequent work in the same laboratory (50b).

It is only fair to add that most workers in the field would consider this approach too critical. They would agree that Sayers's theory of the "ubiquitous role" of the adrenocortical steroids (629) is not tenable today in its original form, but they

would accept the idea that a very large number of types of cell possess receptors for both glucocorticoids and sodium-retaining steroids. To the reviewer, this seems undoubtedly to be a sensible working hypothesis, but apart from the one or two clear pieces of evidence considered above there seems to be considerable room for further work before it can be established firmly. Certainly, few would disagree that the evidence is insufficient to demonstrate the existence of a definite number of recognizable classes of receptor for these two types of steroid hormones.

An important topic in this field is the question of the possibility of dissociating the undesirable "side-effects" from the therapeutically useful anti-inflammatory effects of cortisone-like steroids. There is no doubt that the sodium-retaining effects of such steroids can be reduced or eliminated by chemical modifications, while their glucocorticoid and anti-inflammatory effects are retained or enhanced (5, 8, 16, 63, 64, 65, 116, 509, 578, 667, 681, 727, 728, 737). It is unlikely that this dissociation is due to the effect of the chemical modifications upon factors such as the distribution or elimination of such analogues; it is almost certainly the result of differences in the interactions of these steroids with the two or more types of receptor that are involved in the two gross effects under consideration. On the other hand, the vast majority of the cortisone-like steroids show little or no dissociation of their glycogenic and anti-inflammatory activities. This is true for many hundreds of compounds with activities ranging from 0.1 to 200 times that of cortisol itself (Table 1). One or two compounds, however, do show significant and regular alterations in the ratio of these two activities. In the case of the 9α,11β-dihalogeno-11-deoxyprednisolones, a complete dissociation has been obtained, but their anti-inflammatory activity is obtained only with local application, a finding which is subject to reservations. Among a number of analogues the removal of the 21-hydroxyl group reduces the glycogenic activity of the analogue much more than it reduces its anti-inflammatory activity. Probably the most striking effect of this sort is seen with 17α-acetoxy-21-deoxy-9α-fluoro-6α-methylprednisolone (467, 633, 683), although, again, the evidence drawn from local anti-inflammatory activity (633) is of questionable interpretation. The range of solubility properties possessed by the known 21-deoxysteroids which show this partial dissociation of activities seems to be too great for the dissociation to be entirely explicable in terms of differences in their distribution or metabolic elimination, but further investigation of this point is urgently needed. Even if such factors turned out to be the main reason for the dissociation of activities, a fuller understanding of them might well lead to the synthesis of analogues with therapeutic advantages.

With the steroid sex hormones and their synthetic analogues, there is far stronger evidence that receptors for them exist in many tissues. This is clear from the local application of estrogens and androgens to the vagina of many species (347, 348, 350), and of minute doses of estrogens and gestagens to segments of the uterus that have been isolated by ligatures (see 675). Similarly, androgens and estrogens produce effects confined to the skin (33), and androgens produce enlargement of the capon's comb (507) when these compounds are applied locally in very small doses. It could reasonably be held that the clear-cut demonstration

of widespread receptors for steroid sex hormones is a justification for accepting the hypothesis that the same is probably true of the receptors for glucocorticoids and sodium-retaining steroids.

In the case of major types of activity, the dissociation of the biological activities possessed by natural steroid hormones is fairly easily understood in terms of the presumed natural receptors for these types of activity. However, even with such major types of activity, the assumption that interaction or lack of interaction with natural receptors is the cause of the dissociations that are seen is only an assumption until proven by a detailed analysis. When the biological activity in question is a minor one, or one which has not been subjected to detailed analysis, this assumption is particularly doubtful. With extensive chemical modifications it becomes likely that steroid (or other) analogues interact with receptors which are not affected by the natural steroid hormones. Thus, for instance, one cannot be sure that the receptors for the "lipid-shifting" activity of such analogues as 16α-methylestr-5,10-en-17β-ol-3-one are the same as those for the "lipid-shifting" activity of estradiol itself (*cf.*, *e.g.*, 196, 562, 761).

III. THE INTERPRETATION OF THE RESULTS OF BIOLOGICAL ASSAYS OF STEROIDS

The complications that arise in the interpretation of biological assays of steroids have been discussed in detail elsewhere. When assays are carried out using slow responses in the whole organism, their design has a very considerable influence on the relative importance of the numerous factors that determine the magnitude of the response (124a, 559, 660, 661, 737).

Many of the factors involved have been discussed above. Briefly, most steroids are relatively insoluble substances, but their solubility in water and their partition coefficients between water and nonpolar solvents or lipids vary very considerably. Hydroxylic steroids can be esterified to yield a variety of lipid-soluble esters which are very poorly soluble in water and usually very slowly absorbed after subcutaneous or intramuscular injection. In general, the larger the molecular weight of the monocarboxylic acid the lower the solubility and rate of absorption of the resulting steroid ester (*e.g.*, 271, 338, 339, 340, 607). Steroids can also be esterified with succinic or phosphoric acid to give hemisuccinates or monosteroid phosphates which are very soluble in water and extremely rapidly absorbed (*e.g.*, 729a).

Most assays for steroids are standardized so as to obtain the maximum precision, sensitivity, reproducibility, and specificity of the response that is compatible with a procedure that is convenient and economical. An excellent example of this process is the paper of McPhail (463) on the Clauberg assay for progestational steroids.

While the usual criteria of parallelism and linearity of log-dose/response curves have been employed, the relative potencies of different steroids reported by different groups of workers are often very different, and there are several examples of gross differences between the successive reports of the same group of workers (*e.g.*, 182, 500). A more important defect is that certain assay procedures are designed so that factors other than those connected with the interaction of

the agent with its receptors are maximized rather than minimized. Since such factors are likely to play a greater role in the assay of steroids than in other classes of substances which are more water-soluble, these assays convey a very distorted picture of the effectiveness of certain synthetic steroids in interacting with their receptors. Thus, for instance, Stafford and his group have usually carried out the liver glycogen assay by giving one subcutaneous injection of the steroid in aqueous suspension and then killing the animals and removing their livers for analysis 7 hours later (682, 683). This assay maximizes the influence of the absorption and metabolic elimination of the steroid upon its apparent biological activity; this influence is further increased by the use of the relatively rapidly absorbed and metabolized steroid cortisol as the standard, while the majority of the analogues the authors have assayed were administered in the form of their acetates which are usually, although not invariably, more slowly absorbed (660, 661). Silber and his group on the other hand have used a similar period for the deposition of liver glycogen but have divided their dose into hourly or half-hourly injections, thus minimizing the influence of the rates of absorption and elimination of the different steroids under test (559, 660, 661). The relative potency of certain analogues has differed by as much as 20- to 30-fold when assayed by these two types of procedure. Dorfman *et al.* (191a) have shown that the thymolytic potency of 14α-hydroxycortisol is 0.12 $\times$ cortisol in the rat but 1.0 $\times$ cortisol in the mouse, when given subcutaneously. When given orally in the rat, its potency is 0.19 $\times$ cortisol. Leathem and Wolf (434) found that 9α-chlorocortisol acetate was eleven times as potent as deoxycorticosterone acetate in a single dose, and of equal potency in daily doses, in the life-maintenance assay in rats.

The effect of chemical modifications of a natural steroid upon these secondary factors is not always predictable (559). It is fairly clear, however, that a detailed analysis of the relative potencies of steroid hormones and their analogues needs far closer attention to this sort of detail than can be expected in routine screening assays. In general, amorphous suspensions or solutions in a hydrophilic oil or solvent will be absorbed more rapidly than crystalline suspensions or solutions in viscous hydrophobic oils or waxes (90, 618). Again, it is usually true that the more hydrophobic the steroid the less rapidly it is absorbed parenterally. This was very thoroughly examined and correlated with variations in biological activity in the studies by Kochakian (423, 424) on the renotrophic actions of very nonpolar (hydrophobic) androgens and their derivatives. It is not uncommonly found that very hydrophobic steroids are better absorbed by the oral route than by parenteral administration (424).

Differences in absorption can have striking effects upon assays using local applications of steroids, such as in the local anti-inflammatory assay in which the cotton-wool used to stimulate granuloma formation is soaked in a solution of the steroid to be assayed. Thus, sodium prednisolone phosphate is quite inactive in this test unless doses are used which are large enough to give a systemic effect, because it is rapidly absorbed from the pellet (Silber, personal communication). On the other hand, the racemic synthetic *dl*-cortisone acetate, which has half the activity of natural *d*-cortisone acetate in the liver glycogen assay (*systemic*

administration) is much more active than *d*-cortisone acetate in the *local* granuloma assay. Sarett and his colleagues (558) pointed out that this is almost certainly due to the very low solubility of the racemate, since the activities of different esters of *d*-cortisone are correlated inversely with their relative solubilities in water (558, 583). Bush and Truelove (unpublished) found that although sodium cortisol hemisuccinate, which is highly effective in the local treatment of ulcerative colitis (Truelove; see 769), was well absorbed from the colon after administration as an enema, there was little evidence of any systemic effect as judged by the absence of any suppression of urinary excretion of dehydroepiandrosterone and other metabolites of adrenocortical steroids. Wade *et al.* (769) obtained some suppression of adrenocortical secretion by rectal administration of cortisol but used much larger doses; this apparent reduction of the usual systemic effect of cortisol may well have been due to the metabolic degradation of cortisol in the colon by the bacteria normally present (769).

IV. THEORIES OF THE NATURE OF THE ACTIONS OF HORMONES

The hope that direct effects of hormones on enzyme reactions would be discovered remains unsatisfied in the sense that although some direct actions have been found *in vitro* with hormones in concentrations close to those found *in vivo*, there is considerable doubt as to whether these actions represent the molecular basis for their characteristic biological activities *in vivo*. It is a common view of biochemists that this type of action must be the basis of hormonal activity because the rate-limiting steps in metabolic pathways are most likely to be the points at which regulators of metabolic activity would exert their effect. On the other hand, physiologists and pharmacologists have always emphasized the importance of the rate-limiting steps in metabolism caused by barriers to the transport of substances from one part of the organism to another (see 124a).

A large number of workers in this field are now tending to search for hormonal effects on the permeability of cellular and intracellular membranes, rather than to pursue the search for direct effects upon enzymic reactions (172, 308a, 309a). A considerable impetus to this return to earlier concepts has been given by the demonstration that a large number of the effects of insulin (602) and of vasopressin (7, 756) are probably due to effects upon the permeability of cells. The work discussed earlier on the stimulation by estrogens of the uptake of water by the uterus, the profound effects of the adrenocortical steroids on the distribution and excretion of water and electrolytes, and the effects of steroids and other agents on the permeability of cells to the nonmetabolizable α-aminoisobutyric acid (524) have all supported this trend of thought. It is still true, however, that these effects upon the permeability of cells may themselves depend upon enzymes or upon transport systems which have many of the properties of enzymes.

A more specific type of theory of how steroid hormones might affect cellular permeability to ions has been developed in great detail by Wilbrandt and his colleagues (791–796; *cf.* 638). This theory postulates that the sodium-retaining corticoids act as lipid-soluble carriers for metal ions. It is generally supposed that the specific permeability properties of cellular membranes for different types of

cations are due to the existence of substances with specific affinities for these cations. The compound thus formed is usually supposed to be hydrophobic in character in order to enable it to penetrate the lipid membrane of the cell. A number of naturally occurring substances have been proposed as possible carriers, but none of them has sufficient specificity of its affinities for sodium and potassium to account for the observed properties of cell membranes with respect to these ions.

As stated, Wilbrandt's theory seems to be untenable. First, there is no good evidence that the ketol side-chain of the natural sodium-retaining steroids can form the sort of chelates that he postulates, or that their stability or solubility properties would be sufficient for a carrier function. Secondly, a fair number of 21-deoxy steroids are now known which have considerable sodium-retaining

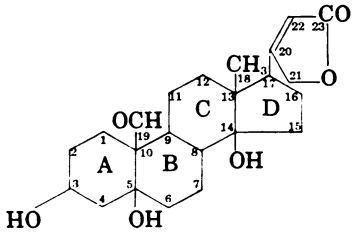

FIG. 1. Strophanthidin

Notice the 5β-hydroxyl and the 14β-hydroxyl. Because of the *cis* A/B and C/D ring-junctions, rings A and D are strongly bent back to the α-side (*i.e.*, as if below the plane of this page).

activity (*e.g.*, compounds 117, 120); while it is possible that a small part of their activity might be due to metabolic conversion to the related 21-hydroxysteroids, it is very unlikely that this conversion would be sufficient in magnitude to explain their biological activity on this basis (see below). It thus appears that the sodium-retaining activity of steroids is not dependent upon the possession of the α-ketol side-chain of the natural corticoids. Chelates could not be formed with simple steroid 20-ketones. Finally, a simple feature of steroid stereochemistry has been overlooked. It was suggested that the unsaturated lactone ring of the cardiac glycosides has the same configuration as the supposed chelate formed by the α-ketol side-chain of sodium-retaining steroids, and that hence it could block receptors in cell membranes which combined with the first chelate to produce a completely lipid soluble metal-carrier complex. Since the final complex was supposed to be produced by the addition of a second chelating substance to the first chelate, thus forming a chelate of the X.M.X. type, the theory gave a consistent and reasonable account of the supposed competitive process. However, the stereochemistry of the A/B and C/D ring junctions is quite different in the two types of steroid; in the cardiac glycosides both these junctions are *cis* (*i.e.*, 5 β-H; 14 β-OH) while in the natural steroid hormones they are both *trans* (*i.e.*, 5 α-H or $\Delta^{4,5}$; 14 α-H). This means that, despite the apparent resemblance of the structural formulae as usually written, the cardiac aglycone is a bunched up molecule with both the A- and B-rings strongly "bent" back towards the α-side of the plane of the C- and D-rings (Fig. 1). In three-dimensional structure, then, the

cardiac aglycone molecule is quite unlike the more or less planar molecule of the natural steroid hormones, and from what we know of the biological activities of these steroids, it is unlikely, although not impossible, that competitive interactions will occur between these two classes of steroid. At present, then, it seems unlikely that sodium-retaining steroids act by playing the part of membrane carriers for metal ions. The idea is even more improbable as an explanation of the activities of other classes of steroid hormone in which no potentially chelating groups exist in most of the active compounds of the series.

Another interesting theory is that of Willmer (802a) in which it was proposed that the arrangement of lipid molecules of different types in cellular membranes might be altered by the incorporation of steroid molecules into the membranes. Using the bimolecular lipid leaflet theory of the structure of such membranes, he suggested that steroid hormones could be incorporated into the leaflets as pairs arranged end-to-end with their lengths roughly parallel to the fatty acid chains of the membranes. While there is no evidence for or against such a picture, the original theory lays considerable emphasis on the dipolar nature of most steroid hormones and the stability this would give to an end-to-end arrangement. It must be pointed out that monofunctional analogues of several classes of steroid hormones have now been shown to be biologically active under circumstances in which metabolic conversion to dipolar compounds is highly unlikely to have occurred to any great extent (175, 347, 424; compounds 328, 344, 355, 356, 466).

A highly original theory of the mode of action of steroid hormones which does depend upon a direct interaction with an enzyme system is that of Talalay and Williams-Ashman and their colleagues (724).[1] These workers showed that a relatively crude preparation of isocitric dehydrogenase from placenta was coupled with triphosphopyridine nucleotide (TPN) and not diphosphopyridine nucleotide (DPN) as had been previously thought by Villee, who first demonstrated that this system was activated by minute concentrations of estradiol (763). They also found that the preparation contained a steroid-17β-ol dehydrogenase capable of converting estradiol to estrone with either DPN or TPN as a coenzyme. They therefore suggested that by coupling the two coenzymes with the dehydrogenase and estradiol the system could act as a transhydrogenase according to the following equations:

$$\begin{aligned} &\text{estradiol} + \text{DPN} \rightleftarrows \text{estrone} + \text{DPNH} \\ &\text{estrone} + \text{TPNH} \rightleftarrows \text{estradiol} + \text{TPN} \\ \text{Resultant} \quad &\text{DPN} + \text{TPNH} \rightleftarrows \text{DPNH} + \text{TPN} \end{aligned}$$

Talalay and his group found that this transhydrogenation was very sensitive to the conditions employed, in particular that it was very prone to substrate in-

[1] Here and in the sections which follow the following abbreviations are used.
DPN: Coenzyme 1, or diphosphopyridine nucleotide.
DPNH: Reduced diphosphopyridine nucleotide.
TPN: Coenzyme 2, or triphosphopyridine nucleotide.
TPNH: Reduced triphosphopyridine nucleotide.
ADP, ATP: Adenosine diphosphate, and triphosphate.
UDPG: Uridinediphosphoglucose.

hibition at relatively low concentrations of coenzyme and steroid. The system was quite active with concentrations of estradiol of around 10^{-7} M. These authors proposed that this action could well be the molecular basis of the action of estrogens and that, since other oxidation-reductions of steroid oxygen functions could be coupled with either DPN or TPN as coenzyme, the same type of transhydrogenation could be catalyzed by other steroid hormones. They were subsequently able to show that transhydrogenation could indeed be catalyzed by the steroid-3α-ol dehydrogenases (721, 722, 724a). Talalay and Hurlock later isolated a highly purified steroid-11β-ol dehydrogenase from rat liver (352a); this preparation was similarly effective with either DPN or TPN as coenzyme and cortisol as the steroid, but they were unable to find conditions in which transhydrogenation was achieved.

Talalay and Williams-Ashman therefore made the imaginative proposal that at least some of the enzymes that had previously been thought to be concerned only with the metabolic inactivation of steroid hormones, and which had little or no coenzyme-specificity, were in fact transhydrogenating systems in which the supposed steroid *substrate* was playing the role of *coenzyme* and the supposed pyridine nucleotide coenzymes were in fact the substrates. Numerous objections have been raised to the idea that this type of effect is the molecular basis of the actions of the steroid hormones. In the first place, a large number of steroids are now known in which the supposedly specific oxygen function that undergoes the necessary oxidation-reduction is a secondary hydroxyl group known to be resistant to oxidation in the body, or else has been converted to a tertiary hydroxyl group with a similar resistance to oxidation. Typical examples are 17α-methyltestosterone (androgen), 17α-methyl- and 17α-ethinylestradiol (estrogen), 2α-methylcortisol, 9α-fluorocortisol (124a) and other 9α-fluorosteroids, and 12α-fluorocorticosterone (glucocorticoids). Second, Talalay and Hurlock showed that the steroid 3α-ol dehydrogenase is slightly more active in transhydrogenation with the androgenically *inactive* steroid etiocholanolone than with the active androgen, androsterone. Further difficulties are discussed in the reviews by Villee (764a) and Talalay (724a) and in the general discussions after them.

Villee has now succeeded in separating from the placental system three enzymes, two with 17β-ol dehydrogenase activity. One is a strictly DPN-dependent, and another a strictly TPN-dependent 17β-ol dehydrogenase; the third is a transhydrogenase (DPN/H/TPN) which is sensitive to activation by estradiol in minute concentration but which has no steroid 17β-ol dehydrogenase activity (763, 764a). To the reviewer it seems that the biochemical niceties of the enzyme system are of considerable enzymological interest (although confirmation of Villee's findings is needed) but that there is little at present to suggest that this *in vitro* phenomenon has any bearing on the molecular basis of the actions of estrogens or of any other class of steroid hormones.

An important obstacle to the original theory was the estrogenic activity of the many stilbestrol derivatives, and of other estrogens, steroidal and nonsteroidal, which have no secondary hydroxyl groups which would be capable of undergoing oxidation-reduction with the steroid-17β-ol dehydrogenase. Williams-Ashman *et al.* (724a, 802) have now found that a similar transhydrogenation can be per-

formed by a phenolase and by a peroxidase found in extracts of potato and mushroom. The former involves the *ortho*hydroxylation of the phenol followed by catechol-*o*-quinone oxidation-reduction; the latter involves the formation of a free radical. There is no doubt that these authors have made an elegant analysis of the metabolism of various phenolic steroids by such enzymes of vegetable origin and have shown that synthetic nonsteroidal estrogens are metabolized in the same way. Despite the fact that the concentration of peroxidase in the uterus is increased by the action of estrogens, there is as yet no evidence that similar reactions are undergone by estrogens in the uterus, nor is there any evidence that such reactions, if they exist, play any role in the uterine response to estrogenic action (see 372a, 730a).

Another general theory of the molecular basis of the actions of steroid hormones is that they influence the *formation* of enzymes rather than that they affect the rates of enzymic reactions directly. As discussed previously, there is a large number of results which support this supposition at first sight in that large changes in the concentration of many enzymes have been produced by the administration *in vivo* of all classes of steroid hormones. Some of them undoubtedly make physiological sense in that the metabolic changes produced by the steroid could be due simply to an increase in the concentration or "effective activity" of the enzyme in question. This is particularly striking in the case of the effect of glucocorticoids on the dipeptidase of muscle (597) and on the glutamate-pyruvate transaminase of liver (598, 599, 600). There is little doubt, however, that these changes in enzyme concentration are in fact the consequence of other effects of the steroid on metabolism rather than the other way about, and that in many cases they are produced by the simple mechanism of induction by increased production of their substrates. The only case in which a steroid has been shown to increase the concentration of an enzyme in circumstances where substrate-induction is apparently inadequate to explain the results is that of tryptophan peroxidase (420, 421). It is possible that the increase in the activity of proline oxidase of kidney produced by cortisone also falls into this class (755) but this is not yet proven.

It is possible that the theories sketched above attempt to be too precise for the present stage of our knowledge. It is more than probable, for instance, that many processes exist in living organisms which cannot surely be classified as typically enzymic or carrier-like. The present insolubility of the problem of the molecular basis of the actions of steroids may well depend upon our incomplete knowledge of the nature of some of these processes. What does appear to be significant is that despite an enormous amount of work in this field, indubitably specific hormonal effects on tissues *in vitro* are extremely rare compared with the protean manifestations observed *in vivo*, and that no such effects have been found with steroids in cell-free preparations *in vitro*. Those effects which have been obtained *in vitro* are almost all unspecific in the sense that the relative effects of different steroids bear no relation to their relative effects *in vivo*, and the concentrations at which they are achieved are many orders of magnitude greater than the concentrations found under any natural circumstances *in vivo*. While there is no reasonable ex-

planation of this lack of specificity in terms of known metabolic or other secondary factors, there are good reasons for supposing that these actions can be explained in terms of the known physicochemical properties of steroids considered merely as chemical agents.

V. METABOLIC FACTORS AFFECTING THE BIOLOGICAL ACTIVITIES OF STEROID HORMONES

A. Metabolic pathways leading to the elimination of active steroids

For many years the metabolism of the steroid hormones was studied in the hope that some features of their metabolic fate might explain their biological activities. For the last fifteen years, however, it has been generally believed that most of the pathways that have been discovered are essentially "detoxication" reactions, the main biological function of which is to remove the active hormones from the organism. Despite the stimulating theories of Talalay and his colleagues (724, 724a) suggesting otherwise, the latter view prevails today; there are excellent reasons for this (488). As with most natural humoral agents, the steroids are no exception to the rule that the biological activity of any pharmacological agent is very dependent upon the processes that lead to its destruction and excretion from the organism (*e.g.*, 24). It is an essential feature of any control system that it must be able to operate in both positive and negative directions; "switching off" is as important as "switching on." Where humoral agents are concerned, negative influence can be effected only by the production of antagonists or by the removal of the active agent from its site of action (see 24, 398, 448). Natural selection has apparently led to a predominance of the latter mechanism.

The study of the metabolism of steroids in the last ten years is dealt with very thoroughly in the valuable reviews of Lieberman and Teich (448), Gallagher *et al.* (267), Roberts and Szego (588), Callow (132), and of Katzman and Doisy (398). Berliner and Dougherty (54c) have also discussed this field in a recent review (54c). Although the pathways are seemingly complicated when all the manifold stereochemical details are dwelt upon, they can now be reduced to a surprisingly simple set of general rules. Because of this, it is no longer necessary to consider the different classes of steroid hormones separately; instead it is much more helpful to consider the reactions of different types of substituent quite independently of the type of steroid in which they are found.

1. Metabolism of the 4,5-double bond. This double bond is found in all the major steroid hormones, with the exception of the estrogens, in conjugation with a 3-ketone group (Fig. 2). The vast majority of the known metabolites of all non-benzenoid steroid hormones lack this double bond (Fig. 3), and its reduction in the liver probably represents the rate-limiting step in the metabolic inactivation of these hormones (742, 743). Tomkins and his colleagues have studied the responsible enzymes very thoroughly, and have found that crude preparations of rat liver will reduce the 4,5-double bond of many Δ^4-3-ketosteroids when a TPNH regenerating system is present. Further purification led to the isolation of a variety of enzymes with much greater substrate specificity, which could be

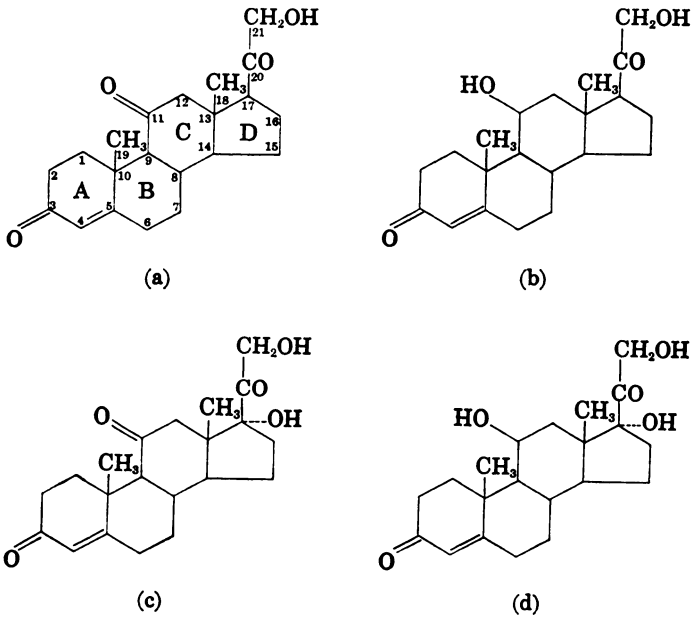

FIG. 2. Four natural adrenocortical steroids showing the Δ^4-3-ketone structure of ring A
(a) 11-Dehydrocorticosterone; (b) corticosterone; (c) cortisone; (d) cortisol.

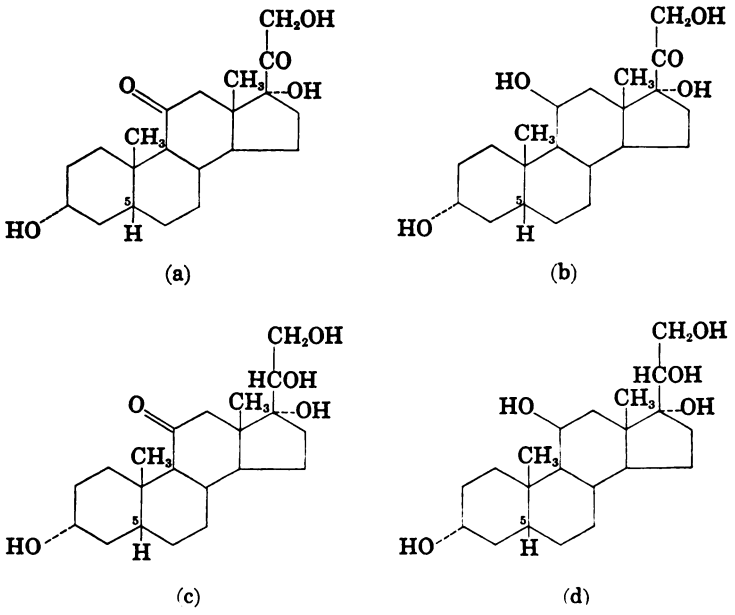

FIG. 3. Four principal urinary metabolites of cortisol in man
(a) Tetrahydrocortisone; (b) tetrahydrocortisol; (c) cortolone; (d) cortol. Note the 5β-hydrogen (A/B junction, *cis*). The 5α-epimers of (b) and (d) also occur in human urine.

shown to be specifically dependent upon TPNH as hydrogen donor. Two types of product result from the reduction of this bond: the 5α-(H)-steroids with a *trans* fusion of the A and B rings, and the 5β-(H)-steroids with a *cis* fusion. The reduction of the 3-ketone group does not occur *in vivo* as far as is known in the Δ^4-3-ketosteroids; it proceeds very rapidly and completely after the reduction of the 4,5-double bond has occurred (742, 743). It is now known that similar reactions are undergone by aldosterone (537, 750a).

The proportions of the two isomers produced by the reduction of any one steroid probably depend largely upon the relative concentrations or "effective activities" of the two classes of enzyme that carry out the reduction. It is known that the enzyme giving the 5α-(H) product ("5α-enzyme") is found in the microsomes of homogenates of rat liver while the 5β-enzyme is found in the cell sap (742, 743, 744). Furthermore, the concentration or "effective activity" of the 5α-enzyme is increased greatly by the administration of thyroxine to rats (461, 462). The same effect is probably one reason for the greatly diminished rate of elimination of cortisol by human beings with hypothyroidism, its restoration to normal in these subjects by the administration of thyroxine, and its greatly increased rate in patients with thyrotoxicosis (*cf.* 124, 310b, 343, 541). This reduction is subject to a number of sources of variation. Thus, the guinea pig is at present unique in showing an almost complete absence of this reaction, so that the major fraction of the recognized urinary metabolites of cortisol is made up of steroids with the Δ^4-3-ketone group remaining intact (118, 119).

An important general rule was proposed by Dorfman (see 132) on the ratio of 5α-(H) to 5β-(H) steroids to be expected with different steroid precursors, to the effect that in man roughly equal amounts of the two epimeric forms would be expected from the C_{19} steroid hormones without 11-oxygen functions; that about 80% of the product from an 11-oxygenated C_{19} steroid would be in the 5α-(H) form; and that about 95% of the product from a C_{21} steroid would be in the 5β-(H) form. This rule has been confirmed on the whole, although the ratio of 5α-(H)- and 5β-(H)-metabolites of 11-deoxy-C_{19} steroids is subject to fluctuations between ratios of 2:1 to 1:2, partly or largely under the influence of the effect of thyroxine (310b). The recent emphasis on the 5α-(H) metabolites of the corticoids does not detract seriously from the validity of Dorfman's rule (125, 240a).

2. Metabolism of secondary hydroxyl and ketone groups. All secondary hydroxyl and ketone groups must be considered as probably or at least potentially interconvertible unless proved otherwise. Such oxidation-reduction reactions are extremely common in all living tissues and occur with a very wide variety of substrates (560a, 643, 720, 721, 723, 724a). There are either a very large number of enzymes carrying out this type of reaction with considerable substrate specificity, or else at least a fair number of such enzymes with rather low substrate specificity. The enzymology of these reactions has been reviewed admirably by Talalay (720, 721) who has dealt with both mammalian enzymes and those found in micro-organisms, and by Tomkins (742) who concentrated on the enzymes of mammalian liver. A most stimulating account of the stereochemistry of this type of reaction in micro-organisms has been given by Prelog (560a).

Reactions of this type are dependent upon the pyridine nucleotides for the transfer of hydrogen. In the case of the steroids, it is usually found that even relatively pure preparations of enzyme carry out the reaction with almost equal facility with either DPN or TPN as hydrogen acceptor in the system (721, 741, 742). The substrate specificity of these dehydrogenases is low and it has not been possible to isolate separate enzymes catalyzing the reaction with one or a few steroids [*cf.* enzymes reducing the 4,5-double bond (741)]. An example of this is the much studied steroid-3α-ol dehydrogenase; even the considerably purified preparations of Talalay and Hurlock have almost the same activity with androsterone and etiocholanolone (724a). The former steroid has the flat A/B ring junction (*trans*; 5α-(H)) and an axial hydroxyl group (3α-), while the latter has the bent A/B ring junction (*cis*; 5β-(H)) and an equatorial hydroxyl group (3α-).

The kinetics of this type of reaction are determined by the redox potential of the hydroxyl/ketone pair. *In vivo*, the ratio of ketonic and hydroxylic products is determined largely by the redox potential of the intracellular pyridine nucleotide systems and the rate at which the hydroxyl group is conjugated with glucuronic or other acids. With relatively unhindered groups, the reaction is rapid and the redox potentials of the ketone/hydroxyl pairs are similar (560a, 721, 724a). The three principal groups of the natural steroid hormones that are subjected to this type of dehydrogenase are the 3-ketone group after reduction of the 4,5-double bond (all natural corticoids, progesterone, testosterone, *etc.*), the 20-ketone group (all natural corticoids, progesterone), and the 17β-hydroxyl group (testosterone, estradiol). In the case of the 3-ketone group, the reaction proceeds almost to completion in the reductive direction, so that only a very small percentage of the urinary metabolites of the Δ^4-3-ketosteroids is in the form of saturated 3-ketosteroids. This is probably due to the over-riding importance of the conjugation with glucuronic (and in some cases sulphuric) acid at position 3 in the excretion of steroid metabolites from the hepatic cells (124d); the formation of the 3-glucuronosides and 3-sulphates is a trapping reaction which continuously removes the 3-hydroxysteroids as they are formed, thus displacing the reductive reaction towards completion (*cf.*, however, VI A 5).

In the case of the 17- and 20-substituents, the reaction appears to reach a ratio of products that is far closer to the equilibrium ratio determined by the redox potential of the ketone/hydroxyl pair. This is probably because there is normally little or no conjugation at these positions, so that the reaction is not drawn in the reductive direction by trapping of the hydroxylic form. Thus, about 50% of the total quantity of known C_{21}-metabolites of cortisol are in the form of 20-ketones while 50% are the related 20-alcohols (Fig. 3) (266a). The ratio for the metabolites of testosterone and progesterone cannot be given with such certainty, but there is probably a rather higher percentage of metabolites of the 17β-ol and 20-ol forms than of the 17-ketone and 20-ketone forms.

As with the reduction of the 4,5-double bond, two epimers can result from the reduction of a steroid ketone group. In man, 3α-ols make up the larger proportion of the saturated metabolites of all the Δ^4-3-ketosteroid hormones and

the 3β-epimers are present in very small quantities. In the rat, the opposite is the case (132, 448, 588). Similarly, the 20α-ols are the predominant metabolites of $C_{21}O_2$ and $C_{21}O_3$ steroids in man although the 20β-epimers are predominant among the $C_{21}O_5$ metabolites (266a). Finally, the 17β-ols are the predominant epimers among the C_{19}-steroid metabolites of human urine.

In the case of the 11β-hydroxyl group of the natural steroids, *i.e.*, cortisol, corticosterone, and 11β-hydroxyandrostenedione, however, the situation is much more complex. In the first place, the redox potential for the 11β-hydroxyl/11-ketone pair is much larger than with any other known position in the steroid nucleus; largely because of steric pressure in this position, the axial 11β-hydroxyl group is even less stable than typical axial groups (see 41) and is much more easily and rapidly oxidized than any other secondary hydroxyl group in the steroid nucleus (226a, 226b). The steric hindrance of this group is well known and affects enzymic attack upon it even more than the attack of ordinary chemical reagents. It was first noticed by Bush (124) that while the 11-ketones predominated among all the known 11-oxygenated steroid metabolites of human urine with the *cis*-A/B ring junction (5β-(H)) (448, 588), three similar 11-oxygenated steroids with the *trans*-A/B ring junction occurred only in the 11β-hydroxy form. Similarly, he found that cortisone absorbed by the oral route was almost entirely reduced to cortisol by the time it reached the systemic blood, as was also observed by Peterson *et al.* (543). Soon afterwards, Hubener *et al.* (345) found that unfortified rat liver homogenates reduced cortisone to cortisol but did not so reduce the 5β-(H) steroid tetrahydrocortisone (3α,17α,21-trihydroxy-5β-pregnane-11, 20-dione). Subsequently it was shown that after oral absorption in man a variety of 5α-(H)-3α-hydroxy-11-ketosteroids were readily reduced to the related 11β-ols while the related 5β-(H)-epimers were excreted entirely in their original 11-ketonic forms. Similarly, the administration of the 11β-ols of either configuration at position-5 (*i.e.*, A/B *cis* and *trans*) led to little excretion of the related 11-ketone in the case of the 5α-(H)-epimer and to none in the case of the 5β-(H) epimer (124c).

It was suggested that these results were most reasonably explained by the hypothesis that among the natural steroids the main factor determining the substrate specificity of the 11β-ol dehydrogenase was the shape of the α-surface of the molecule rather than the presence or absence of specific groups such as the Δ^4-3-ketone group (124c; *cf.* 345). Thus both 5α-(H)-saturated steroids and Δ^4-3-ketosteroids with more or less flat A/B ring junctions were readily reduced by this enzyme *in vivo* while the 5β-(H)-saturated steroids were not. It also appeared from the results that the hepatic system operated *in vivo* very actively indeed in the reductive direction, quite contrary to the direction that would be expected purely from the redox potential of this particular hydroxyl/ketone pair. This has now been confirmed directly by collecting hepatic venous blood from cats during the administration of [4-^{14}C-] cortisone into the portal vein; during the first passage through the liver 88 % of the steroid reaching the hepatic veins had been reduced to cortisol (124f).

The reduction of cortisone to cortisol and the reverse reaction had been

noticed previously by many workers and was first emphasized for man by H. L. Mason, by Burton *et al.*, and by Burstein *et al.* (121, 122), and with *in vitro* experiments on liver by Fish *et al.*, and by Miller and Axelrod (see 47a, 124, 125, 137, 266, 266a, 493, 550). The early workers were struck by the fact that while the adrenal cortex of man secreted predominantly or entirely cortisol, about two thirds of all its recognized metabolites were in the form of 11-ketones. Morris suggested (501a) first that the oxidation of the 11β-hydroxyl group of adrenal steroids might occur peripherally, but the exact site of this oxidation remained obscure. It is of great interest, therefore, that Mahesh and Ulrich have found that the heavier particles of homogenates of rat kidney are quite active in carrying out the oxidation of this group to the 11-ketone group (469, 469a) and that both DPN and TPN can act as hydrogen acceptor in the reaction. It remains to be seen whether the activity of this system and its substrate specificity will account quantitatively for the pattern of 11-ketonic metabolites found in urine.

Talalay and Hurlock have isolated a highly purified 11β-ol dehydrogenase from microsomes of rat liver, although it was not soluble (352a; *v.s.*).

The pattern of 11-ketones and 11β-alcohols in human urine is at present adequately explained by the general scheme proposed by Bush and Mahesh (124c). It is possible that the 11β-ols are reduced preferentially to 5α-(H) metabolites while the 11-ketones are preferentially reduced to 5β-(H) metabolites. This preference, however, cannot be very large and is quite unable on its own to account for the results that have been obtained, since little or no further interconversion of the 11-ketones and 11β-ols can take place once reduction to the 5β-(H) form has occurred (124c). The sizable amount of tetrahydrocortisone and tetrahydrocortisol (*i.e.*, 5β-(H) forms) in human urine must therefore have been derived from cortisone and cortisol, respectively, by the action of the 5β-enzyme.

In a recent and fascinating paper, Gallagher's group (310c) has shown that the extent of oxidation of the 11β-hydroxyl group is also greatly increased in hyperthyroid subjects. While their general conclusions are of the greatest interest, it should be pointed out that the lower three redox reactions depicted in Figure 1 of their paper are very unlikely to occur and are in conflict with the scheme proposed by Bush and Mahesh (124c).

The same type of reaction underlies the ready reduction of 21-aldehydosteroids to 21-ols (565, 595, 634).

3. Oxidation of side-chains to 17-ketone groups. This reaction was first demonstrated in man by Dobriner and his colleagues (see 447) and is now recognized as a common reaction for all C_{21} steroids with a 17α-hydroxyl group. It is the reaction responsible for the conversion of about 4 to 5% of endogenous cortisol to 17-ketosteroids in human urine (448, 588). Little is known about its mechanism or the substrate specificity of the enzyme or enzymes responsible.

4. Hydroxylations and dehydroxylations. Hydroxylation of alicyclic compounds is not a common or dominant metabolic pathway in mammals unless the methylene groups that are oxidized are activated by neighboring groups. It is thus

understandable that the only hydroxylations that occur in the degradation of steroid hormones *in vivo* are at positions close to activating groups. These hydroxylations give rise to compounds which are invariably less active as hormones than the precursor, and usually the reduction in activity is considerable (*e.g.*, compounds 3, 43, 66, 86, 125). The most active system of this type known at present is the 16α-hydroxylation that occurs with both androgens and estrogens. This gives rise to estriol, 16α-hydroxyestrone, and a number of other 16-oxygenated metabolites of the etiocholane and androstane series (398, 448, 588). Lesser amounts of the 16β-epimers are probably formed as well, and these hydroxyl groups are potentially subject to the same rules of oxidation-reduction as others, although complicated of course by the neighboring 17-oxygen function.

Hydroxylations at other positions are known to occur with both corticoids and with estrogens. Thus 2α- and 6β-hydroxy-metabolites of cortisol (118, 119, 120) and testosterone are known to occur, and the same is true for estradiol (240a, 398, 588). 6α-Hydroxysteroids (447) and 18-hydroxyestrone (132, 398) have also been found in human urine. It is further evidence of the predominant role of the two reductions leading to the formation of the saturated 3-hydroxysteroids, and their subsequent conjugation at this position with (in most cases) glucuronate, that the proportion of hydroxylated metabolites is much higher in the guinea pig which lacks the A-ring reducing systems (118, 119, 120, 511), and in man with those synthetic steroids in which the A-ring reductions and conjugation are blocked by substituents such as the 2α-methyl group (124d).

Dehydroxylation at C-21 is known to occur with cortisol and to a greater extent with deoxycorticosterone (see 448, 588). On the other hand, it seems at present unlikely that any tertiary hydroxyl groups such as the 17α-hydroxyl group undergo this reaction (266). Hydroxylation of C-21, while an important biosynthetic reaction in the adrenal cortex (588), is not known to occur to any large extent outside this organ.

5. Conjugation of hydroxyl groups. The principal reaction of this type undergone by the metabolites of the natural steroids is conjugation of the 3-hydroxyl group with glucuronic acid to form the glucosiduronate (448). These conjugates are rapidly excreted by the hepatic cells, and in man and probably some other species their renal clearance is very large (132, 398, 588). It is unlikely that more than a trace of the total amount of glucosiduronate formed *in vivo* is split by glucuronidase in man and other species in which the renal excretion of these compounds is large (see also 266a, 368). On the other hand, the rat and some other species excrete the major proportion of these steroid conjugates *via* the bile into the intestine (267). It is probable that in these species as in man (611, 789a) some hydrolysis occurs due to the action of the intestinal flora, and that a certain amount of the glucosiduronate or of the free 3-hydroxysteroids is reabsorbed to undergo an entero-hepatic circulation similar to that of the bile salts.

The controlling influence of this conjugation is shown by the fact that there is little or no evidence that steroid diglucosiduronates are formed in more than

trace amounts. When conjugation at position 3 is blocked, however, it is quite clear that extensive conjugation occurs with hydroxyl groups in other positions (124d). This feature of the reaction is probably partly due to the rapidity of the excretion of glucosiduronates by hepatic cells and partly to a low affinity of monoglucosiduronates for the uridinediphosphoglucose (UDPG) transferase enzyme.

With certain steroids the 3-hydroxyl group is conjugated to a significant or even predominant extent with sulphuric acid (448, 588). This seems to be the major or even exclusive form of conjugation of Δ^5-3β-hydroxysteroids, such as dehydroepiandrosterone, except when very large doses are administered, and is a variable though sometimes major pathway for saturated C_{19}-3-hydroxysteroids (448, 588). The sulphates differ in two ways from the glucosiduronates which are extremely important from the point of view of the biological activity of steroid hormones. First of all, their renal clearance is low; secondly, they probably undergo a fair amount of hydrolysis back to the free hydroxysteroids, partly because of their relatively inefficient renal excretion. Thus, estrone 3-sulphate is quite active as an estrogen (127) which is almost certainly consequent upon its hydrolysis to estrone and subsequent reduction to estradiol (see below), and the concentration of dehydroepiandrosterone sulphate in human plasma is relatively large in most subjects (398, 588).

In general, therefore, the pathway involving formation of glucosiduronates approximates *in vivo* to an irreversible and efficient route of inactivation and excretion of steroid hormones, while the formation of sulphates is relatively inefficient in that it is relatively reversible. When hydrolysis of the sulphate yields an active compound such as androsterone or estrone, the biological action of the precursor may be prolonged.

Estrogens undergo hydroxylation at all the positions known to be hydroxylated in the nonbenzenoid C_{19}-steroids, but in the case of the 2-position the hydroxyl group is, of course, phenolic in type. In fact, the result of this reaction is the formation of a catechol, and as with other catechols (24) the 2-hydroxyl group of the 2-hydroxyestrone and related compounds is extensively O-methylated resulting in the excretion of 2-methoxyestrone in human urine (240, 240a).

6. Unidentified pathways of steroid metabolism. While the above metabolic reactions are well established and account satisfactorily for the recognized metabolites of all classes of steroid hormones, there remains a sizable fraction of these hormones which cannot be accounted for. Thus, the recognized metabolites of cortisol in human urine account for about 70 to 75% of the hormone, those of progesterone for about 10 to 20% (536), and those of testosterone for about 30% (see 132, 266a, 267, 398, 448). There seem to be several possible explanations for these deficits. Firstly, metabolites may be formed which have undergone extensive hydroxylation and reduction so that they are even more water-soluble than the cortols and similar pentols and have not been extracted by the usual methods for isolating urinary steroids. Secondly, there may be quite different metabolic pathways leading to hitherto unrecognized products. Thirdly, some of the recognized metabolites may be conjugated in a way that is

resistant to the usual methods of hydrolysis of steroid glucuronosides and sulphates. Fourthly, a portion of the metabolites may be excreted in the feces, although in man this route is probably negligible (266a, 267, 545).

Present evidence suggests that in the case of cortisol small contributions to the deficit are probably made by all four of these processes. It seems unlikely that any major pathway has been overlooked. The most complete figures accounting for the metabolism of cortisol in man are probably those of Gallagher and his group (266a). Thus, in the first two days after an oral or intravenous dose of 4-^{14}C-cortisol, 80 to 85% of the total radioactivity was recovered in the urine in six experiments on five subjects. Thus, only 15 to 20% of the dose could have been excreted by extrarenal routes and this figure would probably be reduced if the collection of urine had been made for a longer period. Similarly, 65 to 92% of the total urinary radioactivity was accounted for as neutral lipid-soluble material after hydrolysis with glucuronidase, and of this fraction 90% was composed of 11 recognized metabolites. Allowing for the losses that usually attend the isolation procedures employed for these steroids and the portion of radioactivity recovered as neutral lipid-soluble material after gentle acid hydrolysis under continuous ether extraction, it seems likely that Gallagher's group accounted for over 90% of the urinary metabolites of cortisol as recognized steroids derived from the reactions described above. It is unlikely that more than 10% of this hormone is excreted in the urine as unrecognized steroid metabolites or as steroids conjugated in unknown ways. The exact nature and route of excretion of the 15 to 20% that was not recovered in the urine remain to be discovered.

There is no comparable study of the metabolites of other classes of steroid hormone in man. The work that has been done, however, suggests that by far the greater proportion of the metabolites of these hormones is in the form of lipid-soluble material conjugated with glucuronate or sulphate in the usual way (398, 588). There is, however, plenty of scope for further work in this field.

7. The overall pattern of the metabolic degradation of steroid hormones. The enzyme systems carrying out the reactions described above are largely confined to the liver, and this organ is undoubtedly the main site of inactivation of the steroid hormones of all classes. Although the isolated perfused kidney metabolizes cortisone slowly, it is probably not a major site of steroid metabolism *in vivo* (398, 740). Slices and homogenates of kidney will, however, oxidize the 11β-hydroxyl group of cortisol *in vitro* and reduce the 20-ketone group quite readily (469, 469a).

It seems that there is very little metabolic alteration of the C_{21}-steroids by the peripheral tissues, with the exception of the reduction of the 20-ketone group and the oxidation of the 11β-hydroxyl group (1, 54b–54e, 192, 469a, 745). The reduction at C-20 is, however, found with cortisol, progesterone, and other 20-ketones, and occurs to some extent even in blood *in vitro*, and quite rapidly in the hepatectomized animal (398, 588), in the dog heart-lung preparation (745), and in rabbit muscle *in vitro* (731). Travis and Sayers (745) commented on the possibility that oxidation-reduction reactions might be the basis of a

coenzyme-like action of steroid hormones, pointing out that this should be reversible. Their findings with the heart-lung preparation and the many other unsuccessful attempts to discover an extrahepatic site at which steroid hormones underwent active metabolic transformations are a strong, although not insuperable, argument against this theory of the mechanism of action of these hormones (469a, 536).

The majority of results in normal man and in dogs and some other species suggests that the overall pattern of the excretion of the recognized steroid metabolites varies relatively little when one considers the enormous number of isomeric combinations that result from the reactions described in this section. Thus, the yield of different groups of metabolites of cortisol in man and dogs is relatively constant, and any major group can be used as a reasonable guide to the rate of secretion of cortisol in the subject or animal under examination (125, 659; *cf.* 162, 511). The only exceptions to this rule are that the fractional yield of 11-oxygenated 17-ketosteroids from cortisone or cortisol seems to drop below the usual 4 to 5% when the secretion or administration of these hormones rises above a rate of 120 mg/24 hr (124b), and that the ratio 11β-ols/11-ketones rises quite rapidly as the rate of secretion or administration of cortisol rises above the normal range of about 20 to 40 mg/day (125, 266a, 282a; see 398, 588, 659).

On the other hand, the rate of removal of cortisol from the blood is remarkably affected by disorders of the liver. Thus, it has been found that cirrhosis or other hepatic diseases in man increase the half-time of the disappearance of cortisol from the blood very considerably (67, 542; see 448, 588) and the same is true for other types of steroid hormones, especially estrogens (*e.g.*, 40). Even more striking are the results of Yates and his group who found that passive venous congestion of the livers of male and female rats leads to decreased efficiency in the inactivation of deoxycorticosterone, cortisone, cortisol, and aldosterone, which may last for as long as nine months after the onset of the congestion (813, 814). This deficiency is probably mainly due to a considerable reduction in the rate of the two reductive reactions of ring A.

The rates of various steps in the metabolic degradation of the steroid hormones are also known to be affected by endocrine and dietary factors. Thus, there is a large sex-difference in the relative rates of reduction of the A-ring and the 20-ketone group of cortisone by liver homogenates from rats (747) and steroid dehydrogenases are very susceptible to inhibition both by substrates and competitively by other steroids (*e.g.*, 461, 471, 721, 724a).

It is by no means certain, however, whether these differences observed in experiments *in vitro* always give rise to similar differences *in vivo*. There is little evidence at present that their effects on metabolism *in vivo* are at all large, and they are far less noticeable than the very considerable effects of variations in thyroid function (50, 343, 462). The quantitative significance of many of the results of experiments *in vitro* is open to doubt because little or no attempt has been made to standardize the biochemical conditions that were employed for the incubations. Thus, the activation of the 5α-enzyme (4,5-dehydrogenase) by thyroxine that is seen with experiments *in vitro* may well depend upon the

activity of endogenous TPNH-regenerating reactions rather than upon a change in the effective activity of the 5α-enzyme itself (461, 744). The significance of the effect of thyroxine *in vivo* is demonstrated by the increased ratio, 5α-(H)/5β-(H) metabolites, found in the urine of human subjects (310b), the increased rate of removal of cortisol from the blood (542; *cf.* 310c), and the compensatory increase in the secretion rate of the adrenal cortex of the dog (484).

The effect of harmful stimuli ("Stress") upon the rate of removal of cortisol from the blood of animals has been investigated by several groups. Most authors are agreed that such stimuli decrease the rate of removal of cortisol from both blood and tissues very considerably (214, 237, 238, 684, 752) in rats, dogs, and men. In such experiments the effect of large changes in the distribution of blood flow probably plays a very large part. Firschein and his colleagues (238) rejected the idea that blood flow plays any part in the phenomenon; their argument for doing so, however, is unconvincing. The proportion of the cardiac output passing through the liver is probably the most important single variable determining the rate of elimination of any given steroid; even in the absence of large changes in cardiac output, the hepatic blood flow can vary very greatly with fluctuations in digestive activity, external temperature, the introduction of pyrogen and other foreign substances into the blood, and following shock (see 214). Kägi (387) found that the rate of removal of cortisol from the blood of patients with Addison's disease was increased by muscular exercise; this could be largely due to the increased cardiac output that must have resulted. There has been little further work that would lead to modification of the conclusions of Sandberg *et al.* (610) that epinephrine caused no detectable change in the rate of elimination of "cortisone" (*i.e.*, Porter-Silber chromogens) from the blood during an infusion of the steroid (see also 29).

Much less work has been done on the influence of similar factors on the metabolic elimination of other classes of steroid hormone. There is much direct and circumstantial evidence, however, to suggest that changes in hepatic, thyroid, and circulatory function lead to significant effects on the metabolism of all classes of steroid hormones *in vivo* (*e.g.*, 40, 50; see 398, 588).

B. Biosynthetic and other metabolic reactions of steroids

The metabolic reactions that have been discussed so far are typical "detoxication" reactions which have been selected by the usual processes of evolution to serve the biological function of eliminating active hormones from the body. Two other types of metabolic reaction may play a part in determining some features of the biological activities of steroids. These are, first, the biosynthetic reactions that lead to the formation of the natural steroids, and second, the reactions that may occur in micro-organisms or by the agency of their enzymes, particularly in the intestines. It is worth discussing these briefly since although little is known about them, they have sometimes been postulated in terms which pay little attention to certain factors which may limit the extent to which they can be effective.

The reason for considering biosynthetic reactions is that progesterone is now

known to be the principal intermediary metabolite in the biosynthetic pathways leading to the production of *all classes* of steroid hormone (310, 398, 588). It is possible that progesterone and some other steroids may be taken up by a steroid-secreting organ and converted to active steroids of another class of hormones. Judging by studies on adrenal glands perfused with steroid precursors, the efficiency of conversions of this type may be considerable (310); there is no evidence at present, however, which gives any indication of the efficiency of such conversions *in vivo*. Most biological assays are carried out with either adrenalectomized or gonadectomized animals; some, however, use immature instead of gonadectomized animals, and some workers have tried to use intact animals for the assay of corticoids and their analogues on the grounds that an active steroid will in any case suppress the secretion of ACTH and thus that of the adrenal cortex. This can be done as long as the suppression of the adrenal cortex is checked by estimating its steroid content at the end of the assay (Silber, personal communication). There are, however, a number of reactions which are catalyzed by both the gonads and the adrenal cortex, so that with suitable compounds there will remain the possibility that active derivatives will be formed from administered steroids which would not themselves show activity in an appropriate local or *in vitro* assay.

There is one major factor, however, that will limit the extent to which this type of artifact will affect the results of biological assays, and this is the proportion of the cardiac output passing through the steroid-secreting organs. This is unlikely to exceed 0.5 to 1.0%, and it is probable that steroids with short half-lives in the blood will suffer little conversion to products with different activity unless the efficiency of conversion at one passage through the organ is in the range 50 to 100%. When the effect of association of steroids with protein carriers in the plasma is taken into account, it is unlikely that efficiencies would commonly rise above 10% per passage. Where, however, groups have been introduced which greatly increase the half-life of the steroid in the blood or extrahepatic tissues, there is a definite possibility that conversions will take place that affect materially the apparent biological activity of the administered steroid. This factor needs experimental assessment in some cases in which an analogue lacking particular groups is found to have activity in a typical bioassay (see below).

The biosynthetic reactions are largely, and some would say wholly, confined to the steroid secreting organs. Most of the experimental evidence, however, is sufficient to state only that the activity of the enzyme systems in other tissues is less than 1 to 2% of their activity in adrenal cortex or gonads, and one cannot neglect the minimal activities that may be found to exist in liver and other tissues (398, 588) when powerful activating groups are present in the steroid that is being assayed (see below).

Similar uncertainties attend the question of the possible metabolic alterations of steroids by micro-organisms in the alimentary canal (see 785). Little is known about this, but some evidence exists that the flora of the human colon is capable of inactivating cortisol (769). Most of the hydroxylations that are carried out by molds would be expected to give derivatives that are less active than the

precursor but one cannot rule out the possibility that microbiological hydroxylations at positions 3, 11, 17 and 21 occur *in vivo*.

At present, one would suggest that the influence of these two classes of reaction is probably small or negligible. Steroid analogues possessing inactivating groups, however, may be converted to significant amounts of other products by such reactions.

C. Factors affecting the distribution of steroids in the body

The numerous possible factors of this type have been discussed previously (124a). It is quite clear that with very hydrophobic steroids, simple solution in the body fat is a significant factor and that progesterone, for instance, is taken up extensively by this component of the tissues (818; see 398, 588). The most influential factor, however, is the existence of strong association between steroids and substances of large molecular weight, particularly the plasma proteins (79, 123, 167a, 167b, 215, 286, 534, 612, 632, 660). The most striking example of this is found with cortisol which associates fairly strongly with plasma albumin and very strongly with what appears to be a fairly specific protein carrier which has been named "transcortin" by Slaunwhite and Sandberg (666). The association constants for a variety of steroids with this protein have been determined by Slaunwhite and Sandberg; they are large for cortisol and much smaller for all other active steroids. On the other hand, the association with albumin is minimal for very hydrophobic steroids (*e.g.*, monoketones and monohydroxysteroids), strong with moderately hydrophobic steroids (*e.g.*, testosterone, estradiol) and weak with the more hydrophilic steroids such as cortisol (215, 632). The concentration of transcortin in normal plasma is low, so that the distribution isotherm of cortisol between plasma and saline solution across a dialysis membrane *in vitro* at physiological temperatures is curved. Up to concentrations of cortisol of about 15 to 20 μg/100 ml, there is a distribution ratio of about 12:1 in favor of plasma, while above this concentration the distribution ratio tends towards a figure of about 4:1 (123). The concentration of cortisol in various types of extracellular fluids suggests that much the same distribution is found between plasma and extracellular fluid (123, 163, 609).

The physiological importance of the carrier protein is demonstrated by the interesting findings of Bartter and his colleagues that the increase in the concentration of cortisol in the plasma of pregnant women or of men treated with estrogens is entirely in the protein-associated fraction because the concentration of transcortin is rapidly and considerably increased by estrogens (see 542; *cf.* 770). These findings explain why the very large concentrations of cortisol found in the plasma of pregnant women, which are similar to those found in moderately severe cases of Cushing's syndrome, do not give rise to the signs of the disease (153, 490, 542). It should be noted that an entirely similar rise in the concentration of plasma proteins responsible for binding thyroxine and possibly other hormones occurs in pregnancy (195).

The effect of such carrier proteins on the biological activities of steroids is not easy to predict. One effect is to reduce the rate at which the steroid is eliminated

from the organism (490, 542), thus increasing the apparent activity in an assay dependent upon infrequent or single doses of steroid. But such carriers also prevent the steroid from reaching the extravascular fluids, which should reduce the observed activity (123, 153). Further discussion of this topic has been given by Peterson (542) and others (see 398).

D. The effects of modifying the structures of natural steroids upon their metabolism and distribution

The vast majority of structural modifications that result in an increased biological activity of steroids probably produce their effect by reducing the rate of elimination of the steroid from the body, by altering their distribution within the body, or by a combination of these factors (124a). Only in a minority of cases is it possible to suggest with any confidence that such structural modifications cause an increase in the association constant of the steroid with its receptors or an increase in its intrinsic action (14, 250) after combination with the receptors. As might be expected from the foregoing discussion, modifications of the A-ring are very prone to give rise to considerable reductions in the rate at which the Δ^4-3-ketone group is reduced to the saturated 3-hydroxyl group.

A large number of studies have shown that the 1,2-dehydrosteroids such as prednisolone (1,2-dehydrocortisol) have a much longer half-life in the blood (between 2- and 2.5-fold) than cortisol (218, 394, 613, 661), and that the A-ring is much more slowly metabolized both *in vivo* in man and *in vitro* by rat liver slices or homogenates (110, 111, 138, 139, 278, 279, 291, 639, 666, 742). There is evidence, too, that the reduction and removal of the side-chain are also slowed down both *in vivo* and *in vitro* (278, 279, 291, 666).

Similar evidence has been obtained with 2α-methylsteroids in which reduction in the A-ring is almost completely prevented by the methyl group (110, 124d, 279) both *in vivo* and *in vitro*.

Another group of substituents can either be expected on stereochemical grounds, or are actually known, to reduce very considerably the rates of various metabolic reactions of the side-chain of C_{21} steroids. Thus the introduction of 16α-substituents generally inhibits these reactions (660, 729a), and recently it has been possible to show that while 9α-fluoroprednisolone undergoes a fair amount of reduction at C-20, triamcinolone acetonide (16α-hydroxy-9α-fluoroprednisolone-16,17-acetonide) is recovered practically unchanged from incubations of homogenates of rat liver which remove over 50% of the side-chain of cortisol or 9α-fluorocortisol under the same conditions (124e).

Some substituents affect not only the metabolism of neighboring groups but that of distant groups. Thus, the reduction and elimination of the side-chain are almost certainly slowed down by the introduction of the 1,2-double bond of prednisolone (above), and the metabolism of the 11-oxygen function is greatly affected by the 1,2-double bond (124a, 639), by the 2α-methyl group (124d), and by both 16α- and 16β-methyl groups (124e). All these substituents reduce the rates of both the oxidation of the 11β-hydroxyl group and the reduction of the 11-ketone group. The effects are additive. Thus, the rate of oxidation of the

11β-hydroxyl group of prednisolone (1,2-dehydrocortisol) is about 0.3 times that of the same group in cortisol *in vitro* (rat liver microsomes), and the oxidation of the 11β-hydroxyl group of 16α-methylcortisol is about 0.2 times that of cortisone. Under the same conditions, no detectable oxidation of the 11β-hydroxyl group of 16α-methylprednisolone was observed (124e).

Although there is as yet little information on the matter, it can confidently be expected that the same features will be found with similar substituents in other classes of steroid. Thus, the great increase in oral activity that is seen with 17-acyloxyprogesterone analogues is almost certainly due to the effect of this large 17α-ester group upon the reduction of the 20-ketone group; the 17α-alkyltestosterones probably owe their oral activity to the protection of the 17β-hydroxyl group from oxidation to the 17-ketone. All the large substituents at C-6 (*e.g.*, chloro-, methyl-) may well reduce the rate of reduction of the Δ^4-3-ketone group (*e.g.*, 278a).

The influence of 9α- and 12α-halogen substituents is complicated by the interaction of the steric and electronic effects of this type of group. This has been fully discussed before and only a brief summary will be given apart from mentioning further work that confirms the arguments given previously (124a). The strongly electronegative fluorine atom increases the redox potential of the ketone-hydroxyl pair in the direction of stabilizing the 11β-hydroxyl group. This is seen in simple chemical reactions and is probably a feature common to all α-fluoroketones (226a, 226b, 662). The results of Bush and Mahesh (124a) were striking in that on the administration of either 9α-fluorocortisone or 9α-fluorocortisol to man, the expected family of metabolites was recovered in the urine as far as modifications of the A-ring and the side-chain were concerned. In contrast, there were practically no 11-ketonic metabolites after giving 9α-fluorocortisone, and none at all after 9α-fluorocortisol. It has now been shown that rat liver homogenates reduce both 9α- and 12α-fluoro-11-ketonic steroids of several types much more rapidly than their nonfluorinated analogues, and that under oxidizing conditions, rat liver microsomes fail to oxidize the 11β-hydroxyl group of the related 9α-fluoro-11β-hydroxysteroids (124e).

With the larger halogens, the electronegativity and hence the negative inductive effect (249, 250, 258, 259) is much less than with fluorine, and in contrast to fluorine their size is sufficient to produce steric hindrance of a considerable magnitude. Bush and Mahesh (124d) obtained evidence that the reduction of the 11-ketone group was inhibited both by steric hindrance around the α-surface of carbons 1 and 2 of the steroid nucleus, and by the side-chain of adrenocortical steroids. They suggested that the steroid associated with the 11β-dehydrogenase by the "upper" half of its α-surface; close contact was postulated on the α and "upper" surfaces of the steroid at carbons 1, 2, 11, 12, and 21. All subsequent evidence has confirmed this supposition, and in particular the prediction that the reaction would be strongly inhibited by a 12α-chloro- or 12α-bromo- group has been fulfilled (124e). There is now strong evidence that in those analogues of cortisone in which this reaction is inhibited, the 11-ketone is inactive or of greatly reduced activity while the related 11β-ol is of the expected activity (144,

319, 729). One of the major effects of the 9α-fluorine atom in 9α-fluoro-11-oxygenated steroids is thus to "maintain" the steroid in its active 11β-hydroxylic (*i.e.*, reduced) form and prevent its oxidation to the inactive 11-ketonic form (124a, 133). Consistent with this is the fact that the ratio of glycogenic activities of 9α-fluorocortisone/9α-fluorocortisol is very close to 1.0 (124a, 252) instead of the more usual 0.5. This protective effect can be expected to produce at least a 2-fold increase in biological activity on its own, but is unlikely to explain all of the 10-fold increase that is actually caused by the 9α-fluorine atom (249, 250; see below).

In contrast to the effects of the structural modification discussed above, Engel *et al.* (225) have shown that 19-nortestosterone is metabolized in a manner very little different from testosterone itself.

The effects of chemical modifications upon the distribution of adrenocortical steroids are not easy to predict in detail because of the overwhelming influence of "transcortin" and other plasma proteins upon their distribution between the intra- and extravascular spaces. It is probable that the majority of substituents of all kinds reduce the affinity of cortisone-like analogues for "transcortin" (*e.g.*, 660), but this is not certain. On the other hand, a number of general rules can be proposed with reasonable confidence for less polar steroids on the basis of existing results and simple chemical inferences.

In the first place, the association of steroids with plasma albumin is subject to general rules which are not very different from those for other classes of crystalloids (396, 415, 416, 631, 632). This protein associates strongly with an enormous range of substances (396) and in general the strongest associations are with nonpolar neutral substances containing two polar groups (632), or with anions (415, 416, 631). Very hydrophobic steroids, such as progesterone, 17α-acyloxyprogesterones, and alkylated testosterones, can be expected on the other hand to associate only weakly with albumin (632). Similar rules probably apply to the association of steroids with other large molecules such as tissue proteins and polysaccharides.

Very hydrophobic steroids are probably taken up largely by fat in adipose tissue and by chylomicrons. This is a pronounced feature of the distribution of progesterone (see 816, 818).

Polar groups added to a given steroid (*e.g.*, hydroxyl) or groups affecting the effective polarity of existing polar groups will produce a general effect increasing the water-solubility of the steroid and tending to decrease both its solubility in lipids and its association with most other molecules in solution or existing in separate phases (124a). If the polar group is concerned specifically with the association of the natural steroid with its receptors, or if an added polar group is fortuitously capable of forming specific links with the receptors or other molecules, then this general rule will break down.

In trying to understand the effects of chemical modifications of steroid hormones, it is impossible to assess their exact influence on all the possible associations these substances may enter into with specific and nonspecific receptors. Considerable insight into these factors can, however, be gained if a sufficient

number of analogues is available for consideration, and if positive evidence of biological activity is given greater weight than occasional instances of absent or reduced biological activity (124a).

Fried (249, 252, 254) was the first to point out that similar modifications of different cortisone-like steroids produced almost constant changes in activity, and that in many analogues combinations of such modifications caused increases in activity that were simply additive. This rule depends, however, upon the design of the assay and breaks down with many of the more highly substituted cortisone-like analogues (see, *e.g.*, 253). Nevertheless, it remains true that for most of the substituents and modifications that have been tried out in cortisone-like analogues, there now exist a large number of compounds in which the effect of a given substituent is seen to be a nearly constant multiple of the biological activity of the root steroid. Many of these effects have been described before, and the best discussion is that of Fried and Borman (252). In the subsequent parts of this review, more recent developments will be dealt with and a fuller interpretation will be attempted.

VI. STRUCTURE-ACTIVITY RELATIONSHIPS OF STEROID HORMONES AND THEIR ANALOGUES

A. The chemistry and structure of the steroids

A few general points must be emphasized here; an admirable account is given by Fieser and Fieser in their monograph (see 507). The natural steroid hormones are roughly planar or slab-shaped molecules, the greater volume of which is occupied by a fused four-ring hydrocarbon skeleton. There is good evidence that in most steroids this skeleton is relatively rigid and invariant in compounds of similar skeletal structure (124a, 507). The most important steric distinctions to be kept in mind are: (a) the epimeric α- and β-substituents which in the usual representations are conceived as lying below and above the plane of the paper, respectively, the α-bonds being drawn as dashed, and the β-bonds as solid lines, (b) the distinction between *equatorial* bonds lying close to the plane of the ring to which they are attached, and *axial* bonds lying more or less perpendicularly to the main plane of the ring (41), and (c) the "*chair*" form of cyclohexane rings which is found in nearly all natural steroids, and the "boat" form which is thermodynamically less stable (41) but which can certainly occur in certain steroids or under certain circumstances (179, 181) (see Figs. 4, 5).

Because of their fused-ring structure, the steroids are relatively inflexible molecules and their detailed shapes, or "conformations" (41), are probably much nearer to those given by geometrical construction and molecular models than with most other classes of compound. The exact shape and electronic structure of each molecule is, however, subject to many subtle modifications, and such changes can be induced by distant as well as neighboring substituents (12, 259, 409). Yet the shapes of steroid molecules in solution are much more accurately known than those of most classes of organic compounds in which there are one or several bonds around which parts of the molecule can rotate. Thus, for instance, the dipole moments of many steroids in solution agree very closely

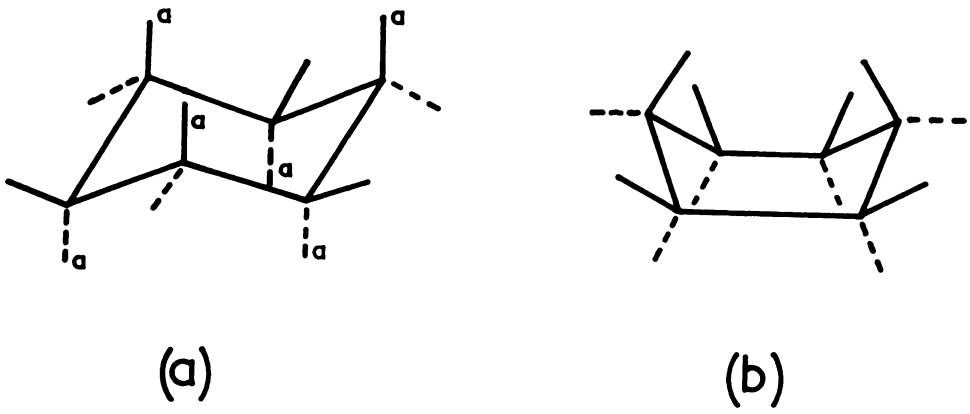

FIG. 4. The cyclohexane ring

(a) The "chair" form; (b) the "boat" form. Axial bonds are marked *a*, and hydrogens are not shown.

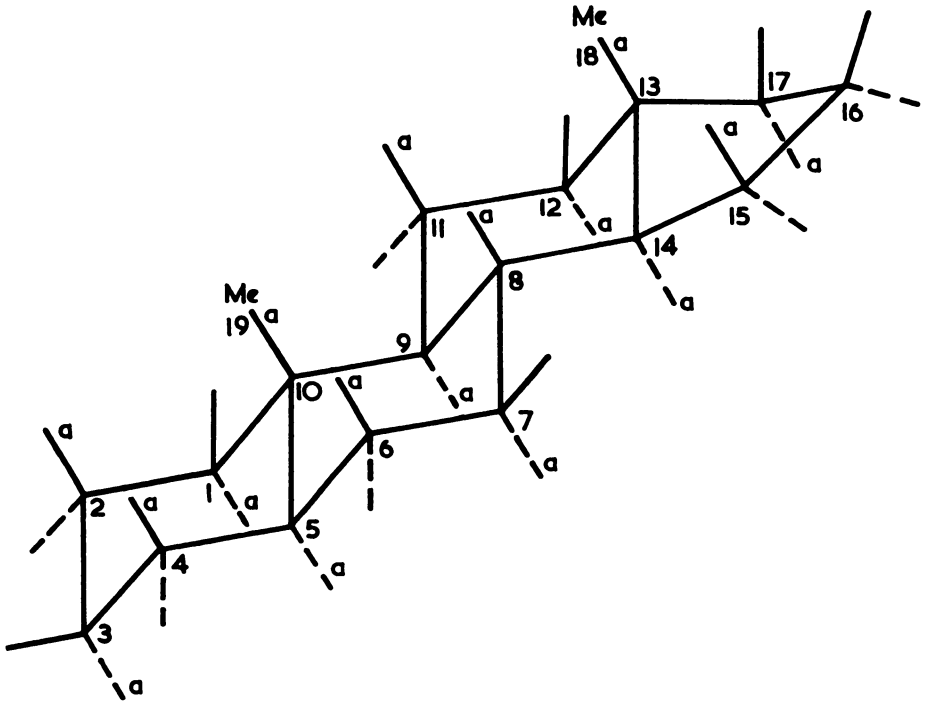

FIG. 5. The carbon skeleton of 5α-androstane

Axial bonds, *a*; α-bonds dashed; β-bonds solid; angular methyl groups, *Me*. Note the intermediate conformations of the 16α- and 16β-bonds. Hydrogens omitted for clarity. In the natural Δ^4-3-ketosteroids the "bottom" half of ring A (carbons 3, 4, and 3-oxygen) is bent slightly "back" towards the α-side.

with those calculated from the molecular shapes and atomic positions derived by calculation and by X-ray crystallography (see 124a).

Certain substituents, usually in axial conformations, are so subject to steric pressure that spontaneous rearrangement to the equatorial epimer occurs, or the molecule is probably forced out of the usual conformation to some degree. This is much more prone to occur with "central" than with terminal substituents. Thus, 6β-substituted steroids are usually rather unstable if the substituent is as large as a methyl group or larger (see, *e.g.*, 765).

Another important factor to be considered is the interaction of steric and electronic forces. These interact mutually and with the solvent so that the thermodynamically most stable form of the molecule is attained. Such interactions are more easily observed in the sterically rigid steroids than with most other classes of alicyclic compounds (*e.g.*, 41, 76, 184).

The conformations of ring D and of the two-carbon side-chain of C_{21}-steroids are, however, less certainly known than those of the C_{18}- and C_{19}-skeletons common to all natural steroid hormones and most of the synthetic analogues. The evidence of X-ray crystallography (see 124a) and optical rotatory dispersion spectra (241) is that ring D is a strained "half-chair" with carbon-15 close to the line projected through carbons 8 and 10, carbon-17 close to the line parallel to this through carbon-12, and carbon-16 behind the plane of carbons 9, 11, 13 and 14 (Fig. 5).

The 17β-side-chain of the natural C_{21}-steroids is capable of rotation around the 17,20-bond (Fig. 6). Inspection of models shows that various conformations are possible, and that with the dihydroxyacetone side-chain common to many glucocorticoids, a number of hydrogen bonded forms may exist (Fig. 7) quite apart from hydrated forms (*cf.* 416a). It is usually held that the preferred structure will be the one with the 20-methyl group (*i.e.*, C-21) to the rear or α-side of the molecule (Fieser; see 507, p. 337) and this will certainly be the preferred conformation in the presence of 16β-methyl groups (727) (see Fig. 8); and in 17α-halogeno-C_{21}-steroids, probably because of the electrostatic repulsion of the 20-ketone group by the electronegative halogen (179a).

From models, however, it is seen that the 20-ketone group common to all natural C_{21}-steroid hormones restricts the rotation of the side-chain about the 17,20-bond because of steric repulsion by the 13-methyl group, and that in the other direction the 20-methyl group (*i.e.*, C-21) is repelled by the 12β-hydrogen and 13-methyl group. This, and evidence from chemical reactions (416a), suggests that the preferred conformation of the side-chain is with the 20-ketone oxygen directed towards the β-side of the molecule and lying just "over" C-16 (179a, 416a).

Relatively slight changes in the position and conformation of a substituent may produce considerable changes in the steric hindrance, and hence the reactivity or accessibility of neighboring groups. Thus, the 11β-hydroxyl group is strongly hindered and difficult to acetylate in most glucocorticoids but is readily esterified in 19-nor- or A-aryl analogues in which the 10-methyl group is missing (compounds 39, 403; references 143, 193, 279, 410, 466; *cf.* 233, 246).

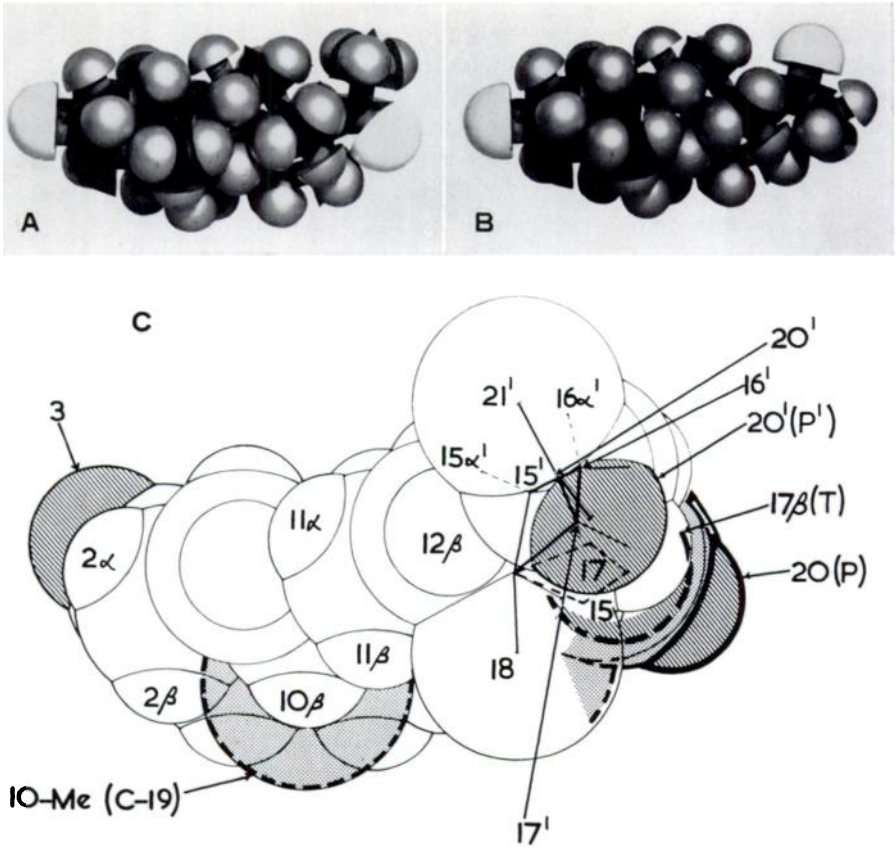

FIG. 6. Rotation of the 17α-side-chain

A model of progesterone photographed from "above" with the β-surface resting on the table. (a) 20-Carbonyl oxygen in β-conformation; (b) 20-carbonyl oxygen in α-conformation. The 3-carbonyl oxygen is to the left, and ring D and the side-chain to the right of the figure. Oxygens, white. Hydrogens, gray. (c) Projection of "top" surface of part of a 19-nor-Δ^4-3-ketone on to a plane perpendicular to the 9,11—C—C bond showing the β-conformations of the side-chains of the active gestagens 19-nor-progesterone (P), 14β,17α-19-norprogesterone (P'), and 19-nor-17α-alkyltestosterone (T) superimposed. (The 17α-alkyl group is omitted, and methyl groups of C-18, C-19, and C-21 are drawn as spheres, for clarity.) A receptor forming a weak polar bond with the 20-carbonyl oxygen of P could do the same with the 20-carbonyl oxygen of P' and with the 17β-hydroxyl of T, from the β-side if the steroid were rotated about an axis through carbons-9 and -17. No other unconstrained conformations of the 17-acetyl side-chain of the two C_{21}-steroids can be found which would allow a similarly close correspondence to a potential receptor site. No similar conformation can be found for the 17-acetyl side-chain of the *inactive* 17α-progesterone.

3, 3-Oxygen in P, P', and T; *10-Me(C-19)*, methyl group as in natural steroids; *15*, *17*, *18*, centers of C-15, C-17 and C-18 in P and T; *15'*, *16'*, *17'*, *20'*, *21'*, centers of respective carbon atoms in P'; *18*, center of C-18; *21'*, center of C-21 in P'; *2α*, *2β*, *10β*, *11α*, *11β*, *12β*—hydrogens; *15α'*, *16α'*—hydrogens in P'; *17β(T)*, oxygen of 17β-hydroxyl group of T; *20(P)*, oxygen of 20-carbonyl group of P; *20'(P')*, oxygen of 20-carbonyl group of P'.

Certain steroids are unstable and may be expected to undergo nonenzymic rearrangements in the body. Thus, orally administered $\Delta^{5,(10)}$-3-ketones will be partly converted to the Δ^4-3-ketones by the acid of the gastric juice (573). Again, the 9α- and 12α-bromo- and -iodo-11β-hydroxysteroids are unstable to alkali (252, 257, 259) and the author and V. B. Mahesh (unpublished) found that con-

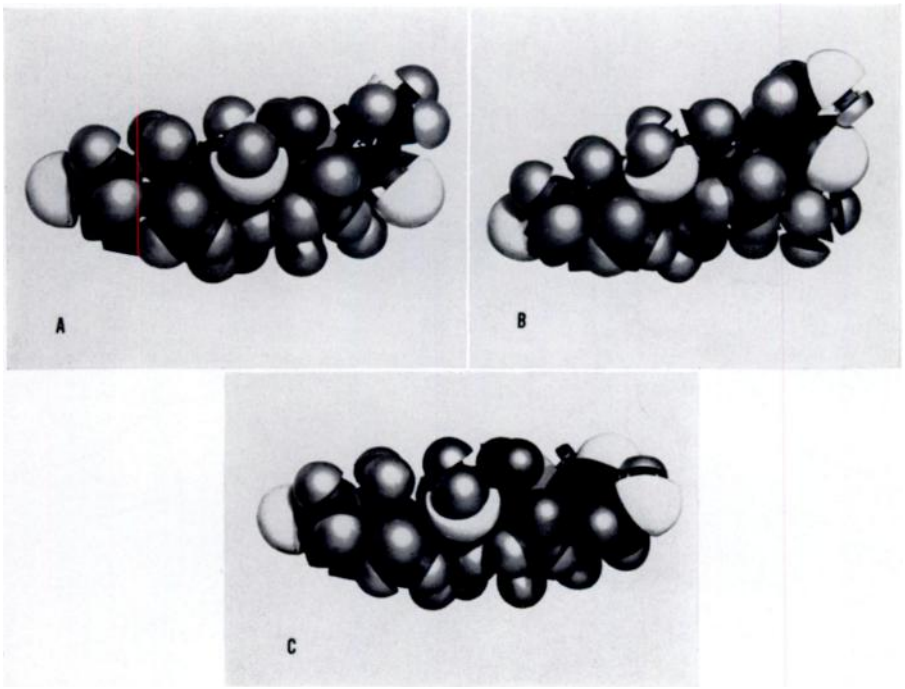

FIG. 7. Hydrogen bonding and rotation of the 17-β-side-chain of cortisol

The model is in the same general position as that of Fig. 6. Oxygen atoms white; hydrogen atoms gray. (a) 20-Carbonyl oxygen on β-side with a 17α,21-hydrogen bond; (b) 20-carbonyl oxygen on β-side with a 20,21-hydrogen bond; (c) 20-carbonyl oxygen on α-side with a 17α,20-, and 20,21-hydrogen bonds.

The preferred conformation and the extent of hydrogen bonding of such side-chains in aqueous solution is at present not known. The 20β-conformation of the 20-carbonyl oxygen is possibly the most likely one for association with the receptors for glucocorticoid activity (see text).

siderable conversion of 9α-bromocortisone to 8,9-dehydrocortisone had occurred after oral administration in man.

B. Stereochemical factors affecting biological activity

1. Optical isomerism. The absolute configuration of all the nonbenzenoid and of most of the benzenoid steroid hormones, and of their analogues, is now established. Cornforth *et al.* (164; see 507) made one of the major contributions to this field and have reviewed the earlier work of J. A. Mills and of Prelog's

group; these authors have correlated the structure of cholesterol with that of *d*-glyceraldehyde (Fischer convention). Fortunately, the absolute configuration of this substance is also established and corresponds with the conventional method of drawing structural formulae. The conventional drawing of the structures of steroids with β-bonds (solid) considered as extending upward from, and with α-bonds (dashed) sticking below, the plane of the drawing thus corresponds with the real configurations of the molecules themselves (Fig. 8).

The total synthesis of racemic *dl*-cortisone (558) and of *dl*-aldosterone (633a, 766a) and their resolution has shown that the natural *d*-compounds are active and that the racemates have one-half this activity so that the *l*-compounds are inactive (558). The anomalously high activity of racemic *dl*-cortisone by *local* application has been explained above (558). The work of Johnson's group has

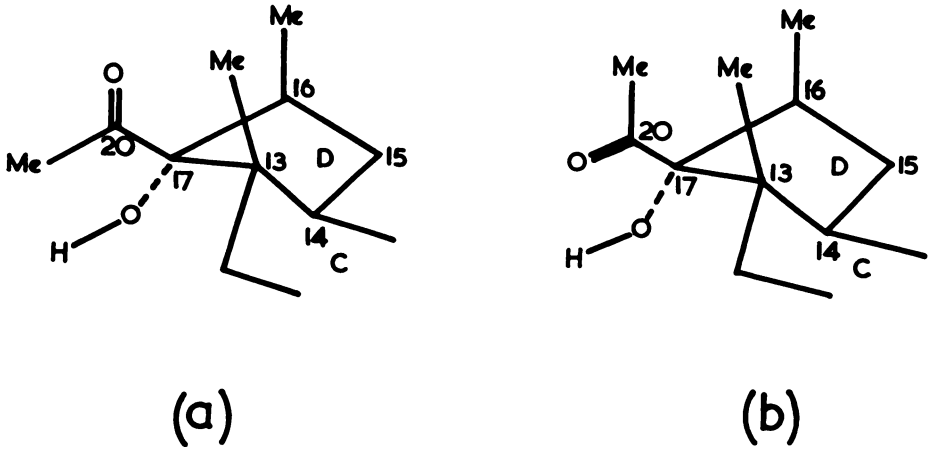

FIG. 8. The 17β-side-chain in the presence of a 16β-methyl group

(a) The preferred conformation. (b) The strong repulsive interactions to be expected between the 13β-, 16β- and 20-methyl groups in the opposite conformation [after Taub *et al.* (727)].

established that androgenic activity of C_{19} steroids is also confined to the natural optical isomers (376a). It is probable but not yet proven that the same is true of progestational activity. Johnson's group have also synthesized the racemates of all the 8 possible stereoisomers of estrone (376b). The activity of the racemate of estrone-b suggests that only the natural enantiomer possesses full biological activity. In contrast, the unnatural *l*-equilenin has a definite though low biological activity (26). The available evidence thus suggests that the biological activities of nonbenzenoid steroid hormones and their analogues are confined to one family of optical isomers, and that the same optical family embraces all known types of steroid hormones.

2. cis/trans-Isomerism of the carbon skeleton. The all-*trans* carbon skeleton of most natural steroid hormones (Fig. 5) seems essential for the possession of biological activity of nearly all types of steroid hormone. Thus the vast ma-

jority of active steroids are either Δ^4-3-ketones or 5α-(H)-steroids (507, 606; Tables 1 to 7). The exceptions are, however, extremely interesting, and some types of biological activity which will not be discussed here are evident with the 5β-(H) (A/B *cis*) series of steroids [*e.g.*, anaesthetic (439) and pyrogenic (393) activity]. The requirements for geometrical specificity are generally less strict for the relatively hydrophobic androgens and gestagens than they are for the relatively polar glucocorticoids and salt-retaining steroids.

A number of inactive compounds are known which differ from the natural configuration at only one centre of asymmetry. Since the available evidence suggests that if anything such changes would *decrease* the rate of inactivation and elimination of the steroid and since the natural epimers are of considerable potency, it is reasonable to conclude that these structural changes interfere with the receptor-steroid association or with the intrinsic action (14) of the steroid.

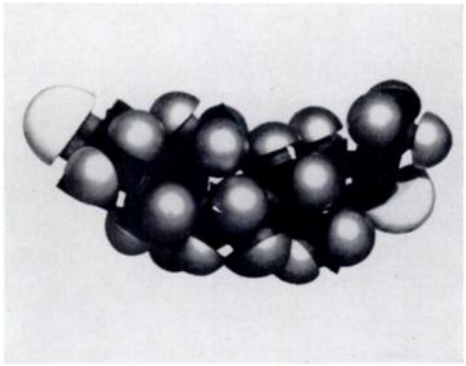

FIG. 9. A model of 9β,10α-progesterone ("retro"-progesterone)

Note the bending of the A and B rings to the α-side (upwards in the figure). The angulation is prevented from being greater than it is by the 10α-methyl group, and space-filling models suggest that ring A takes a half-boat conformation (*cf.* 637). Oxygens white. Orientation is same as in Fig. 6.

Examples of this type of epimerism are 17α-progesterone (130, 324; compound 308), 13α-androsterone (128; compound 407), 17α-deoxycorticosterone (656; compound 209), and 14β-equilenin (26; compounds 551, 552). A number of doubly-inverted epimers are also inactive: *e.g.*, 14β,17α-progesterone (556; *cf.* compound 303) and 14β,17α-deoxycorticosterone (212; compound 210).

The most important exceptions to this general rule are 8α-testosterone (181; compound 391) which has 0.4 $\times$ the activity of testosterone, 19-nor-14β,17α-progesterone (212, 213; compound 303) which is several times as active as progesterone itself, and 9β,10α-progesterone and its derivatives, the so-called "retro-steroids," which are several times as active as progesterone itself (637. 780a; compounds 263, 265) (Fig. 9).

3. Geometrical isomerism of substituents. In the natural steroids there is good evidence that combination with the receptors or intrinsic action is possible only with certain crucial substituents in one of the two epimeric configurations. Thus,

11-epicortisol (*i.e.*, 11α-OH, 11β-H) and 11-epicorticosterone are inactive (252, 616, 786, 787); there is no known 11α-hydroxysteroid dehydrogenase in mammalian tissues. Similarly, 17-epitestosterone (*i.e.*, 17α-OH, 17β-H; compound 423) is even less active in most assays than androstenedione (605) and its weak activity can be attributed to its partial conversion *in vivo* to testosterone by the reactions: 17-epitestosterone $\overset{A}{\rightleftharpoons}$ androstenedione $\overset{B}{\rightleftharpoons}$ testosterone, reaction A being catalyzed by a 17α-hydroxysteroid dehydrogenase, and reaction B by the 17β-hydroxysteroid dehydrogenase (721). When reaction A is blocked as in 17β-methyl-17-epitestosterone (488; compound 421) or is greatly slowed as in 3-deoxy-17-epitestosterone (347), or reaction B is greatly slowed as in 3-deoxyandrostenedione (347, 350), then it is found that only the 17β-ol is active as an androgen. Similarly, 17-epiestradiol (compound 561) is very weakly active, and estradiol itself with a 17β-hydroxyl group is analogous with testosterone in this respect (347).

In the natural steroid hormones, these are the only substituents that are potentially epimeric. A number of steroids with saturated A rings, however, are metabolites of the Δ^4-3-ketosteroids, and being biologically active are possibly of endocrine significance (*e.g.*, 329, 605). It is clear that the androgens provide the vast majority of such examples. Thus, a large number of 5α-(H)-C_{19}-steroids are active androgens and in these cases the epimeric 3α- and 3β-ols are both active (see Table 5). The differences in the activities of the epimers show several irregularities and could well be due to factors affecting their distribution and metabolism. This is probably the basis for the regular finding that 3-ketones are usually more active in the chick comb assay than the related 3β-ols (507). Epimers of this sort are also interconvertible in most cases by 3α- and 3β-steroid dehydrogenases (721, 741) *via* the 3-ketone. There is not yet sufficient evidence upon which to decide whether one or both epimers of 3-hydroxysteroids can combine with receptors for steroid hormones. All the natural nonbenzenoid steroids with undoubted status as hormones possess the Δ^4-3-ketone group.

4. Other geometrical changes in the carbon skeleton. Under this heading are considered the geometrical factors which are affected by the addition to, or subtraction from, the carbon skeleton.

The first major discovery in the field of synthetic analogues of steroid hormones was that removal of the 10β-methyl group (*i.e.*, C-19) of several classes of steroid hormone provided compounds with enhanced and sometimes altered biological activity (75, 182). It is now known that these 19-norsteroids are more active than their parent steroids in the case of progesterone (182, 235, 500, 625, 750; compounds 259, 263, 264, 303), corticosterone (compound 42), and deoxycorticosterone (compound 185) (23, 382, 614, 615). The same is true for 17α-alkyltestosterones both in respect of their androgenic activity, and even more of their levator or progestational activity (compounds 369–372, 462–466, 468–487, 492, 493, 506–519) (320, 419, 614; *cf.* 158). The evidence for testosterone itself is somewhat conflicting (74, 158, 177): the activity of 19-nortestosterone (compounds 336, 337) is less than that of testosterone in several assays, but is still considerable

19-Norcortisol (compounds 38, 39) has definite though reduced biological activity: earlier reports of greatly reduced or absent activity (193, 279) have been firmly contradicted by others (382, 466, 817) who found activities of 0.1 to 0.3 × cortisol in tests for glycogenic and anti-inflammatory activity, and greatly increased sodium-retaining activity (466).

The 18-norsteroids, in which the 13β-methyl group has been removed, appear to be inactive or very weakly active. The work is incompletely published but suggests that a number of steroids of this type have been synthesized in unpublished work with the same result (75, 374). Thus, 18-nordeoxycorticosterone (compound 200) has 0.1 × the sodium-retaining activity of deoxycorticosterone (374), 18,19-bisnorprogesterone (compound 299) is inactive (518, 688), 18-norandrostenedione is inactive (75; compound 408), and so is 18,19-bisnortestosterone (374; compound 406).

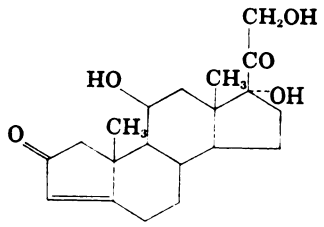

FIG. 10. A-nor-cortisol

A-nor-cortisone is biologically inactive: this may be due to prevention of the reduction of the 11-ketone by the displaced oxygen of the natural 3-ketone group.

It was thought by Johnson (75, 376) among others that the conformation of the strained 5-carbon D-ring was stabilized in the natural steroids by the 13β-methyl group. Such stabilization would not be necessary if ring D were enlarged to a 6-carbon ring. In accord with this line of argument, it was found that certain 18-nor-D-homosteroids possessed significant or even full biological activity while their 18-nor analogues were inactive. A striking example is 18-nor-D-homo-5α-androstane-3,17-dione (compound 409), which is slightly more active than androstanedione itself (376), but in contrast a number of 17α-alkyl-18-nor-D-homotestosterones were found to be inactive (122a; compound 461).

Enlargement or contraction of various rings of the steroid nucleus has now been achieved for a number of types of hormone. In the first place, a number of D-homosteroids possess activities comparable to or even much greater than their parent steroids including those derived from testosterone, androstenedione, androsterone, 3-epiandrosterone, and several androstane-diols (325; compounds 409, 424, 425, 440–445). D-Homocortisone (152; compound 128) has 0.4 × the activity of cortisone. On the other hand, D-homodeoxycorticosterone (compound 208) was said to have no sodium-retaining activity and only 0.1 × the life-maintenance activity of deoxycorticosterone (186).

Contraction of ring A to give A-nortestosterone, A-norprogesterone (compound 254), and A-norcortisone (778; Fig. 10) abolishes biological activity. On

the other hand, B-homo-5α-dihydrotestosterone has slightly greater activity than 5α-dihydrotestosterone itself (574a; compounds 385, 386).

Rupture of the fused-ring skeleton has effectively been carried out by the synthesis of various analogues, usually in racemic form, from nonsteroid precursors. Such compounds appear to be very weakly active (799) or inactive (*cf.* 525a), for instance the 11,12-bisnor-C-seco-progesterone (compound 309) and -deoxycorticosterone made by Wilds and Shunk (798).

There is undoubtedly much unpublished work which has achieved the same results in other fields (see also 797). Earlier work is referred to by Wilds and Shunk (798) and by Birch (75) (*cf.* 508 for estrogens).

A large number of analogues with extra double-bonds in the carbon skeleton have been made. Introduction of a 1,2-double bond produces compounds of enhanced biological activity in the case of cortisol and cortisone-like steroids (see Table 1). The activity of progestational (54, 566; *cf.* 42), androgenic (126, 481) and nonhalogenated sodium-retaining steroids (70, 601) is usually reduced to 0.1 to 0.5 of that of the parent steroid by this modification. Levator activity is sometimes increased (*e.g.*, 481, 816a). It will be seen from Tables 1 and 3, however, that this modification apparently *increases* the sodium-retaining activity of many 9α-halogenated steroids.

It is also well established that 6,7-unsaturation (ring B) reduces glucocorticoid activity (62, 252, 289), progestational activity (783), and androgenic activity (783) of typical parent steroids, but increases the sodium-retaining activity of 11-oxygenated steroids (252) while reducing that of deoxycorticosterone (783). 8,9-Dehydroprednisone (compound 79) has 0.15 × the activity of prednisone. More germane to later arguments, however, are the facts that 16,17-dehydrocorticosterone (compound 130) is appreciably active (5) while the related 11-ketone (compound 140) is not (466), and that 16,17-dehydroprogesterone (compound 306) is inactive (783). The introduction of a 9(11)-double bond into deoxycorticosterone leaves sodium-retaining activity relatively unchanged (23) while 11,12-dehydroprogesterone (compound 294) is three times as active as progesterone itself (297). More interesting still, the A-aryl analogue of cortisol, 3,11β,17α,21-tetrahydroxy-19-nor-pregna-1,3,5(10)-triene-20-one (compound 40), has about 0.3 × the glucocorticoid activity of cortisol itself (466). The potent antiestrogen activity of the 19-nor-$\Delta^{4,9\,(10)}$-3-ketosteroids is interesting (538): their other activities (androgenic, levator) seem to be similar to those of the 19-nor-Δ^{4}-3-ketosteroids.

The size of the 17β-side-chain of C_{21}-steroids has also been subjected to modifications. This side-chain projects more or less in the plane of the skeleton since the 17,20-bond is equatorial in conformation (Fig. 5). Adding a 21-methyl group to 9α-fluoroprednisolone causes a moderate reduction in glucocorticoid activity (3a), while 23-acetoxy-norchol-4-ene-3,22-dione (compound 216) is devoid of sodium-retaining activity (784). Similarly, 21-norprogesterone (compound 317) is biologically inactive (489), while 21-methylprogesterone (compound 314) has very weak, and 21-ethylprogesterone (compound 312) no detectable activity (782).

Substitution of the 21-hydroxyl group of corticoids with fluorine increases their glucocorticoid and sodium-retaining activities, while similar substitutions with chlorine and bromine abolish activity. These effects are almost certainly due to complicated secondary factors. Similar effects on progestational activity are seen with 21-halogenated derivatives of 17α-acetoxyprogesterone (54, 725; *cf.* 221).

A full analysis of the role of these side-chains will need much further work to be done on the steric, electronic, and metabolic factors that are probably involved in the biological effects of modified side-chains.

It is not easy at first sight to see any rhyme or reason in the effects of these changes of the carbon skeleton on the biological activities of steroids. From the inspection of models, however, and by laying more weight on the possession of activity than on small decreases in activity, it is possible to suggest with reasonable confidence that changes of the configuration of the "upper" half of rings C and D have a far greater effect on all types of biological activity than many changes in the configuration of rings A and B. If the receptor concept is accepted, then it seems likely that the closest and most specific attachments of steroid hormones occur over the surface of the steroid around carbon atoms 11, 12, 13, 16, 17, 18, and in C_{21} steroids, 20 and 21. The strength of this argument will be seen to be increased by a consideration of the effect of the bulky substituents found in some steroid analogues.

5. The effects of bulky substituents. It is now clear that biological activity, either in full or even greatly increased, is present in a wide range of steroids possessing very bulky substituents which are not found in the natural steroids. Sarett and others (124a, 616, 821) have pointed out independently that β-substituents abolish the glycogenic activity of cortisone-like analogues while α-substituents do not. In Figure 11, two composite projections illustrate most of the known α-substituents which are known to be compatible with considerable and often very greatly enhanced glucocorticoid activity in cortisone-like analogues. The very large protuberances on the normal α-surface that are produced by the 9α- and 12α-bromine and iodine atoms (133, 249, 703), by the angulation of the planes of the A and B rings in 1,2-dehydrosteroids (252), and by the various ketal groups of the 16α, 17α-ketals (252, 253) are notable. It seems incontestable that the association of these steroids with their receptors must be by some part of the "upper" and β-surfaces of rings C, D and the 17β-side-chain (124a, 616).

Certain findings are at first sight anomalous. Thus 7α- and 7β-methyl-cortisol (compounds 51, 52) are less active than cortisol itself and this might suggest that the "lower" parts of rings B and C (C-6, C-7) are closely opposed to the receptor surface. The large glycogenic activity of many 6α-methyl and 6α-chloro steroids (*e.g.*, compounds 50, 53, 60) makes this unlikely and there are reasons for preferring other explanations of the influence of 7α- and 7β-methyl groups. The 7-position is peculiar in that the equatorial 7β-hydrogen and other 7β-substituents are more hindered than the axial 7α-position. This reversal of the usual relationship (41) is due to steric hindrance by the "lower" edge of rings C and

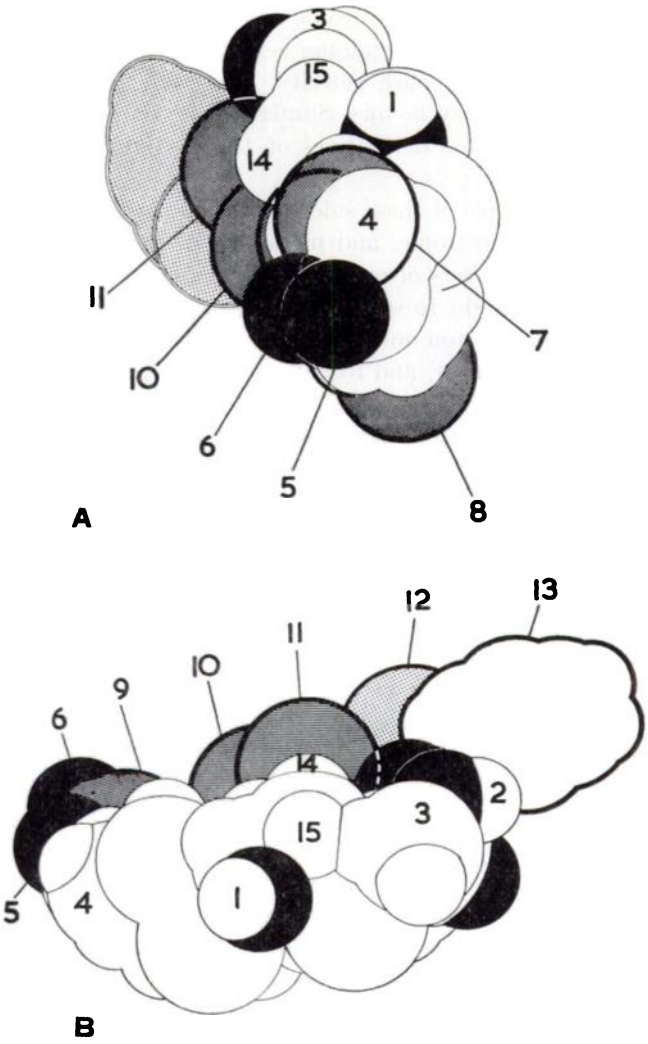

FIG. 11. The major bulky α-substituents which leave intact or greatly enhance the glucocorticoid activity of cortisol

(a) Projection on to a plane perpendicular to the line through carbons 8 and 10. The 3-carbonyl oxygen is nearest the reader.

(b) Projection from "above" on to a plane perpendicular to the 9,11-carbon-carbon bond. C-21 at the right is nearest to the reader.

1. 11β-Hydroxyl (H-atom labelled); *2*. 21-hydroxyl (H-atom labelled); *3*. carbon-21; *4*. carbon-2 (in Δ^4-3-ketones); *5*. 3-oxygen (in Δ^4-3-ketones); *6*. 3-oxygen (in $\Delta^{1,4}$-3-ketones); *7*. 2α-methyl group; *8*. 6α-methyl group; *9*. carbon-2 ($\Delta^{1,4}$-3-ketones); *10*. 9α-bromine; *11*. 12α-bromine; *12*. methyl group of 16α,17α-diol acetophenonide; *13*. phenyl group of (12); *14*. 12α-hydrogen; *15*. 12β-hydrogen. Oxygen atoms black. Extra volume of groups in the analogues are shaded or stippled, except for (13) in (b).

D (C-14, C-15). It is likely that methyl groups in this position produce a fair amount of distortion of rings C and D by their steric pressure. At any rate, the loss of activity produced by these substitutions is not large enough to exclude the role of secondary factors, and it is not justifiable at present to attribute it to an influence on the steroid-receptor interaction.

The reduced activity of 6β-chlorocortisone (816a; compound 54) and similar analogues (578) might also suggest that close apposition of the whole of the β-side of ring B with the receptors was necessary. While this is possible, the effect may well be due to distortions transmitted to the rest of the β-side of the molecule and to hydrogen bonding with the 11β-hydroxyl group.

16β-Methylsteroids have the same glycogenic potency as their 16α-methyl-epimers (660, 727). As Sarett has pointed out, however (616), these substituents are at the sterically unique 16-position (Fig. 5) where both epimeric configurations have an intermediate conformation which is not strictly axial or equatorial. The protuberance of the 16β-methyl group beyond the β-surface of the natural steroids is therefore small.

The position is much more confusing with sodium-retaining steroids. Thus, the 2α-methyl group enhances very greatly the sodium-retaining activity of 11-oxygenated steroid analogues (252; compounds 218, 221) but reduces the activity of deoxycorticosterone to 0.05 of that of the parent steroid (446; compound 180). This low activity, however, may well be due to the very low solubility of this extremely hydrophobic analogue. What is certain, however, is that both 16β- and 16α-methyl, 16α-methoxy-, and 16α-hydroxyl groups abolish or reverse this activity *both* of 11-oxygenated steroids (Tables 2, 3) and of deoxycorticosterone (23, 156, 464a, 546). Since the 16-methyl and 16-hydroxyl groups have opposite effects on the solubility properties of the compound, and since they cause a small decrease (hydroxyl) and a small increase (16α-methyl) in the glycogenic activity of 11-oxygenated steroids, it is likely that the loss of sodium-retaining power is due to an effect on the steroid-receptor interaction rather than solely upon secondary factors. The same arguments can reasonably be applied to the 17α-hydroxyl and 17α-methyl substituents, both of which abolish the sodium-retaining activity of deoxycorticosterone (23, 222, 375, 382, 555, 663; compounds 207, 211).

Until more is known about the identity of the receptors for the sodium-retaining activity of deoxycorticosterone and 11-oxygenated steroids, it is difficult to make any specific suggestions as to the nature of the steroid-receptor interaction for this type of activity. It is at least possible, however, that close association with the α-surface of rings A, C and D, and the side-chain is essential for this type of activity.

Progestational activity shows many of the features of glucocorticoid activity. Thus, considerable activity is possessed by the 6α-methyl-, 17α-capryloxy-, 6β-methyl-, 6α-chloro-, 6,7-dehydro-6-methyl-, 17α-methyl-, and 17α-chloro- derivatives of 17α-acetoxy-progesterone, and by a number of 9α-bromo-11β-hydroxysteroids (53, 252, 255). The even greater distortion of the whole of the

A and B rings in the 9β,10α-progesterones ("retro"-progesterones) (Fig. 9; Table 4) is also compatible with biological activity much greater than that of the parent steroids of the natural series (637, 780a). Finally, the considerable and often greatly enhanced activities of many 17α-alkyl-19-nortestosterones (Table 6) suggest strongly that the α-surface of these steroids is not involved in any structurally specific links with their receptors (Figs. 12, 13; Table 8).

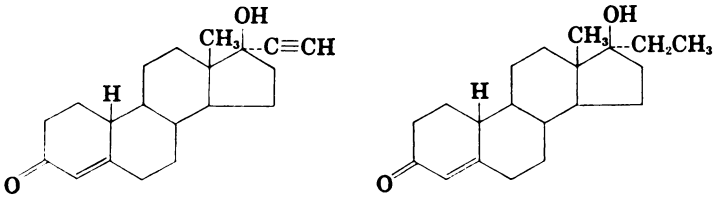

FIG. 12. Typical 17α-substituted 19-norsteroids

(a) 17α-Ethinyl-19-nortestosterone ("Norethisterone"); (b) 17α-ethyl-19-nortestosterone.

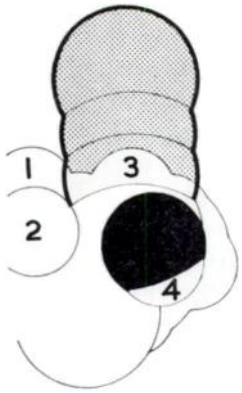

FIG. 13. A typical bulky 17α-substituent giving a compound with enhanced progestational activity

The area of the D-ring of a 17α-(2-methallyl)-17β-hydroxysteroid in the same projection as in Fig. 11 (b). *1*. 12α-Hydrogen; *2*. 12β-hydrogen; *3*. 17α-hydrogen in natural steroids; *4*. 17β-hydroxyl (hydrogen labelled). The extra bulk of the 17α-(2-methallyl) group is stippled.

It is reasonable, therefore, to suggest that the steroid-receptor interaction that is responsible for progestational activity involves the "upper" and β-surface of rings C and D and the side-chain of the steroid. Other features will be discussed below.

Androgenic activity presents a more complicated picture. Zaffaroni (816a) has given an excellent brief review. Inspection of Table 5 will show that bulky substituents have been studied in positions 1, 2, 4, 6, 7, 9, 11, 16, and 17, and that a wide variety of structures have been tried out in ring A which appear to produce very varied results. Another difficulty is that most of these compounds

are very hydrophobic in character, so that bulky nonpolar substituents may produce gross effects on their absorption and distribution (see, *e.g.*, 423, 424). Unlike steroids with other types of biological activity, the androgens are remarkable for the very large number of natural or synthetic steroids in which a saturated structure in ring A of the 5α-(H) configuration (but not the 5β-(H)-epimers) is associated not only with substantial activity but often with greatly enhanced activity (*e.g.*, 97, 101, 574, 606). The possession of considerable activity by 6α-methyl- and 4-methyl-testosterone (21, 577, 580) suggests that close apposition of these steroids to their receptors does not occur over the "lower" edge of rings A and B.

Protuberances from the α-side of ring A, however, appear to cause considerable losses of biological activity. Judging by existing evidence from other classes of steroid, these cannot reasonably be ascribed to secondary factors. Thus, a 1,2-double bond (126), a 2α-methyl group (481, 579), a 2-methylenehydroxy group (576), a 1α-acetylthio group (188), or a 3α-methyl group (424) causes great reductions in, or the disappearance of, androgenic activity. On the other hand, various 9α-bromosteroids have fair or considerable androgenic potency (592; *cf.* 819).

Another source of difficulty is the controversy over the effect of 17α-alkyl substituents. The 17α-methyl group, while conferring oral activity on a typical C_{19}-17β-ol, reduces androgenic potency by parenteral routes while leaving levator activity intact. Larger 17α-alkyl groups (*e.g.*, -ethyl, -propyl, -vinyl, -propargyl) cause the appearance of considerable progestational activity while reducing or abolishing androgenic activity (Tables 5, 6). Most clinical workers, however, have found that androgenic activity is still present. However, it is clear that substitutions and modifications in ring A have much more complicated and striking effects on biological activity than in other classes of steroids. It is difficult to point with any certainty to a specific area of these steroids which might be most closely and specifically opposed to their receptors. One possible reason for the present confusion is suggested by the low substrate specificity of β-steroid dehydrogenase (721). It is clear that this enzyme, while associating specifically with the α-side of C_{19}-steroids, can attack both 3- and 17-ketone groups. It is possible that such steroids can fit the active site in either of two positions in which the line from carbon-3 to carbon-17 is rotated through 180° in the main plane of the carbon skeleton. If this were true for the steroid-receptor association responsible for androgenic activity, then groups added to ring A of certain androgen analogues might be interacting with the part of the receptor site normally associated with ring D and *vice versa*. The possibility of such "reversed" associations may well be the reason for the activity of symmetrical diphenols of the stilbestrol family of synthetic estrogens. On the other hand, they would be unlikely to confuse the picture with C_{21}-steroids in which the 17β-side-chain confers a pronounced asymmetry upon the carbon skeleton.

The appreciable androgenic activity of 8α-testosterone (compound 391) suggests, however, that close apposition of the β-surface of ring B is not involved in the steroid-receptor association for androgen activity. Djerassi *et al.* have

provided strong evidence from the optical rotatory dispersion spectrum of this compound that the B ring is in the unusual boat form (181). This produces a large β-sided protuberance on the molecule and leaves the ring A in a similar conformation relative to rings C and D to that found in testosterone itself.

At present then, the balance of the evidence is in favor of an association of the α-side of the steroid with the receptors for androgenic activity, but more work is needed before the uncertainties about the role of substituents at C-17 will be resolved. A β-sided association is suggested, for instance, by the activity of epimeric 17-hydroxysteroids (124a; see below). In a recent article Ringold (574b) has made a closer analysis and favors α-sided attachment of androgens to their receptors. His arguments, however, do not deal completely with the uncertainty of the effects of 17α-substituents.

The steroid estrogens will not be dealt with fully. It is clear, however, that considerable or full activity is possessed by 17α-ethinylestradiol (364; compound 562) and by the 17α-methyl- and 17α-vinyl derivatives (83, 196, 364; compounds 555–557), while 17α-hydroxy-epimers, like the androgens, are of low or negligible activity (347). Unlike the C_{21}-steroids, but like the androgens, estradiol has its activity very considerably reduced by a number of alkyl substituents in rings A and B (366, 759), and by all 16-substituents (196, 348, 406, 503, 590) that have been tried.

C. The role of the polar groups of steroids

Polar groups exert their effect in two ways. First, they fill space in the same way as nonpolar substituents, and secondly, they are sites of electrical forces between themselves and other polar groups around them. This second feature of polar groups is associated with an ability to undergo chemical reactions with a facility not possessed by nonpolar groups. In aqueous media, truly electrostatic forces are weak compared with the London, or "exchange," forces common to all atoms, except those due to hydrogen bonds.

Contrary to previous suppositions, it is now known that many of the polar groups of steroid hormones are not strictly required for the possession of biological activity, at any rate in the form found in the natural hormones. The best working hypothesis at present is that certain polar groups of steroids are probably involved in structurally specific links with the receptors for these hormones; that a larger number are involved in much less specific links; and that many or possibly all of them can be dispensed with, while retaining biological activity, if the steroid-receptor association is strengthened by modifications of other parts of the steroid molecule (124a, 250; *cf.* 157).

1. The Δ^4-3-ketone group. It is generally held that the Δ^4-3-ketone group is essential for all types of corticoid activity and for progestational activity. While this question needs further investigation, this view has been based largely upon a relatively limited number of tests with saturated 5β-(H) steroids, and with 3-hydroxysteroids for which the rates of conjugation and elimination from the body are very high. At present, the main "function" of the Δ^4-3-ketone group seems to lie in the rate-limiting effect of the 4,5-double bond on the metabolic

inactivation of the nonbenzenoid steroid hormones (616). It is unlikely that this structure plays more than a minor role in contributing to the steroid-receptor association constant, or to the specificity of this association, at the receptors for all types of steroid activity.

While equally striking evidence is not yet available for sodium-retaining and glucocorticoid activity, it is clear that a number of modifications of ring A are compatible with the possession of considerable biological activity (124a). The chief examples are:

5α,11β,17α,21-tetrahydroxy-5α-pregnane-3,20-dione	1.0 × cortisone acetate (61)
4,5α-dihydroprednisone	0.15 × cortisone acetate (805)
9α-fluoro-4,5α-dihydroprednisolone	0.6 × cortisone acetate (331)
5α,6β-dihydroxy-4,5α-dihydrocortisone	0.16 × cortisone acetate (739)
3,11β,17α,21-tetrahydroxy-19-nor-pregna-1,3,5-trien-20-one	0.13 × cortisol (466)
Prednisolone	4 to 5 × cortisol (323).

Prednisolone is included here because although it contains the Δ^4-3-keto group, the introduction of the 1,2-double bond produces such a large change in both the polar and steric properties of ring A, that any interaction of this part of the natural steroid with its receptor would suggest that such interactions were of low specificity.

In the field of androgens, it is clear that a number of 3-deoxy- and 3β-fluoro-3-deoxy-steroids possess significant androgenic activity (175, 347, 350, 370, 423, 424). There is no evidence for and much against the supposition that their activity is due to the introduction of the missing 3-ketone or 3-hydroxyl group *in vivo*. Even more striking are the biological activities reported by de Winter *et al.* (175; compound 466) for a series of 3-deoxy-17α-alkyl-19-nortestosterones which were as potent as oral gestagens as the related 3-ketones.

2. The 17β-hydroxyl group. There are some grounds for believing that the 17β-hydroxyl group of testosterone plays a specific role in the association of this steroid with the receptors for levator and androgenic activity (124a, 605). In the first place, a number of steroids lacking any oxygen function at C-17 are inactive (347, 350) both by systemic and local administration. 3-Deoxysteroids of similarly low solubility are active in the same tests, so that it is unlikely that this is due to secondary factors. Again, all steroids active in assays for androgenic and levator activity possess a 17β-hydroxyl group or else a group (17α-hydroxyl or 17-ketone) which can be converted to it. A small number of 17α-hydroxy- and 17-keto-steroids in which this conversion is blocked are inactive (see above). At present, no other type of substituent at C-17 is known which will replace the 17β-hydroxy group or its precursors, although Dorfman has found that androstane itself is active both in the chick comb assay and the rat seminal vesicle assay (190a). However, large doses were used and oxidation at C-17 may have occurred. The polar groups of testosterone, therefore, may not be essential for androgenic activity, although the 17β-hydroxyl group of testos-

terone itself probably forms a fairly specific link with the receptors for androgenic activity.

3. The 11β-hydroxyl group. A very similar position holds for the 11β-hydroxyl group and glucocorticoid activity (124a). All but two types of known active steroids possess this group or an 11-ketone group which is known to be, or is probably, reducible. A small number of 11-ketones which are not reduced *in vivo* to the 11β-ols are inactive, while the related 11β-ols are active (see above). A large number of steroids lacking this group are essentially devoid of glucocorticoid activity. It seems likely, however, that even this *doyen* of "specifically required" groups can be dispensed with. The first example of a definite glucocorticoid lacking this group was the weakly but definitely active 1α-acetylthio-17,21-dihydroxypregn-4-ene-3,20-dione (Dodson and Tweit, 187). The reviewer suggested that the 1α-acetylthio group might replace the 11β-hydroxyl group by supplying a lone pair of electrons favorable for hydrogen bonding with a proton-donor group on the receptor (124a). The conformation of this group is in fact accurately known because of the steric pressure to which it is subjected (187), and it is seen in Figure 14 that the carbonyl oxygen is remarkably well placed to play this role.

The second exceptional type of 11-deoxysteroid is more striking still, in that there is no polar group available to play the role of the 11β-hydroxyl group. The best example is 16α,17α-isopropylidenedioxy-6α-methylpregna-1,4-diene-3,20-dione which was synthesized by Petrow's group (73a) after a related progestational analogue was found to be active, in addition, in the liver glycogen and systemic anti-inflammatory assays (see also 278a). The related Δ^4-3-ketone (*i.e.*, the 1,2-perhydro derivative) was twice as active as cortisone, and the $\Delta^{1,4}$-3-ketone itself nearly as active as prednisolone. In local assays on the skin of human volunteers, its anti-inflammatory activity was equal to that of cortisol. The authors suggested that this proved that its systemic activity was not due to conversion *in vivo* to an 11-oxygenated derivative.

It seems indeed probable that this compound is itself possessed of glucocorticoid activity and that its spectrum of activities is similar to that of cortisol. One cannot, however, accept the argument of these authors given above as a convincing proof of their contention. It is clearly established that a number of steroids, especially heavily substituted and hydrophobic compounds, have local but not systemic anti-inflammatory activity. As discussed earlier, one cannot assume without further analysis that local anti-inflammatory activity is pharmacologically identical with the systemic anti-inflammatory activity of glucocorticoids. The local activity of this compound may well be nonspecific in type, a possibility which is made all the more likely by the fact that its relative potency in the local assay is only ¼ to ⅕ of its systemic potency. It is probable that the 11β-hydroxy-derivative of this synthetic steroid has a potency of about 100 times that of cortisol (*cf.* 16, 253, 683); according to the type of assay (and Petrow's group used an aqueous suspension by subcutaneous injection for their assay), from 1 to 4% conversion to the 11β-hydroxy derivative would suffice to give potencies in the range reported by Petrow's group. The crucial impor-

tance of this compound and of similar 6α-methyl-11-deoxysteroids (278a) for theories of the nature of the intrinsic action of cortisone-like steroids is obvious, and it is very desirable that a complete analysis of its action should be carried out.

Despite these interesting exceptions, it is highly likely that the interaction of most glucocorticoids with their receptors involves a structurally specific link

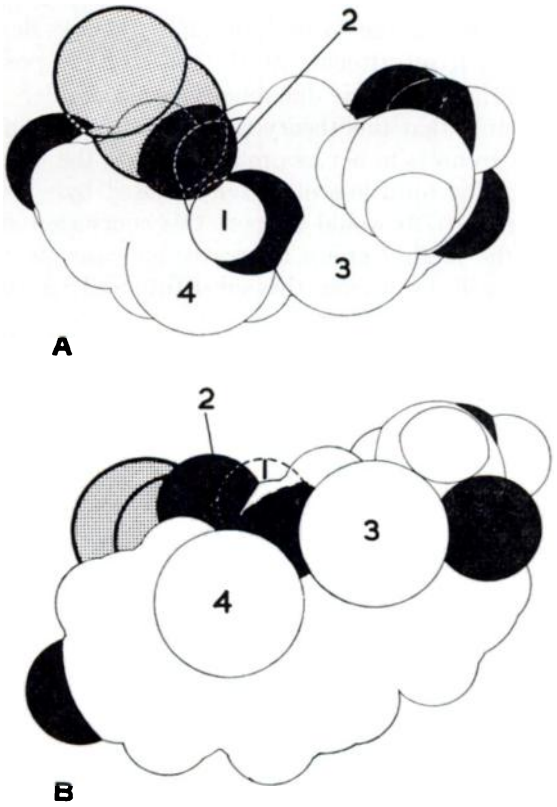

FIG. 14. Projections of 1α-acetylthio-11-deoxycortisol

The 11β-hydroxyl group of *cortisol* is shown for comparison. (a) Projection on to a plane perpendicular to the 9,11-carbon-carbon bond with β-surface at bottom of figure. (b) Projection on to a plane parallel with that of carbons 5, 7, and 8 with β-face towards reader. *1*. 11β-Hydroxyl group (hydrogen labelled); *2*. carbonyl oxygen of 1α-acetylthio group; *3*. 13-methyl; *4*. 10-methyl. Oxygens black: rest of 1α-substituent stippled.

with the 11β-hydroxyl group which cannot be satisfied by an 11-ketone group (*e.g.*, 354). The nature of this link is suggested by the role of halogen substituents at positions 9 and 12 (Fig. 15).

It was suggested above that the metabolic influence of the 9α-fluorine atom was likely to explain only part of the 10-fold increase in glucocorticoid activity that it causes in 11β-hydroxysteroids. Fried (252, 258, 259), who discovered these biological effects incidentally while exploring the possibilities of 9α-fluoro-

steroids as intermediates in chemical syntheses, suggested that the increased activity was due to the negative inductive effect of the halogen which would increase the acid dissociation of the 11β-hydroxyl group (459) and thus also the strength of a postulated hydrogen bond between this hydroxyl group and the receptor. In support of this theory, he went on to show that electropositive 9α-substituents caused marked decreases in the glucocorticoid activity of 11β-hydroxysteroids (252, 259), and that the effect of 12α-halogens was identical with those of the same halogen in the 9α-position (252, 319). As he pointed out, the inductive effects of such substituents at the 9α- and 12α-positions should be negligibly different, while the steric difference is considerable.

An important point is that this theory can be correct only if the normal role of the 11β-hydroxyl group is to act as proton *donor* to the hydrogen bond with the receptor (124a). The total loss of activity caused by substituting an 11α-methyl group in cortisol (72) would support this conclusion since the positive inductive effect of the methyl group is greatly increased in the tertiary 11β-hydroxyl group that will result from this substitution. This would decrease the

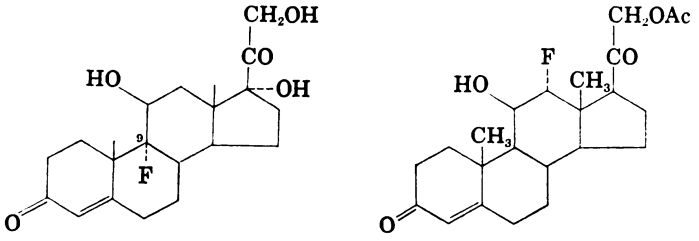

FIG. 15. (a) 9α-Fluorocortisol; (b) 12α-fluorocorticosterone-21-acetate

availability of the proton for hydrogen bonding even more markedly than in the 9α-methyl- and 9α-methoxy- analogues (249).

Fried noted (252) that the larger 9α-halogens caused far smaller increases in the glucocorticoid activities of 11-ketone analogues than were expected. These are explained by the diminishing inductive effect of the larger halogens on the redox potential of the ketone/hydroxyl pair and the probable partial steric hindrance of the reduction of the 11-ketone to the 11β-ol (124a). On the other hand, Fried (250a, 252) explained the anomalous lack of activity of 12α-chlorocortisone on the basis of hydrogen bonding between the 17α-hydroxyl and the 12α-chloro group. This might be necessary in the case of 12α-chlorocortisol but the inactivity of the 12-chloro-11-ketone would be readily understood as due to the almost complete inhibition of reduction of the 11-ketone group by large 12α-substituents (124e).

Fox *et al.* (246) found that the 11β-acetate of triamcinolone acetonide was about twice as active as cortisol in the liver glycogen and other assays. It is highly probable that this was due to hydrolysis despite the fact that the usual 11β-acetates are almost impossible to hydrolyze chemically. In the first place, esters of α-fluorocarbinols are much more rapidly hydrolyzed than esters of non-

fluorinated carbinols (*e.g.*, 310a), and in the second it is known that certain micro-organismal enzymes will hydrolyze the 11β-acetoxyl group very readily (143; see 507, p. 701). Only 2% of this steroid would need to be hydrolyzed *in vivo* to give the activity these authors obtained (see 253).

Present evidence, then, is satisfactorily accounted for by the theory that the steroid-receptor interaction for glucocorticoid activity involves a highly specific link between the 11β-hydroxyl group and the receptor, which is almost certainly a hydrogen bond the proton of which is derived from the 11β-hydroxyl group itself. Only two types of compounds are known which lack this substituent, or a reducible 11-ketone group, where the evidence is strongly suggestive that they possess full glucocorticoid activity.

4. The 17β-side-chain. The 9α-halogenated steroids have also illuminated the problem of the role of the side-chain in glucocorticoid activity. Since both 9α- and 12α-fluoro-11β-hydroxyprogesterone are approximately as active as cortisol (252, 319), it is clear that contrary to previous suppositions the 21-hydroxyl group is not essential for glucocorticoid activity. The best theory is probably that the α-ketol side-chain of the natural glucocorticoids forms hydrogen bonds which strengthen the steroid-receptor association but which are not critical to the structural specificity of the association. In the presence of a greatly strengthened link with the 11β-hydroxyl group of 9α- and 12α-fluoro-11β-hydroxysteroids, the links with the side-chain are no longer necessary for effective interaction (250, 252). The indispensability of the 20-ketone group is undecided (1; *cf.* 279).

The role of the polar groups of the side-chain is probably also unspecific in both progestational and sodium-retaining steroids, much the same as it is for glucocorticoid activity. Apart from the competence of the 17β-hydroxyl group to endow 17α-alkyl-testosterone analogues with full or greatly enhanced progestational activity, it is also known that both the 20α- and 20β-dihydro-progesterones are fully potent in the local Hooker-Forbes assay (816).

Djerassi's (179a) analysis of the preferred conformation of the 17β-side-chain of 17α-halogeno-20-ketones suggests that the 20-carbonyl oxygen of progesterone is normally in the β-conformation with the 21-carbon to the α-side. Engel and others have shown that a number of 17α-halogenoprogesterones (221, 476) and 17α-halogenocorticosterones (173, 220) have appreciable or full biological activity as gestagens and glucocorticoids, respectively. (The inactivity of some 17α-halogeno-11-dehydrocorticosterones may well be due to inhibition of the 11β-ol dehydrogenase.) In these analogues the 20-ketone must have been predominantly in the β-conformation according to Djerassi's findings (see also 416a).

The position is not equally clear for sodium-retaining steroids, but full or enhanced biological activity is possessed by a large number of 9α-fluoro-11β-hydroxy-21-deoxysteroids and 21-deoxy-21-fluorosteroids. On the other hand, it has been found that removal of the 21-hydroxyl group of deoxycorticosterone itself and of most 11-oxygenated sodium-retaining steroids reduces their activity quite considerably (252). The exact role of the 21-hydroxyl group will be understood only when the pharmacological identity of the sodium-retaining actions of 11-deoxy- and of 9α-halogeno-11β-hydroxysteroids has been proved

or disproved. Certainly it does not appear to be essential for all types of sodium-retaining activity.

5. Additional hydroxyl groups. A large number of analogues with extra hydroxyl groups have been synthesized. All of them show less activity than the unsubstituted parent steroids and this is true for all the main classes of steroid activities (see Tables 1 to 7). Such polar groups probably produce their effect mainly by decreasing the chemical potential of the substance in water. This will reduce nonspecifically the association constants of the steroids with all substances excepting those having groups placed favorably for forming hydrogen bonds or other links with them (124a). Such groups will also offer points of attack for the detoxicating enzyme systems of the body, particularly those forming conjugates. Other factors probably play some part, but there is as yet little information about them.

6. The isolated 9α-fluoro group. In an interesting paper, Bergstrom and Dodson (52) found that 9α-fluorodeoxycorticosterone has twelve times the sodium-retaining activity of deoxycorticosterone. They therefore challenged Fried's interpretation of the influence of this substituent in 11-oxygenated steroids, and suggested instead that the 9α-fluorine atom inhibits the metabolic inactivation of such steroids, "possibly 9-hydroxylation." While it is true that Fried's interpretation cannot be extended to this steroid which lacks a hydroxyl group α to the fluorine atom, one must dispute these conclusions of Bergstrom and Dodson. In the first place, 9-hydroxylation must be a very minor metabolic pathway if it exists in mammals; secondly, the metabolic inactivation of 9α-fluorocortisol is qualitatively and quantitatively similar to that of cortisol with the exception of the effect on the 11β-dehydrogenase reaction described above (124a); finally, Fried's analysis of the glucocorticoid activities of various 9α- and 12α-substituted 11β-hydroxysteroids is the only existing one capable of explaining all the facts and is not really affected by the evidence of Bergstrom and Dodson.

It is, however, possible that part or all of the increased sodium-retaining activity of 9α-fluoro-11-hydroxysteroids is due to a different mechanism which is similar to the unknown one underlying the enhanced activity of 9α-fluorodeoxycorticosterone. As with other sodium-retaining steroids, a complete analysis will be impossible until the homogeneity or heterogeneity of receptors for this type of activity has been determined.

D. Miscellaneous compounds

A number of interesting steroids have been reported with either no biological information (405, 641) or unusual types of activity.

Gould *et al.* (288) found that 16α-N-piperidino-3β-hydroxypregn-5-en-20-one lowered the blood pressure of dogs in doses of 1 to 2 mg/kg, and was pharmacologically similar to veratrine. Drill and Riegel (198) found that several 3β-hydroxyandrost-5-ene-17β-sulfonium halides had ganglion-blocking activity and atropine-like actions. Antaki and Petrow (11) made heterocyclic tetracycline-like compounds with cholic acid, but they lacked antibiotic activity [see also the cyclic trithiol of Kincl (405)].

Finally, Petrow's group (304) found that 6β-hydroxy-3,5-cyclopregnan-20-one relieved patients with depression and obsessional disorders, but not those with "anxiety hysteria" or schizophrenia. Confirmation of these findings is desirable with a larger number of patients and controls.

Kallistratos and Voigt (390) found that cortisone 21-glycinate was inactive in hamsters. This suggests that, unlike most 21-acetoxysteroids, some other types of ester and the 21-acetoxyl group of certain 16-substituted steroids (729a) may not be hydrolyzed at all rapidly *in vivo*.

E. The structural requirements for the hormonal activity of steroids

Recent work has shown in general that most types of steroid activity can be obtained with synthetic steroids which differ greatly from the natural hormones in the shapes of certain regions of their molecules. It is reasonable to conclude that these regions of the natural hormones are either not associated closely with their receptors, or that they associate with a flexible part of their receptors in a way that confers little or no specificity upon the interaction. The information for glycogenic and anti-inflammatory activity is very complete, as it is for progestational activity. The picture is confusing for sodium-retaining activity, because of the probable heterogeneity of the receptors for this activity; for androgenic activity, because of the inconclusive evidence on the role of 17α-alkyl groups and the emphasis in the published literature on levator and other types of activity; and for estrogenic activity, because of the wide range of nonsteroidal estrogens. A summary of the available information is given in Tables 8 and 9.

These conclusions are based largely on active compounds possessing alkyl, alkyloxy, or halogen substituents which are very unlikely to be modified to any great extent by metabolic reactions. It should be emphasized, however, that much more work is needed in this field: thus, it is very likely that 17α-alkenyl and 17α-alkynyl groups, and many bromo- and chloro- steroids in which the halogen is close to a polar group or a double bond, will undergo many metabolic and also nonenzymic reactions in living organisms.

It is less easy to point to regions of the molecules of natural steroid hormones the shapes and constitutions of which are essential for biological activity, since here one must rely largely upon a correct interpretation of the *loss or reduction* of biological activity. In many cases, the essential compounds, being naturally less interesting to the pharmaceutical firms, have either not been made or not reported upon. For reasons given above such arguments are more likely also to be upset by factors which have nothing to do with the steroid-receptor interaction under consideration. Table 9 is an attempt to derive the best hypotheses from the existing evidence. The crucial importance of *limited* regions of the molecules of certain hormones and drugs is not confined to the steroids (see, *e.g.*, 305).

VII. THE NATURE OF THE INTRINSIC ACTIONS OF STEROID HORMONES

The best quantitative analyses of the pharmacology of hormones suggest that steroid-receptor interactions are similar to those of other hormones and of drugs (*e.g.*, 245, 687), and hence that they are similar in nature to enzyme-substrate interactions (see 14). The first important question is whether the intrinsic

action (14) which is consequent upon the association between steroid and receptor involves a chemical reaction or not (124, 124a). It is possible to generalize with reasonable confidence at present that it does not.

For all types of steroid hormone, highly active analogues are known in which one or all of the polar groups available for possible metabolic reactions have been either rendered unreactive by substituents introduced into the molecule, or else removed altogether. The great activity of some of these analogues, and the very low or absent reactivity of the polar groups or vacant positions available for consideration, make it very unlikely that such biological activities are due to metabolic modification of the analogues. Until recently, it seemed possible that the 20-ketone group of C_{21}-steroids was a potential site of a redox reaction at their receptors, particularly since this is one of the few reactions occurring in peripheral tissues as well as in liver. It is now known that this reaction is blocked completely in triamcinolone acetonide (Bush and Hunter, unpublished observations) which is approximately ninety times as active as cortisol as a glucocorticoid, and hence almost certainly blocked in the active *gestagen*, 16α,17α-isopropylidenedioxy-6α-methylpregn-4-ene-20-one (73a). Such a redox reaction remains a possible but unlikely basis for sodium-retaining activity. This reaction is of course impossible as a basis for the progestational activities of the 17α-alkyl-19-nortestosterones.

The intrinsic action of steroid hormones is therefore most likely to be due to some effect of a so-called "physical" nature on their receptors or their surroundings (124a). Sarett (616) has already pointed out that multi-point molecular interactions are of little importance in ordinary chemistry because of the low probability of their occurrence. He envisages a glucocorticoid as finding a loose one-point attachment to the receptor by one of its polar groups and then making further contacts by rotation around the point of first attachment, analogous to the berthing of a large ship by getting a line ashore and then swinging her around into place. In this way it is possible that the apparently crucial polar groups in natural steroids [*e.g.*, 11β-hydroxyl (corticoids), and 17β-hydroxyl (androgens)] increase the specificity of their receptor-associations by increasing the probability that the steroid attaches itself by the correct "face" and in a position in which rotation can bring it into correct apposition with the rest of the receptor surface. This concept is attractive in that polar groups, like ships' lines, exert forces of longer range than the London forces which will be mainly responsible for the final specificity of the close apposition of the steroid to the receptor.

Once full apposition has occurred, it seems likely at present that the area of contact will be too large, and the association too dependent upon short-range forces, to permit more than very minor rotation and oscillation of the steroid about its preferred position on the receptor (124a). One has to ask what physicochemical events could arise from such an association of a steroid with conceivable receptors.

Many interactions of steroids with other substances have been studied in the search for ones which might have physiological significance. The studies of Munck, Scott and Engel are particularly valuable in that precise physicochemi-

cal methods were used (504, 505, 642). These and the studies of Schellman *et al.* (215, 631, 632) have provided a wealth of valuable physicochemical information on the interactions between steroids with proteins (215, 632), nucleotides (225b, 505, 642), and water-oil interfaces (504). Most of these studies, however, suggest that association occurs at the α-side of the steroid. Yet it is very unlikely that α-sided associations are involved in the receptor-interactions of glucocorticoids and gestagens. The work of Talalay and his colleagues (471, 721, 722, 723) has shown that steroids are much more prone to cause substrate-inhibition at relatively low concentrations with steroid alcohol dehydrogenases than other classes of compounds. These findings are in accord with the supposition that the association of steroids with their receptors probably involves a relatively firm and immobile attachment of the steroid.

In the case of glucocorticoids and gestagens, it is fairly clear too that ternary complexes (*i.e.*, X-steroid-Y) are very unlikely to occur at the receptor site. There is room for only a one-sided (or a "one-sided + top") association of the steroid. The highly substituted glucocorticoid analogues and the 9,10-di*iso*-progesterones could not form a ternary complex matching those that might be possible with the natural steroids.

These and earlier considerations therefore render the following mechanisms rather unlikely as the basis of the intrinsic action of steroid hormones: (a) a coenzyme-like action in which a reversible reaction occurs with a polar group on the steroid (372a, 724, 724a); (b) the formation of chelates for the transport of metal ions across cell membranes (793, 794); (c) replacement of, or association with cholesterol in cell membranes (802a); (d) a transport or carrier function with the steroid rotating about a single point of attachment to a receptor.

The very large size of the α-sided distortions of the natural glucocorticoids and gestagens that are compatible with full or greatly enhanced biological activities also suggest that the chemical system in which the intrinsic action is produced contains a large volume of empty solvent on the side of the steroid distant from the receptor surface. If any part of the receptor fills this space before or after association with the steroid has been established, it must be extremely flexible (*e.g.*, 396) and its conformation cannot be of critical influence on the intrinsic action of the steroid.

The conclusion is reached, then, that the intrinsic action of glucocorticoids, gestagens, and probably of other types of steroid hormone (372a) is accomplished by an association with their receptors that involves *little or no movement of the parts of the molecules that are in close apposition.* The most likely conceivable consequence of such an association which could be of physiological significance is that that part of the receptor which would otherwise interact with other molecules or groups (or with other parts of the same molecule, if large enough) is prevented from doing so *by the mere presence of the steroid.* The biochemical and physiological consequences of this sort of molecular event could be of many types. The following can be suggested as reasonable possibilities:

(a) The steroids would be large enough to block "holes" or channels the walls of which contain their receptors.

(*text continued on page 418*)

TABLE 1[1]

Structure-activity relations: glucocorticoids

The biological activity (columns 4 and 5) of the analogue resulting from the modification (column 2), relative to the parent steroid (column 1), is given as a figure where possible. Where figures are not available, a reduction in activity is signified by "−", an increase by "+", and zero activity by "abolished." Ambiguity or absence of specific comments on biological activity is indicated by "?." Parentheses around reference or in columns 4 and 5 indicate that results were probably affected seriously by secondary factors mentioned in text. *E.g.*: For analogue No. 4: the activity of 2α-methylcortisol is 10 × that of cortisol by a liver glycogen assay likely to be seriously affected by secondary factors, and 4.5 × that of cortisol in a granuloma-inhibiting assay subject to the same reservations. S.C. = subcutaneous. Asterisks, after the letter indicating the ring of the steroid nucleus which is affected by the modification in question, indicate that the steric properties of other parts of the molecule may be affected by the changes in that ring.

Root Steroid	Modification or Analogue	Ring (A, B, C, D) or Side-chain (S) Affected by Modification	Change in Main Activity (Liver Glycogen Assay)	Change or Appearance of Minor Activity	References
1 Cortisol	1,2-dehydro-	A		× 1.8 (skin, local)	633, 738, (766)
4 Cortisol	2α-methyl-	A	(× 10)	(× 4.5)(granuloma)	131, 248, 333, 445, 446
6 Cortisol	3-enol ether	A	−− (systemic) ++ (oral)	−− (systemic) ++ (oral)	226
7 Cortisol	4-hydro-5α-hydroxy-	A		(× 0.5)(thymus)	61
8 Cortisol	4-methyl-	A	Abolished		684a
9 Cortisol	4,5α-dihydro-	A(*)	Abolished	− (local, clinical)	251, 338, 340, 739
12 Cortisone	2α-methyl-	A	(× 0.1)	(× 0.1)	446
14 Cortisone	4-hydro-5α,6β-dihydroxy-	A	× 0.16		739
17 11-Deoxycortisol	1α-acetylthio-	A		Anti-inflammatory +++ (local) Liver glycogen ++	187 187

[1] The compounds listed in Tables 1 through 7 have been selected as the most appropriate examples for the present review from a similar but more complete list entitled "Structure-Activity Relations of Cortical Steroids." The latter includes all the numbered compounds which are missing from the sequential listing in the left-hand columns of Tables 1 through 7. The complete list has been deposited as Document number 7173 with the ADI Auxiliary Publications Project, Photoduplication Service, Library of Congress, Washington 25, D. C. A copy may be secured by citing the Document number and by remitting $5.00 for photoprints, or $2.25 for 35 mm microfilm. Advance payment is required. Make checks or money orders payable to: Chief, Photoduplication Service, Library of Congress.

18 6α-Fluorocortisol	1,2-dehydro-	A	×	× 2 (granuloma) × 8 (thymus)	16, 92, 254, 331, 335
23 9α-Fluorocortisol	2α-methyl-	A	(× 3)	× 1.3 (granuloma) ++ (electrolyte)	131, 248, 333, 446, 705, 706, 707, 730
31 Triamcinolone	2-methyl-	A	× 0.55		59, 63
34 Cortisol	2α-ethyl-	A, AB	Abolished		279
38 Cortisol	19-nor-	A/B*	× 0.10	++ (electrolyte) × 0.3 (granuloma)	382, 466, 817
39 Cortisol	19-nor-	A/B	Abolished (279)	× 0.005 (local anti-inflammatory)	193, 279
40 Cortisol	19-nor-1,2,3,4,5,10-dehydro-3-ol (A-ring phenol-3)	A, A/B	× 0.13	−− (granuloma) 0 (electrolyte)	466
41 Cortisone	4,5α-dihydro-1,2-dehydro-	A, A/B	× 0.15		805
42 Corticosterone	19-nor-	A/B		+ (electrolyte)	382
45 Cortisol	6α-chloro-	B		× 5 (thymus)	578
46 Cortisol	6α-fluoro-	B		× 10 (granuloma)	92
50 Cortisol	6α-methyl-	B	× 4.0		681
54 Cortisone	6β-chloro-	B		× 0.4 (thymus)	578
79 Prednisone	8,9-dehydro-	B/C	× 0.15		739
81 Cortisol	9α-bromo-	C	× 0.17	× 0.05 (thymus)	249
82 Cortisol	9α-chloro-	C	× 2.4	++ (electrolyte) × 1.4 (thymus) + (clinical)	249, 703 133 283
84 Cortisol	9α-fluoro-	C	× 5.5	× 5–10 (clinical) ++ (electrolyte)	85, 249, 257, (259), 283, 338, 346, 444, 510, 570, 593, 682, 703
85 Cortisol	9α-hydroxy-	C	× 0.1	× 0.3–0.5 (thymus)	249, 451
87 Cortisol	9α-iodo-	C	× 0.5		249
88 Cortisol	9α-methoxy-	C	× 0–0.1		249
89 Cortisol	11α-methyl-	C(*)	Abolished	Abolished (granuloma)	72

TABLE 1—*Continued*

Root Steroid	Modification or Analogue	Ring (A, B, C, D) or Side-chain (S) Affected by Modification	Change in Main Activity (Liver Glycogen Assay)	Change or Appearance of Minor Activity	References
90 Cortisol	11β-acetate	C(*)	Abolished	Abolished (thymus, granuloma)	143, 246
91 Cortisol	11-dehydro-	C	× 0.5	× 0.4–0.5 (H_2O, K)	786
				× 0.5 (thymus)	685
				Abolished (eviscerate)	354
				× 1 (local, eye)	292, 438
				× 0.025 (local, chick embryo)	117a
92 Cortisol	11-deoxy-9α-bromo-11β-fluoro-	C		Abolished (granuloma)	95a
93 Cortisol	11-deoxy-9α,11β-dichloro-	C		× 0.28 (granuloma)	95a
95 Cortisol	14α-hydroxy-	C		− (clinical, local, thymus)	338, (657), *cf.* 3, 191a
98 Cortisol, corticosterone	12α-fluoro-	C(*)	× 10	++ (electrolyte)	729, *cf.* 250a
100 Cortisone	12α-chloro-	C	0.01		252, (256), 729
102 Corticosterone	9,11-anhydro-	C		+ (electrolytes)	290
107 Prednisolone	11-deoxy-9α,11β-dibromo-	C		× 1.2 (granuloma)	591
115 Triamcinolone	11β-acetate	C	Abolished	Abolished (thymus, granuloma)	246
116 Triamcinolone acetonide	11β-acetate	C	× 0.01	× 0.05	246
120 11β-Hydroxyprogesterone	12α-fluoro-	C	Glycogenic 0.75 × cortisone acetate	× 200 (= 1 × DOC) (Na)	252
121 11β,17α-Dihydroxyprogesterone	9α-fluoro-	C		++ (clinical, glucose excretion)	284
125 Cortisol	16α-hydroxy-	D	× 0.4	× 0.3 (thymus)	5a, 54a, 64, 65
126 Cortisol	16β-methyl-	D	× 0.6	× 4 (granuloma)	616, 660, 727, 728

127 Cortisol and others	16α-methyl-	D	× 4	× 3 (granuloma)	616, 635, 660
128 Cortisone	D-homo-	D	× 0.4		152
129 Cortisone acetate	16β-chloro-	D	Abolished	Abolished (thymus)	71
131 Corticosterone	17α-hydroxy-	D		× 10 (thymus) × 2,440 (local anti-inflammatory)	655 193
133 Corticosterone	17α-methyl-	D	× 0.23		218b
137 16α-Hydroxycortisol and others	16,17-ketals (various)	D	× 1–10	+++ (granuloma)	252, 253
138 9α-Fluorocortisol acetate	16β-acetoxy-	D	Abolished		60
141 11-Dehydrocorticosterone	16α,17-epoxy-	D	Abolished		(380), 460
142 11-Dehydrocorticosterone	17α-bromo-	D	–	– (eosinophils)	173, 220
143 11-Dehydrocorticosterone	17α-chloro-	D*	–	Abolished (eosinophils)	220, 322
145 11-Dehydrocorticosterone	17α-methyl-	D	× 0.23		218a, 222
154 Triamcinolone	16,17-ketonides (various)	D	× 3.5–20	× 3–8 (granuloma) × 10 (local, skin)	253, 572 572
155 Triamcinolone	16α,17-cyclic orthoesters	D	× 0.5–1.5	0.5–1.5 (thymus)	667
157 21-Deoxy-9α-fluorocortisol	17-deoxy-	D	× 0.45		249, 252, 255
158 9α-Fluorocortisol	17,21-bis-deoxy-	D, S	× 0.08		249, 252, 255
161 Cortisol	20,20-dihydro-(20β-hydroxyl)	S	× 0.1		1
163 Cortisol, *etc.*	21-deoxy-	S	– –	– – – (electrolyte)	254, 739
164 Cortisol, cortisone	21-dehydro- (and various condensation products)	S	× 1.0		433. 595, 634
165 Cortisol	21-deoxy-21-bromo-	S	Abolished		739
166 Cortisol, and others	21-deoxy-21-chloro-	S	Abolished		318, 739
167 Cortisol, and others	21-deoxy-21-fluoro-	S	× 3–5	× 3–5 (granuloma)	318, 680
169 Cortisone	21-glycinate	S	Abolished		390
171 9α-Fluorocortisol	17,21-epoxide	S	× 1.0		330
175 9α-Fluoroprednisolone	21-methyl-	S		× 0.6 (granuloma)	3a

TABLE 2

Structure-activity relations: deoxycorticosterone and related mineralocorticoids

Conventions as in Table 1

Root Steroid	Modification or Analogue	Ring	Change in Main Activity (Sodium-Retention)	Change or Appearance of Minor Activity	References
179 Deoxycorticosterone (DOC)	1,2-dehydro-	A	× 0.25		70, (601), (766), *cf.* 708
180 Deoxycorticosterone	2α-methyl-	A	× 0.05		446
181 Deoxycorticosterone	2α-hydroxy-	A	? Abolished		671
182 Deoxycorticosterone	2β-acetoxy-	A	× 0.17		23
184 Deoxycorticosterone	4,5α - dihydro - 1,2 - dehydro-	A; A/B	0.15		23
185 Deoxycorticosterone	19-nor-	A, B	× 5.1	× 2.0 (Na/K)	382, 614, 615, *cf.* 23, 382
187 Deoxycorticosterone	19-hydroxy-	A, B	× 0.04		37, (388)
188 Deoxycorticosterone	19-oxo-	A, B	× 0.50		38
189 Deoxycorticosterone	19-nor-14-iso-17-iso-(*i.e.*, 14β,17α-)	A, B; C/D; D/S	Abolished		212
191 Deoxycorticosterone	6,7-dehydro-	B		× 0.25 (survival)	783
192 Deoxycorticosterone	6α-hydroxy-	B	× 0.04		23
194 Deoxycorticosterone	7-oxo-	B	× 0.3		436a
196 Deoxycorticosterone	9α-fluoro-	C	× 12		52
197 Deoxycorticosterone	9,11-dehydro-	C	× 1.2		23
198 Deoxycorticosterone	11β-hydroxy-	C	× 0.01	× 0.1 (Na/K)	382, 663
199 Deoxycorticosterone	14α-hydroxy-	C/D	? Abolished		(785)
200 Deoxycorticosterone	18-nor-	C, D	× 0.1		374
201 Deoxycorticosterone	14α-; 15α-; 15β-; 16α-; 17α-; 18-hydroxy-	C, D	Abolished or − − −		23, 156, 375, 382, 388, (487), 663
205 Deoxycorticosterone	16α-methyl-	D	Abolished		464a, (546)
208 Deoxycorticosterone	D-homo-	D	Abolished	× 0.1 (survival)	186
209 Deoxycorticosterone	17-iso-	D/S	Abolished?		656
211 Deoxycorticosterone	17α-methyl-	S	? Abolished	Gestagen	(222), 555
213 Deoxycorticosterone	21-deoxy-	S	0.01	DOC-antagonist	23, 381
214 Deoxycorticosterone	21-dehydro-(aldehyde)	S		× 0.04 (Everse-de Fremerey)	565
215 Deoxycorticosterone	21-glucoside	S	× 1		270, 293, 294
216 23-Acetoxy-norchol-4-ene-3,22-dione	(DOC + branch and lengthened side-chain)	S	Abolished		784

TABLE 3

Structure-activity relations: 11-oxygenated mineralocorticoids

Conventions as in Table 1

Root Steroid	Modification or Analogue	Ring	Change in Main Activity (Glucocorticoid)	Change or Appearance of Minor Activity	References
217 Cortisol	1,2-dehydro-	A	× 4–5 (glycogen)	− − − (Na)	509
218 Cortisol	2α-methyl-	A	× 5–10 (glycogen)	× 50 (Na; Na/K)	16, 131, 333, 446
221 9α-Fluorocortisol	2α-methyl-	A	× 3 (glycogen)	× 20	131, 333, 706, 707
222 Cortisol	19-nor	A/B	× 0.1 (glycogen)	× 50 (Na)	466
223 Cortisol	19-nor-A-aryl-3-OH	A/B; A	× 0.13 (glycogen)	× 1.0 (Na)	466
224 Cortisol	6α-fluoro-	B	× 8 (granuloma)	× 1.0 or reversed	92, 96
225 Cortisol	6α-methyl-	B	× 4 (glycogen)	(− −Na)	681
227 9α-Fluorocortisol	6α-fluoro-	B	× 10 (granuloma)	− − (Na)	92, 210, 211
228 9α-Fluorocortisol, 9α-fluoroprednisolone, *etc.*	6α-methyl-	B	× 2–5 (glycogen)	Abolished (Na)	680
229 9α-Fluorocortisol, *etc.*	6,7-dehydro-	B	× 0.5 (glycogen)	× 20 (Na)	254
231 9α-Fluoroprednisolone	6α-chloro-	B	× 0.5 (thymus)	− − − (Na)	578
232 Cortisol	9α-hydroxy-	C	× 0.4 (thymus)	Reversed (Na/K)	451
233 Cortisol	9α-chloro-	C	× 4.7 (glycogen)	× 100 (Na)	23, 89, 283
235 Cortisol	9α-fluoro-	C	× 10.7 (glycogen)	× 50 (Na, man)	89, 283
238 Cortisol, corticosterone	12α-fluoro-	C	+++ (= 9α-fluoro) (glycogen)	+++ (=9α-fluoro) (Na)	729
240 Corticosterone	9α-chloro-	C	× 4.7 (glycogen)	× 10 (Na)	255
241 Corticosterone	9α-fluoro-	C	× 10 (glycogen)	× 300 (Na)	80, 85, 255
243 Cortisol, prednisolone	16α-hydroxy-	D	× 0.4 (glycogen)	Abolished (Na)	64, 65
244 Cortisol, prednisolone	16α - hydroxy - 16,17 - acetonide	D	× 3–5 (glycogen)	Reversed (Na)	65
245 Cortisol, prednisolone, 9α-fluoroprednisolone	16β-methyl-	D	× 0.6 (glycogen)	Abolished or reversed (Na)	727, 728, 737
250 9α-Fluorocortisol, 9α-fluoroprednisolone	16α-methyl-	D	× 1.3–2.0 (glycogen)	Abolished or reversed (Na)	16, 116, 737
251 9α-Fluoro-16α-hydroxyprednisolone	16,17-cyclic-orthoesters	D	× 0.3 (glycogen)	Reversed (Na)	667
252 9α-Fluoroprednisolone	21-methyl-	S	× 0.6 (granuloma)	Abolished (Na)	3a

TABLE 4

Structure-activity relations: gestagens based on progesterone

Conventions as in Table 1

Root Steroid	Modification or Analogue	Ring	Change in Main Activity (Endometrial Response)	Change or Appearance of Minor Activity	References
253 Progesterone	1,2-dehydro-	A	× 0.55 (Clauberg, S.C.)		566, *cf.* 42
255 Progesterone	3-enol ethers	A	× 10 (Clauberg, oral)	−− (narcosis)	226
256 Progesterone	5-en-3β-ol	A	× 0.13 (inhibition of estrogen)		349
258 Progesterone	9β,10α- ("retro")	A	+ (S.C.) +++ (oral)		637, (780a)
259 Progesterone	19-nor-	A	1–8 (Clauberg, S.C.)	× 1 (local), × 20 (oral)	182, 500, 750 235, 625
260 Progesterone	19-hydroxy-	A	× 0.01 (Clauberg)		37
269 6α-Methyl-17α-acetoxy-progesterone	1,2,6,7-bis-dehydro-	A	× 8.0 (Clauberg, oral) × 1.0 (Clauberg, oral)		42, 95 582
274 Progesterone	6α-acetoxy-	B	× 0.1 (Corner-Allen)		35
275 Progesterone	6β-acetoxy-	B	× 0.3 (Corner-Allen)		35
276 Progesterone	6β-acetyl-	B	Abolished (Clauberg, S.C.)		822
279 Progesterone	6α-methyl-	B	1.0 (copulation Cavy) × 5 (Clauberg, S.C.)		577 42, 216
281 Progesterone	6β-methyl-	B		× 1.0 (copulation)	577
	6β-ethinyl (see also ethyl, vinyl, 1-chlorovinyl)			× 0.25 (copulation)	822
282 Progesterone		B	Abolished (Clauberg, S.C.)		575
285 17-Acetoxyprogesterone	6α-methyl-	B	× 55 (Clauberg, oral)	++ (adrenal atrophy)	25, 208, *cf.* 582
294 Progesterone	11,12-dehydro-	C	× 3		297
296 Progesterone	17α-bromo-	C	× 2.0 (Clauberg, S.C.)		221, 476
298 Progesterone	17α-methyl-	C	× 2.0		297, 324, 555
299 19-Norprogesterone	18-nor-	C/D	Abolished		518

300	17α-Acetoxyprogesterone	9α-fluoro-11β-acetoxy-	C	× 1.0 (Clauberg, S.C.)		53
301	17α-Acetoxyprogesterone	9α-fluoro-11β-hydroxy-	C	× 2.5 (Clauberg, S.C.)	Glycogenic	53, 284
302	*dl*-Progesterone	18,19-bisnor-	C	Abolished		518, 688
303	19-Norprogesterone	14-iso-17-iso-(14β,17α)	C	× 2 (*cf.* abolished)		38a, 212, 213, (*cf.* 556)
304	Deoxycorticosterone	17α-methyl-	C	× 20 (?)		555
310	Progesterone	20-thio-	S	Active		185
317	Progesterone	21-nor-(17-formyl-androst-4-en-3-one)	S	Abolished		489
318	Progesterone	23-acetoxy-norchol-4-ene-3,22-dione	S	Inactive		784
319	17-Acetoxyprogesterone	21-bromo-	S	× 0.1 (Clauberg, S.C.)		54, *cf.* 221
321	17α-Acetoxyprogesterone	21-fluoro-	S	× 1.0 (Clauberg, S.C.)	× 2–4 (Corner Allen)	54, 725

TABLE 5

Structure-activity relations: testosterone and androgens

Conventions as in Table 1

Root Steroid	Modification or Analogue	Ring	Change in Main Activity (Rat Seminal Vesicle, Prostate, or Bird's Comb)	Change or Appearance of Minor Activity	References
324 Testosterone	1,2-dehydro-4,5α-dihydro-	A	× 0.08	× 1.0	126
325 Testosterone	2α-fluoro-	A	× 0.2	× 0.5 (anabolic)	209
327 Testosterone	3-deoxy-	A	− (oral)	0, renotropic	423
			Abolished (S.C.)	Abolished (S.C.)	424
				× 0.2 (increase uterine weight)	347, 350
335 Testosterone	4,5α-dihydro-	A/B	× 0.9		605
336 Testosterone	19-nor-	A/B	× 0.2	× 1.0	74, 158, 177, *cf.* 623
343 17α-Methyltestosterone	Δ⁵-3β-hydroxy-analogue	A	× 0.25 (oral)		66
345 17α-Methyltestosterone	3-cyclohexyl enol ether	A	oral × 5 syst. − − − −	× 1.0	226
352 Androst-4-enedione	1α-acetylthio-	A		Topical antiandrogen	188
354 5α-Androstane-3β?,17β-diol	3α?-methyl-	A	Abolished (zero absorption)	Abolished (zero absorption)	424
355 5α-Androstane-3β,17β-diol	3-deoxy-	A		× 0.5 (increase uterine weight)	347, 350
359 19-nor-Testosterone	4,5α-dihydro-	A, A/B	× 8	× 8–10 (antiestrone)	97, 101, 574
360 19-nor-Testosterone	4,5α,3β(OH)tetrahydro-	A, A/B	+	× 1.0	101, *cf.* 606
374 Various C_{19} androgens	5α → 5β-(H)	A/B	− − −		507
375 Androstenedione	18,19-bisnor-11α-OH	A/B, C/D, C	Weak		75
385 Testosterone 386 Androstenedione	B-homo-(5α)-dihydro-	B; A/B	× 1.0	× 2 (anabolic)	574a
388 Allodihydrotestosterone	6β-methyl-	B	× 4	× 8	577
389 Androstenedione	6α-acetoxy-	B	× 0.2		

391 Testosterone	8-iso-(8α-(H))	B/C	× 0.4	–	181
395 Testosterone	9α-bromo-11β-chloro-	C	× 0.04	× 0.34 (anabolic)	592
397 Testosterone	9α-chloro-11β-fluoro-	C	× 0.51	× 0.84 (anabolic)	592
398 Testosterone	11-methyl-9,11-dehydro-	C	Abolished	Abolished (anabolic)	819
399 Testosterone	11α-methyl-11β-hydroxy-	C	Abolished	Abolished	819
402 17α-Methyltestosterone	9α-fluoro-11β-hydroxy-	C	× 9.5 (oral)		313, 378, *cf.* 158, 436
406 19-nor-Testosterone	18-nor-	C/D	× 0.0–0.10		374
407 Androsterone	13-iso-(13α-methyl)	C/D	Abolished		128
409 5α-Androstane-3,17-dione	18-nor-D-homo-	C/D, D	× 1.0 or +		376
411 Testosterone	17α-ethinyl-	D	× 22 (oral)	Gestagen (0.3 × P)	468
412 Testosterone	17α-ethyl-	D	× 22 (oral)	Gestagen (0.2–0.5 × P)	206, 468
417 Testosterone	17α-propargyl-	D	Abolished	Gestagen (0.3 × P)	468
418 Testosterone	17α-propyl-	D	× 22 (oral)	Gestagen (0.5 × P)	206
419 Testosterone	17α-(2-methallyl)-	D	× 22 (oral)	Gestagen (25 × P, S.C. 10 × P, intravenous)	217
421 Testosterone	17β-methyl-cis- (*i.e.*, 17β-methyl-17α-hydroxyl)	D	Abolished		325, 488
422 Testosterone	17-deoxy-	D		Abolished (increase uterine weight)	347, 350
423 Testosterone	17-epi- ("cis")	D	× 0.04		325
424 Testosterone	D-homo-	D	× 0.6		325
441 3-epi-Androsterone	D-homo-	D	× 5.1		325
442 5α-Androstane-3,17-dione	D-homo-	D	× 1.3		325
455 17α-Methyl-19-nor-androst-5-ene-3β,17β-diol	17α-ethyl-	D	× 0.4	× 0.6 (anabolic)	365
461 *dl* - 17α - Methyltestosterone and others	18-nor-D-homo-	C, D	Abolished	Abolished (anabolic)	122a

TABLE 6

Structure-activity relations: 17α-alkyltestosterone and related gestagens

Conventions as in Table 1

Root Steroid	Modification or Analogue	Ring	Change in Main Activity (Endometrial Response)	Change or Appearance of Minor Activity	References
469 17α-Ethinyltestosterone	19-nor-	A/B	× 5 (oral)		320, *cf.* 419
471 17α-Ethyl-19-nor-testosterone	4,5-dihydro-5,10-dehydro-	A/B	× 0.067	× 0.017 (inhibition of estrone on uterus)	206, *cf.* 551, 625
475 17α-Ethinyl-19-nor-testosterone	4,5-dihydro-(10β,5α)	A/B	Abolished		206
476 17α-Ethyl-19-nor-testosterone	4,5-dihydro-(10β,5α)	A/B	× 0.13		206
481 17α-Methyl-19-nor-testosterone	4,5-dihydro-(10β,5α)	A/B	× 0.33	Hypotensive	206, 693
489 17α-Ethinyltestosterone	6β-ethyl-	B	Abolished (oral)		169
490 17α-Ethinyltestosterone	6α-methyl-	B	× 6.5 (oral)		169
491 17α-Ethinyltestosterone (and others)	6β-methyl-	B	× 0.3 (oral)		169
496 17α-Ethinyltestosterone	6α,21-dimethyl-	B, D	× 11.5 (oral)		169
497 17α-Ethinyltestosterone	11β-hydroxy-	C	Abolished	Abolished (androgenic)	473, *cf.* 622
508 19-nor-Testosterone	17α-butenyl-	D	2.5 × progesterone (S.C.)		622
			10 × progesterone (intrauterine)		622
512 19-nor-Testosterone	17α-isopropyl-	D	20 × progesterone	× 55 (inhibition of estrone on uterus)	206
514 19-nor-Testosterone	17α-octyl-	D	? 1.0 × progesterone (intrauterine)		622
519 19-nor-Testosterone	17α-(2-methallyl)-	D	12–25 × progesterone	× 15 (inhibition of estrone on uterus)	206, 217, 621
521 17α-Ethinyltestosterone	21-ethyl-	S	× 1.6 (oral)		169

TABLE 7

Structure-activity relations: steroid estrogens

Conventions as in Table 1

Root Steroid	Modification or Analogue	Ring	Change in Main Activity (Uterine Weight)	Change or Appearance of Minor Activity	References
527 Estrone	2-methyl-	A	× 0.005 (mouse)	Antiandrogen	366
533 Estrone	3-sulfate	A	× 0.14 (i.m.)		127
534 Equilenin	3-deoxy-	A	Abolished		27
536 *d*-Equilenin	*l*-enantiomer	A	× 0.075		26
537 17α-Methylestradiol	3-dehydro-1,2,3,4-tetra-hydro-estr-5(10)-3-one	A	× 0.005	× 0.25 (lipid shift)	161
541 Estradiol (and estrone)	6β-methyl-	B	× 0.002 (mouse)		759
553 Estradiol	16α-hydroxy-; (oestriol)	D	× 0.03 (mouse) × 0.011 (rat)	(impeded)	196, 348
554 Estradiol	17α-ethinyl-	D	× 1.8–2.5 (mouse) × 1.5 (rat)		69, 196
555 Estradiol	17α-vinyl-	D	× 0.2 (S.C., rat)		364
556 Estradiol	17α-methyl-	D	× 1.0 (mouse)		83
557 Estradiol	17α-methyl-	D	× 1.0 (rat)		83
558 Estradiol	17-dehydro- (estrone)	D	× 0.2–0.3 (mouse) × 0.15 (rat)		196
559 Estradiol	17-deoxy-	D	× 0.008 (rat, vagina)		560, *cf.* 347
561 Estradiol	17-epi-	D	× 0.005 (mouse)		347
562 Estradiol (and others)	17α-ethinyl-	D	× 1.0 (S.C.) × 16 (oral)		364
565 Estrone-3-methyl ether	16β-methyl-	D	Abolished		406

TABLE 8

Steroid-receptor associations: areas of the steroid molecules which are probably not involved in a close and structurally-specific apposition to their receptors

References and further examples will be found in Tables 1 to 7. A question mark indicates that the evidence in question is equivocal in one or more ways (see text).

The analogues used in evidence are of indubitable biological activity and steric factors are emphasized. Certain possibly contradictory evidence based on polar substituents has been omitted since alternative explanations are to be preferred (see text).

Hormone and Type of Activity	Areas Not Involved in Close Contact with Receptors	Groups Compatible with Significant Biological Activity in One or More Analogues	Inactivating Groups Modifying, or Contradicting, Conclusions of Column 2
Cortisol (glycogenic)	α-side: Rings A, B, C, D and side-chain	1,2-dehydro-; 1α-acetylthio-; 9α-, 12α-bromo-; 16α,17α-diol ketals; *etc.*	
	β-side: Ring A (?), B (?)	(?) 1,2-dehydro-	? 6β-chloro (× 0.2) *cf.* 6α-chloro (0.5–10)
	"Upper" edge: Ring A, 10-methyl (?)	2α-methyl-; 1α-acetylthio-	2α-ethyl- (× 0)
	"Lower" edge: Rings A (?), B, C, D	6α-methyl-, 6α-chloro-, 14α-hydroxy-; D-homo-	? 4-chloro- (× 0) ? 7β-methyl- (× 0.2)
	"Left" edge: Rings A, C	2α-methyl-; 1α-acetylthio-	? 2α-ethyl- (× 0)
	"Right" edge: Ring D, side-chain	16β-, 16α-methyl; 16α,17α-diol ketals	
Deoxycorticosterone (nonglycogenic, sodium-retaining)		Inadequate information (see text)	
Progesterone (progestational)	α-side: Rings A, B, C, D, side-chain	1α-methyl-; 1,2-dehydro; 9α-bromo-; 9,10-diiso-, (retro); 17α-alkyloxy-, *etc.*	
	β-side; Rings A, B, C	9,10-diiso-, (retro); 6β-methyl-; 11β-acetoxy; 9α-,11β-dichloro-	? 1β-methyl-10-iso-(× 0) ? 6β-acetyl (× 0) ? 6β-ethyl (× 0)
	"Upper" edge: Rings A, B	9,10-diiso (9β,10α)	

	"Lower" edge: Ring B	6α-methyl-, 9,10-diiso (9β,10α)	
	"Left" edge: Ring A	9,10-diiso- (9β,10α)	
Testosterone (androgen)	α-side: Rings C, D	9α-chloro-11β-fluoro-; 17α-methyl-	? 17-iso ? 17α-ethyl, *etc.*
	β-side: Rings B, C, D (?)	9α-fluoro-11β-hydroxy; 6β-chloro-; 6β-methyl-; 8-iso-(8α); B-homo-;	17β-methyl-17-iso-(× 0) ? 13-iso- (× 0)
	"Lower" edge: Rings A, B	4-chloro-; 6α-methyl-; 6α-chloro-; 8-iso-(8α);	
	"Left" edge: Rings A, C	2α-methyl-; 9α-fluoro-11-oxo-;	
Estradiol (steroid, estrogenic)	α-side: Ring D	17α-ethinyl-; 17α-methyl	

TABLE 9

Steroid-receptor associations: areas of the steroid molecules most likely to be involved in close contact with a structurally specific area of their receptors

References, further information, and conventions as in Table 8. Where possible, control evidence has been taken into account: thus steric effects can be identified if analogues with electronegative and electropositive substituents at the same position have been made (*e.g.*, chloro- (r = 2.0 Å, *I*−) and methyl- (r = 2.0 Å, *I*+)).

Hormone and Type of Activity	Areas of Natural Steroid Involved in Close Contact with Receptors	Evidence for Column 2	Considerations Modifying, or Contradicting, Conclusions of Column 2
Cortisol (glycogenic)	β-side: Ring B (?)	6β-chloro- (× 0.2) (?)	H-bonding with 11β-OH (?) Some authors give higher activity (*e.g.*, 578)
	Ring C, 11β-hydroxyl	11-deoxy- (× 0) 11β-acetoxy-(× 0) 11β,12β-diol-acetonide (× 0) 11-ketone (× 0) (when reduction blocked) 11α-hydroxy- (× 0)	Exceptions: 1α-acetyl-thio-11-deoxycorticosterone, 6α-methyl-16α,17α-diol ketals, *etc.* One 11β-acetoxy-steroid (see text) (?)
	Ring D and side-chain	Preferred conformation of 17β-acetyl side-chain	17,21-epoxy- (× 0) (?) 17α-acetoxy- (× 0) (?)
	"Upper" edge: Ring C (?)	12β-hydroxy- (?)	
	"Right" edge: Side-chain (?)	21-glycinate (?)	16α-, 16β-methyl, (?)
Deoxycorticosterone (nonglycogenic, sodium-retention)	α-side: Ring A (?)	1,2-dehydro- (?)	Could be due to hindrance on α-side, *or* increased distance from point of attraction on β-side
	Ring D	16α-methyl-, 16α-methoxy, 16α-hydroxy; 17α-methyl-; 17α-hydroxy-; 16α,17α-diol ketals, *etc.* (× 0–0.05)	
	Side-chain (?)	17α-substitutions: (× 0–0.05)	
	"Upper" edge: Ring D	16α-, 16β-methyl-, 16α-methoxy- (× 0–0.1)	

	Side-chain	21-deoxy- (× 0.05) (?) 23-acetoxynorchol-4-ene-3,-22-dione (× 0)	
	"Left" edge (?)	(?) 2α-methyl (× 0.05)	? Secondary factors
	"Right" edge	16α-, 16β-substituents (× 0–0.05)	
Testosterone (androgenic)	α-side: Ring A	1α-methyl-, 1α-acetylthio- (× 0)	
	Rings B, C (?)		9α-chloro-11β-fluoro- (× 0.51)
	Ring D	(?) 17α-hydroxy-, (× 0.1)	17α-methyl
		(?) 17α-ethyl, *etc.* (× 0.1?)	Controversy over some other large 17α-alkyl groups, *etc.*
Progesterone (progestational)	β-side: Ring B (?)	19-nor (?) (× 1–2)	
	Ring C	9,(11)-dehydro-, (× 0.1)	? 9α,11β-dichloro (× 5.5)
		cf. 11,12-dehydro- (× 3) (?)	? 9α-fluoro-11β-acetoxy- (× 1.0)
	Ring D (?)	(?) 16,17-dehydro- (× 0)	
		(?) 17-iso (× 0)	
	Side-chain	Preferred conformation of 17β-acetyl side-chain:	
		17β-OH in 19-nortestosterones:	
		20-ketone of 14,17-diiso-19-nor-progesterone (× 1–5) has only common conformation with progesterone in the 20-ketone β-conformation	
	"Upper" edge: Ring C (?)	18,19-bisnor- (× 0)	? *via* effect on D-ring
	Side-chain	21-methyl- (× 0.05?)	
		21-bromo- (× 0.1)	
		21-iodo- (× 0)	
		(*cf.* 21-fluoro- (× 1–4))	
		21-nor- (× 0) (?)	*cf.* 17β-OH effective
Estradiol (steroid estrogen)		Insufficient unequivocal information	

(b) The displacement of a polar molecule from the receptor site would convert a polar and/or hydrated region of the receptor into a nonpolar one. This could result in extensive bending or unfolding of parts of the receptor molecules *distant* from the site of attachment.

(c) Another consequence of (b) could be a breakdown of large areas of hydrated gel around the receptor (see 55). Such molecular events could be the basis of a direct effect on the activity of enzymes, but the evidence is at present rather against this possibility. It seems more likely that they will be found eventually to affect cellular functions by influencing those molecular structures (*e.g.*, 455) of the cell or of extracellular materials, the detailed configurations of which determine the availability and transport of intermediary metabolites or other substances.

ACKNOWLEDGMENTS

The author is extremely grateful to Professor Sir George Pickering, F.R.S., and to the Medical Research Council and the Rockefeller Foundation for the support of his own work and during the period of writing this review. He is also extremely grateful to the following for helpful discussions and often advanced information on unpublished work:

Professor A. Albert, Professor A. Neuberger, Dr. L. L. Engel, Dr. Miles Glenn, Dr. J. A. Hogg, Dr. L. H. Sarett, Dr. R. H. Silber, and Dr. E. G. Tomich.

He is also extremely grateful to Miss Sally Tomlinson and Miss Rita Hore who did much of the bibliographical checking and the preparation of the figures.

REFERENCES

1. ABELSON, D., ULRICH, F. AND LONG, C. N. H.: Identification of 20β-hydroxy-hydrocortisone in rat plasma after administration of hydrocortisone. Proc. Soc. exp. Biol., N. Y. **89**: 386–388, 1955.
2. ADAMS, W. J., CROSS, B. G., DAVID, A., HARTLEY, F., PATEL, D., PETROW, V. AND STUART-WEBB, I. A.: 21-Acetoxy-12α,17α-dihydroxypregn-4-ene-3:20-dione and its anticortisone properties. J. Pharm., Lond. **5**: 861–868, 1953.
3. AGNELLO, E. J., BLOOM, B. L. AND LAUBACH, G. D.: A new class of biologically active corticoids. J. Amer. chem. Soc. **77**: 4684–4685, 1955.

3a. AGNELLO, E. J., PINSON, R., JR., FIGDOR, S. K., HUGHES, B. M. K., ORDWAY, H. W., BLOOM, B. M. AND LAUBACH, G. D.: Synthesis of corticoids methylated at C21. Experientia **16**: 357–358, 1960.

4. ALEXANDER, D. P., FRAZER, J. F. D. AND LEE, J.: I. Effect of steroids on maintenance of pregnancy in spayed rats. J. Physiol. **130**: 148–155, 1955.
5. ALLEN, W. S. AND BERNSTEIN, S.: Steroidal cyclic ketals. XII. The preparation of delta^{16}-steroids. J. Amer. chem. Soc. **77**: 1028–1032, 1955.

5a. ALLEN, W. S. AND BERNSTEIN, S.: Steroidal cyclic ketals. XX. 16-Hydroxylated steroids. III. Preparation of 16α-hydroxyhydrocortisone and related compounds. J. Amer. chem. Soc. **78**: 1909–1913, 1956.

6. ALLEN, W. S., BERNSTEIN, S., HELLER, M. AND LITTELL, R.: Steroidal cyclic ketals. XV. 17,21-Oxido steroids. Part I. Preparation. J. Amer. chem. Soc. **77**: 4784–4788, 1955.
7. ANDERSEN, B. AND USSING, H. H.: Solvent drag on non-electrolytes during osmotic flow through isolated toad skin and its response to antidiuretic hormone. Acta physiol. scand. **39**: 228–239, 1957.
8. ANNER, G. AND WETTSTEIN, A.: Steroids with cortical hormone activity. In: Encyclopaedia of Chemical Technology, ed. by R. Kirk and D. Othmer, pp. 849–888. Interscience Encyclopaedia, Inc., New York, 1957.
9. ANON.: Danger of corticosteroids in ophthalmology. Brit. med. J. **2**: 1079, 1959.
10. ANON.: Arthritis and corticosteroids. Brit. med. J. **2**: 1390–1391, 1959.
11. ANTAKI, H. AND PETROW, V.: Steroids and related compounds. Part XII. Some heterocyclic derivatives. J. chem. Soc. **1951**: 901–904.
12. ANTONUCCI, R., BERNSTEIN, S., HELLER, M., LENHARD, R., LITTELL, R. AND WILLIAMS, J. H.: Steroidal cyclic ketals. III. Hydrocortisone and related corticoids. J. org. Chem. **18**: 70–82, 1953.
13. ARCHER, R. K.: The mechanism of eosinopenia produced by ACTH and corticoids in the horse. J. Path. Bact. **74**: 387–395, 1957.
14. ARIËNS, E. J., VAN ROSSUM, J. M. AND SIMONIS, A. M.: A theoretical basis of molecular pharmacology. Arzneim.-Forsch. **6**: 282–293, 1956 (Part I); **6**: 611–621, 1956 (Part II); **6**: 737–746, 1956 (Part III).

15. VAN ARMAN, C. G. AND DRILL, V. A.: Some cardiovascular effects of norethandrolone (Nilevar), testosterone and progesterone. J. Pharmacol. **124:** 59–63, 1958.
16. ARTH, G. E., JOHNSTON, D. B. R., FRIED, J., HOFF, D. R., SILBER, R. H., STOERK, H. C., WINTER, C. A. AND SARETT, L. H.: 16-Methylated steroids. II. 16α-Methyl analogs of cortisone, a new group of anti-inflammatory steroids. 9α-Halo-derivatives. J. Amer. chem. Soc. **80:** 3161, 1958.
17. ASCHKENASY, A.: Pourquoi la cortisone est-elle moins éosinopéniante chez le Rat surrénalectomisé que chez le Rat normal? C. R. Soc. Biol., Paris **151:** 521–524, 1957.
18. ASCHKENASY, A. AND WELLERS, G.: Rétablissement par la cortisone de l'action hépatotrope de la cystine et de la synthèse du glutathion hépatique chez des Rats surrénalectomisés carencés en protéines. C. R. Soc. Biol., Paris **152:** 1080–1083, 1958.
19. ASHMORE, J., CAHILL, G. F., JR., HILLMAN, R. AND RENOLD, A. E.: Adrenal cortical regulation of hepatic glucose metabolism. Endocrinology **62:** 621–626, 1958.
20. ASHMORE, J., HASTINGS, A. B., HESBETT, F. B. AND RENOLD, A. E.: Studies on carbohydrate metabolism in rat liver slices. VI. Hormonal factors influencing glucose-6-phosphatase. J. biol. Chem. **218:** 77–88, 1956.
21. ATWATER, N. W.: 4-Substituted steroids. J. Amer. chem. Soc. **82:** 2847–2852, 1960.
22. ATWATER, N. W. AND RALLS, J. W.: 4-Oxasteroid analogs. J. Amer. chem. Soc. **82:** 2011–2014, 1960.
23. AXELRAD, B. J., CATES, J. E., JOHNSON, B. B. AND LUETSCHER, J. A.: Bioassay of mineralocorticoids: relationship of structure to physiological activity. Endocrinology **55:** 568–574, 1954.
24. AXELROD, J.: Biochemical factors in the activation and inactivation of drugs. Arch. exp. Path. Pharmak. **238:** 24–35, 1960.
25. BABCOCK, J. C., GUTSELL, E. S., HERR, M. E., HOGG, J. A., STUCKI, J. C., BARNES, L. E. AND DULIN, W. E.: 6α-Methyl-17α-hydroxy-progesterone 17-acylates; a new class of progestins. J. Amer. chem. Soc. **80:** 2904–2905, 1958.
26. BACHMANN, W. E., COLE, W. AND WILDS, A. L.: The total synthesis of the sex hormone equilenin and its stereoisomers. J. Amer. chem. Soc. **62:** 824–839, 1940.
27. BACHMANN, W. E. AND WILDS, A. L.: The synthesis of *cis* and *trans* 17-equilenone. J. Amer. chem. Soc. **62:** 2084–2088, 1940.
28. BACHMANN, W. E. AND HOLMES, D. W.: The synthesis of 6-hydroxy-17-equilenone (an isomer of equilenin) and two of its homologs. J. Amer. chem. Soc. **62:** 2750–2757, 1940.
29. BACHUS, H.: Influence of adrenaline on the plasma content of 17-hydroxy-corticosteroids. Amer. J. Physiol. **178:** 437–440, 1954.
30. BAGGETT, B., ENGEL, L. L., SAVARD, K. AND DORFMAN, R. I.: The conversion of testosterone-3-C^{14} to C^{14}-estradiol-17β by human ovarian tissue. J. biol. Chem. **221:** 931–941, 1956.
31. BAKER, B. L.: Mast cells of the omentum. Ann. N. Y. Acad. Sci. **56:** 684–692, 1953.
32. BAKER, B. L. AND ADAMS, G. D.: The physiology of connective tissue. Annu. Rev. Physiol. **17:** 61–78, 1955.
33. BAKER, B. L. AND EVANS, H. M.: Growth inhibition in the skin induced by parenteral administration of adrenocorticotropin. Anat. Rec. **102:** 313–348, 1948.
34. BAKER, B. L., INGLE, D. J. AND LI, C. H.: The histology of the lymphoid organs of rats treated with adrenocorticotropin. Amer. J. Anat. **88:** 313–350, 1951.
35. BALANT, C. P. AND EHRENSTEIN, M.: Investigations on steroids. XX. 6β- and 6α-acetoxy- and hydroxy- derivatives of progesterone and androstenedione. J. org. Chem. **17:** 1587–1596, 1952.
36. BARAN, J. S.: 2-Hydroxy-$\Delta^{1,4}$-3-ketosteroids. J. Amer. chem. Soc. **80:** 1687–1691, 1958.
37. BARBER, G. W. AND EHRENSTEIN, M.: Investigations on steroids. XXIV. 19-Hydroxy-11-desoxycorticosterone and 19-hydroxy progesterone. J. org. Chem. **19:** 1758–1765, 1954.
38. BARBER, G. W. AND EHRENSTEIN, M.: Investigations on steroids. XXV. 19-Oxo-progesterone and 19-oxo-11-desoxy-corticosterone. J. org. Chem. **20:** 1253–1259, 1954.
38a. BARBER, G. W. AND EHRENSTEIN, M.: Untersuchungen über Steroide. XXVIII. Synthese von 19-Nor-tes e. 14β.17α-Progesteron und 19-Nor-11-desoxy-10-e.14β.17α-Corticosteron aus Strophanthidin. Liebigs Ann. **603:** 89–109, 1957.
39. BARGER, A. C., BERLIN, R. D. AND TULENDO, J. F.: Infusion of aldosterone, 9α-fluorohydrocortisone and antidiuretic hormone into the renal artery of normal and adrenalectomised unanesthetised dogs: effect on electrolyte and water excretion. Endocrinology **62:** 804–815, 1958.
40. BARR, R. W. AND SOMMERS, S. C.: Endocrine abnormalities accompanying hepatic cirrhosis and hepatoma. J. clin. Endocrin. **17:** 1017–1029, 1957.
41. BARTON, D. H. R.: The stereochemistry of cyclohexane derivatives. J. chem. Soc. **1953:** 1027–1040.
42. BARTON, S. P., ELLIS, B. AND PETROW, V.: Preparation of 17α-acetoxy-6α-methylprogesterone. J. chem. Soc. **1959:** 478–479.
43. BARTTER, F. C.: The role of aldosterone in normal homeostasis and in certain disease states. Metabolism **5:** 369–383, 1956.
44. BARTTER, F. C. AND FOURMAN, P.: The non-renal effect of adrenal cortical steroids upon potassium metabolism. J. clin. Invest. **36:** 872–873, 1957.
44a. BARTTER, F. C., MILLS, I. H., BIGLIERI, E. G. AND DELEA, C.: Studies on the control and physiologic action of aldosterone. Recent Progr. Hormone Res. **15:** 311–335, 1959.
45. BASS, A. D.: Chemical influences on cell division and development. Annu. Rev. Physiol. **21:** 49–68, 1959.
46. BASTENIE, P. A., IONARD, V. AND FRANCKSON, M. R. M.: Effect of cortisone on carbohydrate metabolism measured by the "glucose assimilation coefficient." Diabetes **3:** 205–209, 1954.
47. BATTEN, J. C. AND MCCUNE, R. M.: The influence of corticotrophin and certain corticosteroids on populations of *Mycobacterium tuberculosis* in tissues of mice. Brit. J. exp. Path. **38:** 413–423, 1957.

47a. Baulieu, E. E. and Jayle, M.: Etude de l'équilibre entre 11β-hydroxy et 11-cétosteroides. Bull. Soc. Chim. biol., Paris **39**: 37–56, 1957.
48. Baumann, E. J. and Kurland, S.: Changes in the inorganic constituents of blood in suprarenalectomised cats and rabbits. J. biol. Chem. **71**: 281–302, 1927.
49. Bazin, S. and Delaunay, A.: Le métabolisme des mucopolysaccharides. Biol. méd., Paris **48**: 351–441, 1959.
50. Beierwaltes, W. H. and Bishop, R. C.: 17-Ketosteroid and pituitary follicle-stimulating hormone excretion in myxoedema, before and during treatment with thyroxine. J. clin. Endocrin. **14**: 928–938, 1954.
50a. Bein, H. J. and Jaques, R.: The antitoxic effect of aldosterone. Experientia **16**: 24–26, 1960.
50b. Bell, J. D.: Effect of adrenal cortical hormones on aspects of liver metabolism. Ph.D. Thesis, Cambridge, England, 1955.
51. Berger, E. Y., Kanzaki, G. and Steele, J. M.: The effect of deoxycorticosterone on the unidirectional transfers of sodium and potassium into and out of the dog intestine. J. Physiol. **151**: 352–362, 1960.
52. Bergstrom, C. G. and Dodson, R. M.: The preparation of 9α-fluorosteroids. J. Amer. chem. Soc. **82**: 3479–3480, 1960.
53. Bergstrom, C. G., Nicholson, R. T., Elton, R. L. and Dodson, R. M.: 17α-Acetoxy-9-halo-11-oxygenated progesterones. A new class of orally active progestins. J. Amer. chem. Soc. **81**: 4432–4433, 1959.
54. Bergstrom, C. G., Sollman, P. B., Nicholson, R. T. and Dodson, R. M.: 21-Halo-17-acyloxyprogesterones. J. Amer. chem. Soc. **82**: 2322–2327, 1960.
54a. Bernstein, S.: The chemistry and biological activities of 16-hydroxylated steroids. Recent Progr. Hormone Res. **14**: 1–22, 1958.
54b. Berliner, D. L., Dougherty, T. F. and Grosser, B. I.: Tissue metabolism of hydrocortisone in eviscerate rats. Arch. Biochem. Biophys. **77**: 81–88, 1958.
54c. Berliner, D. L. and Dougherty, T. F.: Influence of reticuloendothelial and other cells on metabolic fate of steroids. Ann. N. Y. Acad. Sci. **88**: 14–29, 1960.
54d. Berliner, D. L., Swim, H. E. and Dougherty, T. F.: Metabolism of 4-C^{14}-corticosterone by fibroblasts, strain U12-79. Biochim. biophys. Acta **38**: 184–186, 1960.
54e. Berliner, D. L. and Dougherty, T. F.: Hepatic and extrahepatic regulation of corticosteroids. Pharmacol. Rev. **13**: 329–359, 1961.
55. Bernal, J. D.: Geometry of the structure of monatomic liquids. Nature, Lond. **185**: 68–70, 1960.
56. Bernstein, S., Allen, W. S., Heller, M., Lenhard, R. H., Feldman, L. I. and Blank, R. H.: 7α-Hydroxy—Reichstein's substance S. J. org. Chem. **24**: 286–289, 1959.
57. Bernstein, S., Brown, J. J., Feldman, L. I. and Rigler, N. E.: 16-Hydroxylated steroids. X. The synthesis of 21-deoxy-triamcinolone. J. Amer. chem. Soc. **81**: 4956–4962, 1959.
58. Bernstein, S., Heller, M., Littell, R., Stolar, S. M. and Lenhard, R. H.: 16α-Hydroxy-2α-methyl-steroids. J. Amer. chem. Soc. **79**: 4555, 1957.
59. Bernstein, S., Heller, M., Littell, R. Stolar, S. M., Lenhard, R. H., Allen, W. S. and Ringler, R.: 16-Hydroxylated steroids. VII. The synthesis of the 16α-hydroxy derivatives of 2-methyl steroids. J. Amer. chem. Soc. **81**: 1696, 1959.
60. Bernstein, S., Heller, M. and Stolar, S. M.: 16-Hydroxylated steroids. XI. Their preparation and apimerization. J. Amer. chem. Soc. **81**: 1256, 1959.
61. Bernstein, S. and Lenhard, R. H.: Steroidal cyclic ketals. XIV. The preparation of pregnane-5α,21-diol-3,20-dione and pregnane-5α,11β,17α,21-ol-3,20-dione. J. Amer. chem. Soc. **77**: 2233–2237, 1955.
62. Bernstein, S. and Lenhard, R. H.: 16-Hydroxylated steroids. XV. Synthetic corticoids related to 9α-fluoro-16α-hydroxy-hydrocortisone and prednisolone (Triamcinolone). J. Amer. chem. Soc. **82**: 3680–3684, 1960.
63. Bernstein, S., Lenhard, R. H., Allen, W. S., Heller, M., Littell, R., Stolar, S. M., Feldman, L. I. and Blank, R. H.: 16-Hydroxylated steroids. IV. The synthesis of the 16α-hydroxy derivatives of 9α-halo-steroids. J. Amer. chem. Soc. **78**: 5693, 1956.
64. Bernstein, S., Lenhard, R. H., Allen, W. S., Heller, M., Littell, R., Stolar, S. M., Feldman, L. I. and Blank, R. H.: 16-Hydroxylated steroids. VI. The synthesis of the 16α-hydroxy derivatives of 9α-substituted steroids. J. Amer. chem. Soc. **81**: 1689–1696, 1959.
65. Bernstein, S., Littell, R., Brown, J. J. and Ringler, I.: 16-Hydroxylated steroids. XII. The 16α,17α-acetonides of synthetic non-halogenated corticoids. J. Amer. chem. Soc. **81**: 4573–4574, 1959.
66. Bernstein, S., Stolar, S. M. and Heller, M.: Synthesis of 17α-methyl-4-androstene-3β,17β-diol. J. org. Chem. **22**: 472–473, 1957.
67. Bertrand, J., Lauras, B. and Cautenet, B.: Etude du rôle du foie dans la dégradation de l'hydrocortisone chez l'enfant. C. R. Soc. Biol., Paris **152**: 1134–1137, 1958.
68. Bettley, F. R.: Corticosteroids in treatment of skin diseases. Brit. med. J. **2**: 943–945, 1959.
69. Beyler, A. L. and Clinton, R. O.: Uterine growth-stimulating and testicular growth-suppressing activities of 17α-ethinylandrostane-3β:17β-diol, its Δ^4-analogue and derivatives. Proc. Soc. exp. Biol., N. Y. **92**: 404–408, 1956.
70. Beyler, A. L., Neumann, E. D. and Burnham, D. F.: Biological activity of a new deoxycorticosterone derivative, $\Delta^{1,4}$-pregnadiene-21-ol-3:20-dione 3-acetate (Δ^1-DCA). Endocrinology **58**: 471–476, 1956.
71. Beyler, R. E. and Hoffman, F.: Synthesis of 16β-chlorocortisone acetate and related 16-substituted steroids. J. org. Chem. **21**: 572–574, 1956.
72. Beyler, R. E., Hoffman, F. and Sarett, L. H.: Bismethylenedioxy steroids. IV. 11α-Methylhydrocortisone acetate and 9α-chloro-11α-methylhydrocortisone acetate. J. Amer. chem. Soc. **82**: 178–182, 1960.
73. Beyler, R. E., Oberster, A. E., Hoffman, F. and Sarett, L. H.: Bismethylenedioxy steroids. III. The synthesis of 7α- and 7β-methyl-hydrocortisones. J. Amer. chem. Soc. **82**: 170–178, 1960.

73a. Bianchi, C., David, A., Ellis, B., Petrow, V., Waddington-Feather, S. and Woodward, E. J.: 16α,17α-Isopropylidenedioxy-6α-methylpregna-1,4-diene-3,20-dione (B.D.H.3144). A new anti-inflammatory agent. J. Pharm., Lond. 13: 355–360, 1961.
74. Birch, A. J.: Hydroaromatic steroid hormones. Part I. 10-Nortestosterone. J. chem. Soc. 1950: 367–368.
75. Birch, A. J. and Quartey, J. A. K.: Hydroaromatic steroid hormones: the androgenic activity of a D-homobisnorsteroid. Chem. & Ind. 489–490, 1953.
76. Bird, C. W. and Cookson, R. C.: Control of basic strength by steric hindrance to solvation of ammonium ions. Chem. & Ind. 1479–1480, 1955.
77. Birke, G., Domeij, B., Olhagen, B. and Plantin, L-O.: Measures for the avoidance of adrenal atrophy in prolonged corticosteroid therapy. Acta med. scand. 155: 245–252, 1956.
78. Birke, G. and Ström, G.: Influence of increased adrenocortical activity on atrio-ventricular conduction in normal individuals. Acta med. scand. 160: 261–265, 1958.
79. Bischoff, F. and Stauffer, R. D.: Orientation of circulating human oestrogens by albumin. Amer. J. Physiol. 191: 313–318, 1957.
80. Black, R. L., Yielding, K. L., Peterson, R. E., Whedon, G. D. and Bunim, J. J.: Metabolic, hormonal and anti-rheumatic effects of delta-1, 9α-fluorohydrocortisone. Ann. Rheum. Dis. 15: 76–78, 1956.
81. Blecher, M. and White, A.: Effects of steroids on the metabolism of lymphoid tissue. Recent Progr. Hormone Res. 15: 391–418, 1959.
82. Bliss, E. L., Migeon, C. J., Branch, C. H. H. and Samuels, L. T.: Reaction of the adrenal cortex to emotional stress. Psychosom. Med. 18: 56–76, 1956.
83. Bocklage, B. C., Nicholas, W. J., Doisy, E. A., Jr., Elliot, W. H., Thayer, S. A. and Doisy, E. A.: Synthesis and biological studies of 17-methyl-C^{14}-estradiol. J. biol. Chem. 202: 27–37, 1953.
84. Boland, E. W.: Antirheumatic effects of hydrocortisone (free alcohol), hydrocortisone acetates, and cortisone (free alcohol) as compared with cortisone acetate: results from oral administration in patients with rheumatoid arthritis. Brit. med. J. 1: 559–564, 1952.
85. Boland, E. W.: The treatment of rheumatoid arthritis with adrenocorticosteroids and their synthetic analogues: an appraisal of certain developments of the past decade. Ann. N. Y. Acad. Sci. 82: 887–900, 1959.
85a. Boland, E. W.: Antirheumatic potency of chemically modified adrenocortical steroids. Amer. J. Med. 31: 581–590, 1961.
86. Boles, D. J.: Modern ophthalmic therapy by ACTH and similar compounds. Illinois med. J. 109: 12–14, 1956.
87. Bondy, F. K.: The effect of the adrenal and thyroid glands upon the rise of plasma amino acids in the eviscerated rat. Endocrinology 45: 605–608, 1949.
88. Bondy, F. K., Ingle, D. J. and Meeks, R. C.: Influence of adrenal cortical hormones upon the level of plasma amino acids in eviscerate rats. Endocrinology 55: 354–360, 1954.
89. Borman, A., Singer, E. M. and Numerof, P.: Growth-survival and sodium retaining activity of 9α-halo derivatives of hydrocortisone. Proc. Soc. exp. Biol., N. Y. 86: 570–573, 1954.
90. Bost, J. and Fargeas, J.: Action directe du benzoate d'oestradiol sur le récepteur vaginal: influence de la nature du solvant. C. R. Soc. Biol., Paris 151: 1424–1426, 1957.
91. Botticelli, C. R., Hisaw, F. L., Jr. and Wotiz, H. H.: Estradiol-17β and progesterone in ovaries of starfish (*Pisaster ochraceous*). Proc. Soc. exp. Biol., N. Y. 103: 875–877, 1960.
92. Bowers, A., Denot, E., Sanchez, M. B. and Ringold, H. J.: Steroids. CXXI. Synthesis of halogenated steroid hormones. 6α-Fluoro cortical hormones, a new class of powerful corticoids. Tetrahedron 7: 153–162, 1959.
93. Bowers, A., Denot, E., Sanchez, M. B., Sanchez-Hidalgo, M. and Ringold, H. J.: Steroids. CXXIV. Studies in cyano steroids. Part I. The synthesis of a series of C-6-cyano steroid hormones. J. Amer. chem. Soc. 81: 5233–5242, 1959.
94. Bowers, A., Ibáñez, L. C. and Ringold, H. J.: Steroids. CX. Studies in nitrosteroids. Part 2. A new route to 6-nitro steroid hormones, 6α-nitro-17α-acetoxyprogesterone and 6α-nitrocortisone. J. Amer. chem. Soc. 81: 3707–3710, 1959.
95. Bowers, A., Ibáñez, L. C. and Ringold, H. J.: Steroids. CXXVIII. Synthesis of halogenated steroid hormones: 6α-fluoro-17α-acetoxyprogesterone and some unsaturated analogs. A new class of highly active oral progestational hormones. J. Amer. chem. Soc. 81: 5991–5993, 1959.
95a. Bowers, A., Ibáñez, L. C., Denot, E. and Becerra, R.: New fluorination procedures. Part I. The addition of Br–F and I–F to cyclohexene and a variety of unsaturated steroids. J. Amer. chem. Soc. 82: 4001–4007, 1960.
96. Bowers, A. and Ringold, H. J.: Steroids. CIII. A new class of potent cortical hormones, 6α-fluorocorticoids. J. Amer. chem. Soc. 80: 4423–4424, 1958.
97. Bowers, A., Ringold, H. J. and Denot, E.: Steroids. CI. 19-Nordihydrotestosterone derivatives. J. Amer. chem. Soc. 80: 6115–6118, 1958.
98. Bowers, A., Sanchez, M. B. and Ringold, H. J.: Steroids. CIX. Studies in nitrosteroids. Part I. The synthesis of 6α- and 6β-nitrotestosterone and 6α- and 6β-nitroprogesterone. J. Amer. chem. Soc. 81: 3702–3706, 1959.
99. Bowers, A. and Ringold, H. J.: Steroids. XCII. Synthesis of halogenated hormones. II. 6α- and 6β-fluorotestosterone and 6α- and 6β-fluoroprogesterone. Tetrahedron 3: 14–27, 1958.
100. Bowers, A. and Ringold, H. J.: Steroids. CXI. Studies in nitrosteroids. Part 3. The synthesis of 21-nitroprogesterone. J. Amer. chem. Soc. 81: 3710–3712, 1959.
101. Bowers, A., Ringold, H. J. and Dorfman, R. I.: Steroids. LXXXIX. 19-Nordihydrotestosterone derivatives. A potent class of antioestrogenic compounds. J. Amer. chem. Soc. 79: 4556–4557, 1957.
102. Breibart, S. and Engel, F. L.: Influence of cortisone and insulin on the respiratory activity of rat adipose tissue *in vitro*. Endocrinology 55: 70–75, 1954.

103. Brendler, H. and Winkler, B. S.: Effect of norethandrolone on 17-KS excretion in prostatic cancer patients. J. clin. Endocrin. 19: 183–192, 1959.
104. Bristol, W. R. and Drill, V. A.: Saline hydration and diuresis after adrenalectomy. Endocrinology 50: 677–683, 1953.
105. Britton, S. W. and Silvette, H.: Effects of cortico-adrenal extract on carbohydrate metabolism in normal animals. Amer. J. Physiol. 100: 693–700, 1932.
106. Britton, S. W. and Silvette, H.: The apparent prepotent function of the adrenal glands. Amer. J. Physiol. 100: 693–700, 701–713, 1932.
107. Bronstein, J., Sulser, F., Kunz, H. A. and Wilbrandt, W.: Die Wirkung von k-Strophanthosid und Nebennierensteroiden auf den Natriumtransport durch die isolierte Froschhaut. Helv. physiol. acta 16: C7–C10, 1958.
108. Brooks, R. V. and Prunty, F. T. G.: The suppression of adrenocortical secretion with 17α-ethyl-19-nortestosterone. J. Endocrin. 15: 385–392, 1957.
109. Brown, F. K. and Remington, J. W.: Arteriolar responsiveness in adrenal crisis in the dog. Amer. J. Physiol. 182: 279–284, 1955.
110. Brown, J. H. U. and Anason, A.: *In vitro* metabolism of substituted steroids by rat liver. Endocrinology 62: 103–107, 1958.
111. Brown, J. H. U., Anason, A. and Jacobs, J.: *In vitro* metabolism of hydrocortisone in tissues of the adult rat. Amer. J. Physiol. 190: 259–264, 1957.
112. Brunner, H., Kuschinsky, G. and Peters, G.: Die Beeinflussung der renalen Wasser- und Salzausscheidung normaler und hypophysektomierter Ratten durch Nebennierenrindenhormone. Arch. exp. Path. Pharmak. 228: 578–595, 1956.
113. Bukhonova, A. I.: Repair processes taking place in the skin of young dogs treated with cortisone and ACTH. Dokl. Akad. Nauk. 124: 477–480, 1959.
114. Bullough, W. S.: Stress and epidermal mitotic activity. I. The effects of the adrenal hormones. J. Endocrin. 8: 265–274, 1952.
115. Bunim, J. J., Black, R. L., Bollet, A. J. and Pechet, M. M.: The metabolic effects of metacortandralone and metacortandracin. Ann. N. Y. Acad. Sci. 61: 358–366, 1955.
116. Bunim, J. J., Black, R. L., Lutwak, L., Peterson, R. E. and Whedon, G. D.: Studies on dexamethasone, a new synthetic steroid in rheumatoid arthritis—a preliminary report. Arthritis and Rheumatism 1: 313–331, 1958.
117. Bunim, J. J., Pechet, M. M. and Bollet, A. J.: Studies on metacortandralone and metacortandracin in rheumatoid arthritis. J. Amer. med. Ass. 157: 311–318, 1955.
118. Burstein, S.: 2α-Hydroxylation of cortisol in the guinea pig. J. Amer. chem. Soc. 78: 1769–1770, 1956.
119. Burstein, S. and Dorfman, R. I.: Steroid metabolism in guinea pigs. I. Metabolism of cortisol in vivo. J. biol. Chem. 213: 581–595, 1955.
120. Burstein, S., Dorfman, R. I. and Nadel, E. M.: 6β-Hydroxycortisol. A new steroid in human urine. Arch. Biochem. Biophys. 53: 307–308, 1954.
121. Burstein, S., Savard, K. and Dorfman, R. I.: The *in vivo* metabolism of cortisone. Endocrinology 52: 448–452, 1953.
122. Burstein, S., Savard, K. and Dorfman, R. I.: Metabolism of hydrocortisone. Endocrinology 53: 88–97, 1953.
122a. Burtner, R. R. and Gentry, R. E.: *dl*-18-nor-D-Homosteroids. J. org. Chem. 25: 582–584, 1960.
122b. Bush, I. E.: Adrenocortical secretion and its relation to the pituitary gland. J. Endocrin. 7: lxxxiii–lxxxvi, 1951.
123. Bush, I. E.: The physicochemical state of cortisol in blood. In: Hormones in Blood, Ciba Foundation Colloquia on Endocrinology, ed. by G. E. W. Wolstenholme and E. C. P. Millar, vol. 11, pp. 263–281. Little, Brown and Co., Boston, 1957.
124. Bush, I. E.: The 11-oxygen function in steroid metabolism. Experientia 12: 325–331, 1957.
124a. Bush, I. E.: Chemical and biological factors in the actions of steroid hormones. In: The Human Adrenal Cortex, ed. by A. Currie and J. K. Grant. E. S. Livingstone, Edinburgh, 1961.
124b. Bush, I. E. and Mahesh, V. B.: Adrenocortical function with sudden onset of hirsutism. J. Endocrin. 18: 1–25, 1959.
124c. Bush, I. E. and Mahesh, V. B.: Metabolism of 11-oxygenated steroids. 1. Influence of the A/B ring junction on the reduction of 11-oxo groups. Biochem. J. 71: 705–717, 1959.
124d. Bush, I. E. and Mahesh, V. B.: Metabolism of 11-oxygenated steroids. 2. 2-Methyl steroids. Biochem. J. 71: 718–742, 1959.
124e. Bush, I. E., Meigs, R. A. and Hunter, S.: Effects of alkyl and halogen substituents on the oxidation-reduction of 11-oxygen functions in steroids. J. Endocrin. 24: ii, 1962.
124f. Bush, I. E.: (Unpublished.)
125. Bush, I. E. and Willoughby, M.: The excretion of *allo*tetrahydrocortisol in human urine. Biochem. J. 67: 689–700, 1957.
126. Butenandt, A. and Dannenberg, H.: Über das wahre Δ^1-Androsten-ol-(17)-on-(3), ein Isomeres des Testosterons. Ber. dtsch. chem. Ges. 73: 206–208, 1940.
127. Butenandt, A. and Hofstetter, H.: Über Oestronsulfat, ein physiologisches Ausscheidungsprodukt des Follikelhormons. Hoppe-Seyl. Z. 259: 222–234, 1939.
128. Butenandt, A. and Poschmann, L.: Über Lumiandrosteron. Ber. dtsch. chem. Ges. 77: 394–397, 1944.
129. Butenandt, A. and Schmidt-Thomé, J.: Überführung von Dehydroandrosteron in Progesteron: ein einfacher

Weg zur künstlichen Darstellung des Schwangerschaftshormons aus Cholesterin. Ber. dtsch. chem. Ges. **72**: 182–187, 1939.

130. Butenandt, A., Schmidt-Thomé, J. and Paul, H.: Umwandlung des Dehydro-Androsterons in 17-*iso*-Progesteron und Progesteron. Ber. dtsch. chem. Ges. **72**: 1112–1119, 1939.
131. Byrnes, W. W., Barnes, L. E., Bowman, B. J., Dulin, W. E., Morley, E. H. and Stafford, R. D.: Adrenal corticoid activities of 2-methylhydrocortisone acetate and 2-methyl-9-α-fluorohydrocortisone acetate. Proc. Soc. exp. Biol., N. Y. **91**: 67–70, 1956.
132. Callow, R. K.: Metabolism of steroids. Annu. Rep. chem. Soc. **53**: 305–318, 1956.
133. Callow, R. K., Lloyd, J. and Long, D. A.: A chloro-derivative of cortisone with enhanced activity. Lancet (July 3), 20, 1954.
134. Cameron, J.: The effect of Aspirin, Cortisone and other compounds on susceptibility to anaphylactic shock in mice. Brit. J. exp. Path. **38**: 512–514, 1957.
135. Camerino, B., Modelli, R. and Patelli, B.: Sintesi di analoghi di ormoni steroidi sostituiti in posizione 4. Farmaco, Ed. Sci. **13**: 52–63, 1958.
136. Carone, F. A. and Spector, W. G.: The suppression of experimental proteinuria in the rat by compounds that inhibit increased capillary permeability. J. Path. Bact. **80**: 55–62, 1960.
137. Caspi, E. and Bergen, J. R.: The isolation of cortisol and Δ^4-pregnene-11β,17α,20β,21-tetrol-3-one from urine of cortisone acetate-treated rats. Arch. Biochem. Biophys. **59**: 207–212, 1955.
138. Caspi, E. and Pechet, M. M.: The metabolism of 1-dehydrosteroids in man: isolation of 6 urinary products following the administration of 11β,17α,21-trihydroxy-1,4-pregna-diene-3,20-dione. Arch. Biochem. Biophys. **68**: 236–237, 1957.
139. Caspi, E. and Pechet, M. M.: Metabolism of 1-dehydrosteroids in man. I. Isolation of six urinary products after the administration of prednisolone. J. biol. Chem. **230**: 843–851, 1958.
140. Cella, J. A. and Kagawa, C. M.: Steroidal lactones. J. Amer. chem. Soc. **79**: 4808–4809, 1957.
141. Cella, J. A., Brown, E. A. and Burtner, R. R.: Steroidal aldosterone blockers. I. J. org. Chem. **24**: 743–748, 1959.
142. Cella, J. A. and Tweit, R. C.: Steroidal aldosterone blockers. II. J. org. Chem. **24**: 1109–1110, 1959.
143. Charney, W., Weber, L. and Oliveto, E.: The microbiological conversion of 4-pregnene-11β,17α,21-triol-3,20-dione-11,21-diacetate (Compound F 11, 21-diacetate) to Compound F. Arch. Biochem. Biophys. **79**: 402–403, 1959.

143a. Chiu, C. Y. and Needham, D.: Effect of adrenal cortical hormone preparations added *in vitro* upon the carbohydrate metabolism of liver slices. Biochem. J. **46**: 114–120, 1950.

144. Christensen, B. G., Strachan, R. G., Trenner, N. R., Arison, B. H., Hirschmann, R. and Chemerda, J. M.: The partial synthesis of 12α-methyl-11-dehydrocorticosterone. J. Amer. chem. Soc. **82**: 3995–4000, 1960.
145. Christy, N. P., Wallace, E. Z. and Jailer, J. W.: Comparative effects of prednisone and of cortisone in suppressing response of adrenal cortex to exogenous adrenocorticotrophin. J. clin. Endocrin. **16**: 1059–1074, 1956.
146. Civen, M. and Knox, W. E.: The independence of hydrocortisone and tryptophan induction of tryptophan pyrrolase. J. biol. Chem. **234**: 1787–1790, 1959.
147. Clark, I. and Colborn, R. W.: A relationship between Vitamin A metabolism and cortisone. Endocrinology **56**: 232–238, 1955.
148. Clark, I., Geoffroy, R. F. and Bowers, W.: Effects of adrenal cortical steroids on calcium metabolism. Endocrinology **64**: 849–856, 1959.
149. Clark, I. and Umbreit, W. W.: Effect of cortisone and other steroids upon *in vitro* synthesis of chondroitin sulphate. Proc. Soc. exp. Biol., N. Y. **86**: 558–561, 1954.
150. Clark, J. H., Jr. and Pesch, L. A.: Effects of cortisone upon liver enzymes and protein synthesis. J. Pharmacol. **117**: 202–207, 1956.
151. Clark, W. G.: Effect of adrenalectomy upon intestinal absorption of sodium chloride. Proc. Soc. exp. Biol., N. Y. **40**: 468–470, 1939.
152. Clinton, R. O., Neumann, H. C., Manson, A. J., Laskowski, S. C. and Christiansen, R. G.: D-Homosteroids. III. The synthesis of D-homocortisone acetate and related compounds. J. Amer. chem. Soc. **80**: 3395–3402, 1958.
153. Cohen, M., Magdalen, S., Reddy, W. J. and Laidlaw, J. C.: The secretion and deposition of cortisol during pregnancy. J. clin. Endocrin. **18**: 1077–1092, 1958.
154. Cole, D. F.: Chemical changes in the tissues of the rat after adrenalectomy. J. Endocrin. **6**: 245–250, 1950.
155. Cole, D. F.: The effects of deoxycorticosterone acetate on electrolyte distribution in the tissues of the adrenalectomized rat. J. Endocrin. **6**: 251–255, 1950.
156. Cole, W. and Julian, P. L.: Sterols. XIV. Reduction of epoxy ketones by chromous salts. J. org. Chem. **19**: 131–138, 1954.
157. Collins, R. J., Ellis, B., Hansen, S. B., Mackenzie, H. S., Moualim, R. J., Petrow, V., Stephenson, O. and Sturgeon, B.: Some observations on the structural requirements for antibiotic activity in the chloramphenicol series. Part II. J. Pharm., Lond. **4**: 693–710, 1952.
158. Colton, F. B., Nysted, L. N., Riegel, B. and Raymond, L.: 17-Alkyl-19-nortestosterones. J. Amer. chem. Soc. **79**: 1123, 1957.
159. Conway, E. J. and Hingerty, D.: The influence of adrenalectomy on muscle constituents. Biochem. J. **40**: 561–568, 1946.
160. Cook, D. L., Edgren, R. A. and Harris, T. W.: Androgen inhibition of atherogenesis in pullets. Circulation Res. **5**: 54–57, 1957.
161. Cook, D. L., Edgren, R. A. and Saunders, F. J.: Lipid-shifting properties of 17α-methyl-17-hydroxy-5(10)-estren-3-one, a steroid with both estrogenic and androgenic activities. Endocrinology **62**: 798–803, 1958.

162. Cope, C. L. and Black, E. G.: The behaviour of C^{14}-cortisol and estimation of cortisol production rate in man. Clin. Sci. 17: 147–163, 1958.
163. Cope, C. L., Hurlock, B. and Sewell, C.: The distribution of adrenal cortical hormones in some body fluids. Clin. Sci. 14: 25–36, 1955.
164. Cornforth, J. W., Youhotsky, I. and Popjak, G.: Absolute configuration of cholesterol. Nature, Lond. 173: 536, 1954.
165. Coxon, R. V., Korenchevsky, V. and Lawrence, A.: Chemical changes in the heart, liver and kidneys of rats after administration of sex hormones. J. Path. Bact. 72: 613–621, 1956.
166. Creger, W. P., Tulley, E. H. and Hansen, D. G.: Effect of hydrocortisone on microelectrophoretic characteristics of human erythrocyte-antibody unions. J. Lab. clin. Med. 47: 686–690, 1956.
167. Daniel, E. E. and Bass, P.: Influence of sodium, potassium, and adrenal hormones on gastrointestinal motility. Amer. J. Physiol. 187: 253–258, 1956.
167a. Daughaday, W. H.: Binding of corticosteroids by plasma proteins. I. Dialysis-equilibrium and renal clearance studies. J. clin. Invest. 35: 1428–1433, 1956.
167b. Daughaday, W. H.: Binding of corticosteroids by plasma proteins. II. Paper electrophoresis and equilibrium paper electrophoresis. J. clin. Invest. 35: 1434–1438, 1956.
167c. Daughaday, W. H.: Steroid protein interactions. Physiol. Rev. 39: 885–902, 1959.
168. David, A., Fellowes, K. P. and Millson, D. R.: Some biological properties of dimethisterone "Secrosteron," a new orally active progestational agent. J. Pharm., Lond. 11: 491–495, 1959.
169. David, A., Hartley, F., Millson, D. R. and Petrow, V.: The preparation and progestational activity of some alkylated ethisterones. J. Pharm., Lond. 9: 929–934, 1957.
170. Davis, A. K., Bass, A. C. and Overman, R. R.: Comparative effects of cortisone and DCA on ionic balance and fluid volumes of normal and adrenalectomised dogs. Amer. J. Physiol. 166: 493–503, 1951.
171. Deanesley, R. and Parkes, A. S.: Oestrogenic action of compounds of the androsterone-testosterone series. Brit. med. J. 1: 257–258, 1936.
172. De Duve, C., Berthet, J., Berthet, L. and Appelmans, F.: Permeability of mitochondria. Nature, Lond. 167: 389–390, 1957.
173. Deghenghi, R. and Engel, C. R.: Steroids and related products. XII. The synthesis of 17α-bromo-11-dehydrocorticosterone. J. Amer. chem. Soc. 82: 3201–3209, 1960.
174. Desaulles, P.: Comparison of the effects of aldosterone, cortexone, and cortisol on adrenalectomised rats under various salt loads. In: International Symposium on Aldosterone, ed. by A. F. Muller, pp. 29–38. Churchill, London, 1958.
175. de Winter, M. S., Siegmann, C. M. and Szpilfogel, S. A.: 17-Alkylated 3-deoxo-19-nortestosterones. Chem. & Ind., 905, 1959.
176. Dingman, J. F., Finkenstaedt, J. T., Laidlaw, J. C., Renold, A. E., Jenkins, D., Merrill, J. P. and Thorn, G. W.: Influence of intravenously administered adrenal steroids on sodium and water excretion in normal and Addisonian subjects. Metabolism 7: 608–623, 1958.
177. Dirscherl, W., Kraus, J. and Voss, H. E.: Neue Stoffe mit starker Wirkung auf die Vesikulardrüsen der kastrierten männlichen Maus. 9. Mitteilung über Sexualhormone und verwandte Stoffe. Hoppe-Seyl. Z. 241: 1–10, 1936.
178. Dixon, A. St. J. and Bywaters, E. G. L.: The effect of intraarticular injection of cortisone acetate and of hydrocortisone acetate in rheumatoid arthritis. Clin. Sci. 12: 15–31, 1953.
179. Djerassi, C. and Finch, N.: Optical rotatory dispersion studies. XXX. Demonstration of a boat form in a 3-keto steroid. J. Amer. chem. Soc. 81: 4997–4998, 1959.
179a. Djerassi, C., Fornaguera, I. and Mancera, O.: Optical rotatory dispersion studies. XXIII. α-Haloketones. J. Amer. chem. Soc. 81: 2383–2386, 1959.
180. Djerassi, C., Lippman, A. E. and Grossman, J.: 1-Methyl-19-norprogesterone and 1-methyl-19-nor-17α-hydroxyprogesterone. J. Amer. chem. Soc. 78: 2479–2481, 1956.
181. Djerassi, C., Manson, A. J. and Bendas, H.: The synthesis of 8-*iso*progesterone and 8-*iso*testosterone. Tetrahedron 1: 22–27, 1957.
182. Djerassi, C., Miramontes, L. and Rosenkranz, G.: Steroids. XLVIII. 19-Norprogesterone—a potent progestational agent. J. Amer. chem. Soc. 75: 4440–4442, 1953.
183. Djerassi, C. and Nussbaum, A. L.: Studies in organic sulfur compounds. V. Synthesis of 21-thiolacetates of adrenal cortical hormones. J. Amer. chem. Soc. 75: 3700–3703, 1953.
184. Djerassi, C., Riniker, R. and Riniker, B.: Optical rotatory dispersion studies. VIII. α,β-Unsaturated ketones and solvent effects. J. Amer. chem. Soc. 78: 6377–6389, 1956.
185. Dodson, R. M. and Sollman, P. B.: Pregnane-20-thiones. U. S. Pat. 2,837,539, June 3. Chem. Abstr. 53: 3282h, 1958.
186. Dodson, R. M., Sollman, P. B. and Riegel, B.: The preparation of D-homoprogesterone and D-homo-11-deoxycorticosterone acetate. J. Amer. chem. Soc. 75: 5132, 1953.
187. Dodson, R. M. and Tweit, R. C.: Addition of alkanethiolic acids to $\Delta^{1,4}$-3-oxo- and $\Delta^{4,6}$-3-oxo-steroids. J. Amer. chem. Soc. 81: 1224–1227, 1959.
188. Doisy, R. J. and Lardy, H. A.: Effect of certain steroids on metabolic rate of hyperthyroid rats. J. Physiol. 190: 142–146, 1957.
189. Dorfman, R. I.: The bioassay of adrenocortical hormones. Recent Progr. Hormone Res. 8: 87–112, 1953.
190. Dorfman, R. I.: The anti-androgenic activity of a phenanthrene derivative in the chick. Endocrinology 64: 464–465, 1959.

190a. DORFMAN, R. I.: Androgens. In: The Mechanism of Action of Steroid Hormones, ed. by C. A. Villee and L. L. Engel, pp. 148–154. Pergamon Press, London, 1961.
191. DORFMAN, R. I., DORFMAN, A. S. AND LAUBACH, G.: Thymolytic activity of 14α-hydroxycortisol. Proc. Soc. exp. Biol., N. Y. **100**: 63–64, 1959.
192. DOUGHERTY, T. F., BROWN, H. E. AND BERLINER, D. L.: Metabolism of hydrocortisone during inflammation. Endocrinology **62**: 455–462, 1958.
193. DOUGHERTY, T. F. AND SCHNEEBELI, G. L.: The use of steroids as antiinflammatory agents. Ann. N. Y. Acad. Sci. **61**: 328–346, 1955.
194. DOWBEN, R. M.: Increased creatine and creatinine excretion after 17α-ethyl-19-nortestosterone. Proc. Soc. exp. Biol., N. Y. **98**: 644–645, 1958.
195. DOWLING, J. T., FREINKEL, N. AND INGBAR, S. H.: Thyroxine-binding by sera of pregnant women, new-born infants, and women with spontaneous abortion. J. clin. Invest. **35**: 1263–1276, 1956.
196. DRILL, V. A., COOK, D. L. AND EDGREN, R. A.: Effect of new steroids on blood lipids. In: Hormones and Atherosclerosis, ed. by G. Pincus. Academic Press, New York, 1959.
197. DRILL, V. A., EDGREN, R. A. AND COOK, D. L.: Prediction of estrogenic side-effects of steroids in man. In: Hormones and Atherosclerosis, ed. by G. Pincus. Academic Press, New York, 1959.
198. DRILL, V. A. AND RIEGEL, B.: Structural and hormonal activity of some new steroids. Recent Progr. Hormone Res. **14**: 29–67, 1958.
199. DRILL, V. A. AND SAUNDERS, F. J.: Biological activity of norethynodrel. Proc. Soc. exp. Biol., N. Y. **94**: 717–720, 1957.
200. DULIN, W. E.: Anti-inflammatory activity of Δ^1-9α-fluoro-hydrocortisone acetate. Proc. Soc. exp. Biol., N. Y. **90**: 115–117, 1955.
201. DUNN, C. E., BASS, A. D. AND MCARDLE, A. H.: Effect of cortisone on liver nucleoproteins. Exp. Cell Res. **14**: 23–28, 1958.
202. EBERT, R. H. AND BARCLAY, W. R.: Changes in connective tissue reaction induced by cortisone. Ann. intern. Med. **37**: 506–518, 1952.
203. EDGREN, R. A.: The uterine growth-stimulating activities of 17α-ethynyl-17-hydroxy-5(10)-estren-3-one (Norethynodrel) and 17α-ethynyl-19-nortestosterone. Endocrinology **62**: 689–693, 1958.
204. EDGREN, R. A.: The modification of estrogen-induced changes in rat vaginas with steroids and related agents. Ann. N. Y. Acad. Sci. **83**: 160–184, 1959.
205. EDGREN, R. A. AND CALHOUN, D. W.: Iso-response lines for steroid interactions: experimental determination and implications. Anat. Rec. **128**: 542, 1957.
206. EDGREN, R. A., CALHOUN, D. W., ELTON, R. L. AND COLTON, F. B.: Estrogen antagonisms: the effects of a series of relatives of 19-nortestosterone on estrone-induced uterine growth. Endocrinology **65**: 265–272, 1959.
207. EDGREN, R. A., CALHOUN, D. W. AND HARRIS, T. W.: Studies on the uterine growth-stimulating effects of combinations of testosterone propionate and natural estrogens in rats. Anat. Rec. **131**: 547, 1958.
208. EDGREN, R. A., HAMBOURGER, W. E. AND CALHOUN, D. W.: Production of adrenal atrophy by 6-methyl-17-acetoxy progesterone with remarks on the adrenal effects of other progestational agents. Endocrinology **65**: 505–507, 1959.
209. EDWARDS, J. A. AND RINGOLD, H. J.: Steroids. CXXXII. 2-Fluoro and 21,21-difluoro steroids. J. Amer. chem. Soc. **81**: 5262, 1959.
210. EDWARDS, J. A., RINGOLD, H. J. AND DJERASSI, C.: Steroids. CXXII. 6α-Fluoro-16α-methyl-corticoids—synthesis and biological activity. J. Amer. chem. Soc. **81**: 5156, 1959.
211. EDWARDS, J. A., RINGOLD, H. J. AND DJERASSI, C.: Steroids. CXXXVI. Synthesis of a new class of potent cortical hormones. 6α-Fluoro- and 6α,9α-difluoro-16α-methylprednisolone and related steroids. J. Amer. chem. Soc. **82**: 2318–2322, 1960.
212. EHRENSTEIN, M.: Discussion after Drill and Riegel. Recent Progr. Hormone Res. **14**: 71, 1958.
213. EHRENSTEIN, M.: Investigations on steroids. VIII. Lower homologs of hormones of the pregnane series: 9-nor-11-desoxycorticosterone acetate and 9-norprogesterone. J. org. Chem. **9**: 435–456, 1944.
213a. EHRENSTEIN, M. AND DÜNNENBERGER, M.: 19-Hydroxy-14β,17α-progesterone and 19-hydroxy-11-desoxy-14β,17α-corticosterone. J. org. Chem. **21**: 783–790, 1956.
214. EIK-NES, K. B. AND SAMUELS, L. T.: Metabolism of cortisol in normal and "stressed" dogs. Endocrinology **63**: 82–88, 1958.
215. EIK-NES, K. B., SCHELLMAN, J. A., LUMRY, R. AND SAMUELS, L. T.: The binding of steroids to protein. I. Solubility determinations. J. biol. Chem. **206**: 411–419, 1954.
216. ELTON, R. L.: Metrotrophic activity of some 21-haloprogesterone derivatives. Proc. Soc. exp. Biol., N. Y. **101**: 677–680, 1959.
217. ELTON, R. L. AND EDGREN, R. A.: Biological actions of 17α-(2-methallyl)-19-nortestosterone, an orally active progestational agent. Endocrinology **63**: 464–472, 1958.
218. ELY, R. S., DONE, A. K. AND KELLEY, V. C.: Δ^1-Hydrocortisone; plasma 17-hydroxycorticosteroid concentrations following oral and intravenous administration. Proc. Soc. exp. Biol., N. Y. **91**: 503–506, 1956.
218a. ENGEL, C. R.: Steroids and related products. II. The synthesis of 11-dehydro-17α-methyl-corticosterone acetate. J. Amer. chem. Soc. **77**: 1064–1065; V. The synthesis of 11-dehydro-17α-methylcorticosterone, **78**: 4727–4733, 1955.
218b. ENGEL, C. R.: Synthesis of 17α-methylcorticosterone. Canad. J. Chem. **35**: 131–140, 1957.
219. ENGEL, C. R. AND DEGHENGHI, R.: Steroids and related products. XIII. Synthesis of 6α-fluoro-17α-bromoprogesterone. Canad. J. Chem. **38**: 452–456, 1960.
220. ENGEL, C. R., HOEGERLE, R.-M. AND DEGHENGHI, R.: Steroids and related products. XIV. Synthesis of 17α-halogenated 20,21-ketols of the corticoid type. Canad. J. Chem. **38**: 1199–1208, 1960.

221. ENGEL, C. R. AND JAHNKE, H.: Steroids and related products. X. 17α-Bromoprogesterone, a new potent gestogen. Canad. J. Biochem. Physiol. 35: 1047–1055, 1957.
222. ENGEL, C. R. AND JUST, G.: Steroids and related products. I. The synthesis of 17α-methyldesoxycorticosterone. J. Amer. chem. Soc. 76: 4909–4914, 1954.
222a. ENGEL, C. R. AND JUST, G.: The synthesis of 17β-methyl-17-isodesoxycorticosterone. Canad. J. Chem. 33: 1515–1520, 1955.
223. ENGEL, C. R. AND NOBLE, R. L.: Concerning the progestational activity of 21-halogenated progesterones. Endocrinology 61: 318–321, 1957.
224. ENGEL, F. L.: A consideration of the roles of the adrenal cortex and stress in the regulation of protein metabolism. Recent Progr. Hormone Res. 6: 277–308, 1951.
225. ENGEL, L. L., ALEXANDER, J. AND WHEELER, M.: Urinary metabolites of administered 19-nortestosterone. J. biol. Chem. 231: 159–164, 1958.
225a. ENGEL, L. L. AND SCOTT, J. F.: Evening lecture, Ciba Foundation, 1960, unpublished.
225b. ENGEL, L. L. AND SCOTT, J. F.: Effects of steroid hormones upon diphosphopyridine nucleotide-mediated enzymatic reactions. Recent Progr. Hormone Res. 16: 79–92, 1960.
226. ERCOLI, A. AND GARDI, A.: Δ^4-3-Keto steroidal enol ethers. Paradoxical dependency of their effectiveness on the route of administration. J. Amer. chem. Soc. 82: 7478, 1960.
226a. ESCHENMOSER, A. A.: Über die relative Geschwindigkeit der Chromosäureoxydation sekundärer, alicyclischer Alkohole. Helv. chim. acta 38: 1529–1536, 1955.
226b. ESCHENMOSER, A.: Personal communication, 1960.
227. ESSELLIER, A. F., MORANDI, L. AND JEANNERET, P.: Der Mechanismus der Adrenalineosinopenie. Schweiz. med. Wschr. 87: 1062–1065, 1957.
228. EULER, U. S. VON, GEMZELL, C. A., LEVI, L. AND STRÖM, G.: Cortical and medullary adrenal activity in emotional stress. Acta endocr., Copenhagen 30: 567–573, 1959.
229. FAJANS, S. S. AND CONN, J. W.: An approach to the prediction of diabetes mellitus by modification of the glucose tolerance test with cortisone. Diabetes 3: 296–304, 1954.
230. FARRELL, G. L. AND LAQUEUR, G.: Reduction of pituitary content of ACTH by cortisone. Endocrinology 56: 471–473, 1955.
231. FARRELL, G.: The physiological factors which influence the secretion of aldosterone. Recent Progr. Hormone Res. 15: 275–297, 1959.
232. FEDERMAN, D. D., ROBBINS, J. AND RALL, J. E.: Effects of methyltestosterone on thyroid function, thyroxine metabolism and thyroxine-binding protein. J. clin. Invest. 37: 1024–1030, 1958.
233. FENTON, S. W., DE WALD, A. E. AND ARNOLD, R. T.: The steric effect of methylene groups. VII. J. Amer. chem. Soc. 77: 979–984, 1955.
234. FERIN, J.: A new substance with progestational activity. Comparative assays in ovariectomized women—clinical results. Acta endocr., Copenhagen 22: 303–317, 1956.
235. FERIN, J.: Progestational activity of certain 19-norsteroids. Comparative assays in oophorectomised women. J. clin. Endocrin. 17: 1252–1254, 1957.
236. FERREBEE, J., PARKER, D., CARNES, W. H., GERITY, M. K., ATCHLEY, D. W. AND LOEB, R. F.: Certain effects of desoxycorticosterone. The development of "diabetes insipidus" and the replacement of muscle potassium in normal dogs. Amer. J. Physiol. 135: 230–237, 1941.
237. FIRSCHEIN, H. E., DEVENUTO, F., FITCH, W. M., PEARCE, E. M. AND WESTPHAL, U. F.: Distribution of injected cortisol-4-C^{14} in normal and shocked rats. Rep. No. 257, U. S. Office of Naval Research. U.S.A.M.R.L. Project No. 6-64-12-028, 1957.
238. FIRSCHEIN, H. E., DEVENUTO, F., FITCH, W. M., PEARCE, E. M. AND WESTPHAL, U. F.: Distribution of injected cortisol-4-C^{14} in normal and shocked rats. Endocrinology 60: 347–358, 1957.
239. FISHER, J. W.: Anti-inflammatory properties of cobaltous chloride and 20-cobalto-Δ-1-hydrocortisone. Fed. Proc. 19: no. 1, part 1, 158, 1960.
240. FISHMAN, J., BRADLOW, H. L. AND GALLAGHER, T. F.: Oxidative metabolism of estrogens. J. Amer. chem. Soc. 81: 2273, 1959.
240a. FISHMAN, J., BRADLOW, H. L. AND GALLAGHER, T. F.: Oxidative metabolism of estradiol. J. biol. Chem. 235: 3104–3107, 1960.
241. FISHMAN, J. AND DJERASSI, C.: On the conformation of substituted cyclopentanones. Rotatory dispersion and spectral data of some steroidal α-halocyclopentanones. Experientia 16: 138–139, 1950.
242. FLANAGAN, J. B., DAVIS, A. K. AND OVERMAN, R. R.: Mechanism of extracellular sodium and chloride depletion in the adrenalectomized dog. Amer. J. Physiol. 160: 89–102, 1950.
242a. FONTAINE, M. AND HATEY, J.: Sur la teneur en 17-hydroxycorticosteroïdes du plasma de saumon (Salmo salar L.). C. R. Acad. Sci., Paris 239: 319–321, 1954.
242b. FONTAINE, M. AND LELOUP-HATEY, J.: Mise en évidence de corticostéroïdes dans l'interrénal d'un Téléostéen: le Saumon (Salmo salar L.). J. Physiol., Paris 51: 468–473, 1959.
243. FORSYTH, B. T.: A sodium-excreting effect of desoxycorticosterone in adrenalectomized mice. Endocrinology 52: 65–72, 1953.
244. FORTIER, C. AND DE GROOT, J.: Neuroendocrine relationships. Progr. Neurol. Psychiat. 14: 256–269, 1959.
245. FOSTER, F. J., MCRAE, D. H. AND BUNNER, J.: Auxin-induced growth inhibition. A natural consequence of two-point attachment. Proc. nat. Acad. Sci., Wash. 38: 1014–1022, 1952.
246. FOX, S. M., ORIGONI, V. E. AND SMITH, L. L.: 16α-Hydroxy steroids. V. 11β-Esters of triamcinolone. J. Amer. chem. Soc. 82: 2580–2585, 1960.

247. Frawley, T. F.: The role of the adrenal cortex in glucose and pyruvate metabolism in man including the use of intravenous hydrocortisone in acute hypoglycemia. Ann. N. Y. Acad. Sci. 61: 464–493, 1955.
248. Frawley, T. F., Kistler, H. and Shelley, T.: Effects of anti-inflammatory steroids on carbohydrate metabolism with emphasis on hypoglycemia and diabetic states. Ann. N. Y. Acad. Sci. 82: 868–885, 1959.
249. Fried, J.: Biological effects of 9-alpha-fluoro-hydrocortisone and related steroids in animals. Ann. N. Y. Acad. Sci. 61: 573–581, 1955.
250. Fried, J.: Structure-activity relationships in the field of halogenated steroids. Cancer, N. Y. 10: 752–756, 1957.
250a. Fried, J.: Discussion. In: Mechanism of Action of Steroid Hormones, ed. by C. A. Villee and L. L. Engel, pp. 232–234. Pergamon Press, London, 1961.
251. Fried, J. H., Arth, G. E. and Sarett, L. H.: Alkylated adrenal hormones. The synthesis of 5-methylated pregnanes. J. Amer. chem. Soc. 82: 1684–1688, 1960.
252. Fried, J. and Borman, A.: Synthetic derivatives of cortical hormones. Vitam. & Horm. 16: 303–374, 1958.
253. Fried, J., Borman, A., Kessler, W. B., Grabowick, P. and Sabo, E. F.: Cyclic 16α,17α-ketals and acetals of 9α-fluoro-16α-hydroxycortisol and -prednisolone. J. Amer. chem. Soc. 80: 2338–2339, 1958.
254. Fried, J., Florey, K., Sabo, E. F., Herz, J. E., Restivo, A. R., Borman, A. and Singer, F. M.: Synthesis and biological activity of 1- and 6-dehydro-9α-halocorticoids. J. Amer. chem. Soc. 77: 4181–4182, 1955.
255. Fried, J., Herz, J. E., Sabo, E. F., Borman, A., Singer, F. M. and Numerof, P.: 9α-Halo-11β-hydroxy and 11-keto derivatives of progesterone, desoxycorticosterone and 17α-hydroxyprogesterone. J. Amer. chem. Soc. 77: 1068–1070, 1955.
256. Fried, J., Herz, J. E., Sabo, E. F. and Morrisson, M. H.: Synthesis of 12α-chlorocortisone. Chem. & Ind. 1232–1234, 1956.
257. Fried, J. F. and Sabo, E. F.: Synthesis of 17α-hydroxycorticosterone and its 9α-halo derivatives from 11-epi-17α-hydroxycorticosterone. J. Amer. chem. Soc. 75: 2273–2284, 1953.
258. Fried, J. F. and Sabo, E. F.: 9α-Fluoro derivatives of cortisone and hydrocortisone. J. Amer. chem. Soc. 76: 1455–1456, 1954.
259. Fried, J. F. and Sabo, E. F.: Halogenated corticoids. I. 9α-Halogen derivatives of cortisone and hydrocortisone. J. Amer. chem. Soc. 79: 1130–1141, 1957.
260. Friedman, S. M., Nakashima, M. and Friedman, C. L.: The extrarenal effect of adrenalectomy on sodium and potassium distribution in the rat. Endocrinology 62: 259–267, 1958.
261. Friedman, S. M., Scherrer, H. F., Nakashima, M. and Friedman, C. L.: Extrarenal factors in diabetes insipidus in the rat. Amer. J. Physiol. 192: 401–404, 1958.
262. Fritz, I.: Effects of adrenal cortex on release of labelled nitrogen from tissue proteins of rats previously fed labelled glycine. II. Preferential catabolism of recently synthesised protein during starvation. Endocrinology 58: 482–492, 493–500, 1956.
263. Froesch, E. R., Ashmore, J. and Renold, A. E.: Comparison of renal and hepatic effects of fasting, cortisone administration and glucose infusion in normal and adrenalectomised rats. Endocrinology 62: 614–620, 1958.
264. Froesch, E. R., Winegrad, A. I., Renold, A. E. and Thorn, G. W.: Mechanism of the glucosuria produced by the administration of steroids with glucocorticoid activity. J. clin. Invest. 37: 524–532, 1 958.
265. Frost, R. C. and Talmage, R. V.: Fluid and electrolyte shifts in the hydrated adrenalectomised rat. Endocrinology 49: 606–612, 1957.
266. Fukushima, D. K. and Gallagher, T. F.: Metabolism of 21-deoxyhydrocortisone-4-C^{14} in man; 17-dehydroxylation, an artefact. J. biol. Chem. 226: 725–733, 1957.
266a. Fukushima, D. K., Bradlow, H. L., Hellman, L., Zumoff, B. and Gallagher, T. F.: Metabolic transformation of hydrocortisone-4-C^{14} in normal men. J. biol. Chem. 235: 2246–2252, 1960.
267. Gallagher, T. F., Fukushima, D. K., Barry, M. C. and Dobriner, K.: Studies with isotopic steroid hormones. Recent Progr. Hormone Res. 9: 131–152, 1954.
268. Ganong, W. F. and Mulrow, P. J.: Rate of change of sodium and potassium excretion after injection of aldosterone into aorta and renal artery of dog. Amer. J. Physiol. 195: 337–342, 1958.
269. Gaudino, M. and Levitt, M. F.: Influence of the adrenal cortex on body water distribution and renal function. J. clin. Invest. 28: 1487–1497, 1949.
270. Gaunt, R., Leathem, J. H., Howell, C. and Antonchak, N.: Some biological properties of different esters of desoxycorticosterone. Endocrinology 50: 521–530, 1952.
271. Gauthier, M. B., Le Dreff, L. and Aubry, R.: Dérivés d'hormones à longue durée d'action. I. Esters de l'oestradiol. Ann. pharm. franç. 16: 757–766, 1958.
272. Gemzell, C. A. and Sjöstrand, T.: Effect of adrenalectomy and of hydrocortisone on total amount of haemoglobin and blood volume in male rats. Acta endocr., Copenhagen 27: 446–454, 1958.
273. Germuth, F. G., Jr.: The role of adrenocortical steroids in infection, immunity and hypersensitivity. Pharmacol. Rev. 8: 1–24, 1956.
274 and 275. Ghadially, F. N. and Green, H. N.: Effect of adrenal hormones and adrenalectomy on mitotic activity. Brit. J. exp. Path. 38: 100–110, 1957.
276. Girod, C.: Effets d'une injection d'acétate de désoxycorticostérone sur la lignée blanche chez le Singe. C. R. Soc. Biol., Paris 150: 1522–1527, 1956.
277. Gitelson, S.: The effect of ACTH and cortisone on the blood pyruvic acid level. Acta endocr., Copenhagen 15: 225–235, 1954.
278. Glenn, E. M.: *In vitro* and *in vivo* metabolism of prednisolone. Endocrinology 64: 373–378, 1959.
278a. Glenn, E. M., Richardson, S. L. and Bowman, B. J.: Biologic activity of 6-α-methyl compounds corresponding to progesterone, 17-alpha-hydroxyprogesterone acetate and compound S. Metabolism 8: 265–285, 1959.

279. GLENN, E. M., STAFFORD, R. O., LISTER, S. C. AND BOWMAN, B. J.: Relation between biological activity of hydrocortisone analogues and their rates of inactivation by rat liver enzyme systems. Endocrinology 61: 128-142, 1957.
280. GLYNN, I. M.: Sodium and potassium movements in human red cells. J. Physiol. 134: 278-310, 1956.
281. GLYNN, I. M.: The action of cardiac glycosides on sodium and potassium movements in human red cells. J. Physiol. 136: 148-173, 1957.
282. GODING, J. R. AND DENTON, D. A.: Adrenal insufficiency and overdosage with DOCA on bilaterally adrenalectomised sheep. Aust. J. exp. Biol. med. Sci. 35: 301-320, 1957.
282a. GOLD, N. I., SINGLETON, E., MACFARLANE, D. A. AND MOORE, F. D.: Quantitative determination of the urinary cortisol metabolites, "tetrahydro F," "allo-tetrahydro F" and "tetrahydro E": effects of adrenocorticotropin and complex trauma in the human. J. clin. Invest. 37: 813-823, 1958.
283. GOLDFIEN, A., LAIDLAW, J. C., HAYDAR, N. A., RENOLD, A. E. AND THORN, G. W.: Fluorohydrocortisone and chlorohydrocortisone, highly potent derivatives of Compound F. New Engl. J. Med. 252: 415-421, 1955.
284. GOLDFIEN, A., MORSE, W. I., FROESCH, E. R., GANONG, W. F., RENOLD, A. E. AND THORN, G. W.: Pharmacological studies in man of 11-, 17- and 21-hydroxy derivatives of progesterone and their fluorinated analogs. Ann. N. Y. Acad. Sci. 61: 433-441, 1955.
285. GOLDING, D. N. AND BEGG, T. B.: Dexamethasone myopathy. Brit. med. J. 2: 1129-1130, 1960.
285a. GOLDSTEIN, M. S., RAMEY, E. AND LEVINE, R.: Relation of muscular fatigue in the adrenalectomised dog to inadequate circulatory adjustment. Amer. J. Physiol. 163: 561-565, 1950.
286. GOLDZIEHER, J. W., BAKER, R. E. AND MYMAN, M. A.: Distribution of oestrogens between red cells and serum. Endocrinology 65: 310-316, 1959.
287. GOODKIND, M. J., HYATT, R. E. AND DAVIS, J. O.: Failure of large doses of deoxycorticosterone acetate to block mercurial natriuresis in adrenalectomised dogs with thoracic inferior vena cava constriction and ascites. Amer. J. Physiol. 187: 361-364, 1956.
288. GOULD, D., SHAPIRO, E. L., FINCKENOR, L. E., GRUEN, F. AND HERSHBERG, E. B.: Steroidal amines. III. 16α-Amino-substituted pregnanes. J. Amer. chem. Soc. 78: 3158-3163, 1956.
289. GOULD, D., SHAPIRO, E. L., HERZOG, H. L., GENTLES, M. J., HERSHBERG, E. B., CHARNEY, W., GILMORE, M., TOLKSDORF, S., EISLER, M., PERLMAN, P. L. AND PECHET, M. M.: Some new active corticosteroids. J. Amer. chem. Soc. 79: 502-503, 1957.
290. GRABER, R. P., HAVEN, A. C. AND WENDLER, N. L.: $\Delta^{9,(11)}$-Dehydrocortical steroids. Synthesis of $\Delta^{9,(11)}$-anhydro-17-hydroxycorticosterone acetate. J. Amer. chem. Soc. 75: 4722-4724, 1953.
291. GRAY, C. H., GREEN, M. A. S., HOLNESS, N. J. AND LUNNON, J. B.: Urinary metabolic products of prednisone and prednisolone. J. Endocrin. 14: 146-154, 1956.
292. GRANT, W. M.: Ophthalmic pharmacology and toxicology. Arch. Ophthal., N. Y. 56: 299-316, 1956.
293. GREEN, D. M., FARAH, A. AND KLEMPERER, W. W.: Mechanisms of deoxycorticosterone action: renal effects of the water-soluble glycoside. Endocrinology 47: 281-288, 1950.
294. GREEN, D. M., JOHNSON, A. D., BRIDGES, W. E., LEHMANN, J. H. AND GRAY, F.: Mechanisms of deoxycorticosterone action: effects of the water-soluble glycoside on human circulatory and renal functions. Endocrinology 47: 102-107, 1950.
295. GREENBLATT, R. B.: Progestational activity of 17-α-ethinyl-19-nortestosterone (norethisterone). J. clin. Endocrin. 16: 869-875, 1956.
296. GROSS, F.: Nebennierenrinde und Wasser-Salzstoffwechsel unter besonderer Berücksichtigung von Aldosteron. Klin. Wschr. 34: 929-941, 1956.
297. GÜNTHARD, H. H., BERIGER, E., ENGEL, C. R. AND HEUSSER, H.: Eine neue Variante der Synthese des biologisch hochaktiven Gestagens 17α-Methyl-Progesteron. Helv. chim. acta 35: 2437-2444, 1952.
298. HAJDU, S. AND SZENT-GYÖRGI, A.: Action of DOC and serum on the frog heart. Amer. J. Physiol. 168: 159-170, 1952.
299. HAJDU, S. AND SZENT-GYÖRGI, A.: Action of digitalis glucosides on isolated frog heart. Amer. J. Physiol. 168: 170-175, 1952.
300. HALKERSTON, I. D. K., EICHHORN, J., FEINSTEIN, M., SCULLY, E. AND HECHTER, O.: Early permeability effects of estradiol on castrate rat uterus. Proc. Soc. exp. Biol., N. Y. 103: 796-799, 1960.
301. HALLIDAY, R.: Effect of steroid hormones on absorption of antibody by young rat. J. Endocrin. 18: 56-66, 1959.
302. HAMILTON, L. H.: Heparin-induced block of leucocyte response to cortisone. Endocrinology 61: 392-397, 1957.
303. HAOT, J.: Influence de l'acétate de désoxycorticostérone et de la prednisolone sur les ulcères provoqués par irradiation de l'estomac chez le Lapin. C. R. Soc. Biol., Paris 153: 354-356, 1959.
304. HARDWICK, S. W., PEARSE, J. J. AND PETROW, V.: 6β-Hydroxy-3:5-cyclopregnan-20-one in mental states. J. ment. Sci. 103: 835-839, 1957.
305. HARRIS, J. I. AND LERNER, A. B.: Amino-acid sequence of the α-melanocyte-stimulating hormone. Nature, Lond. 179: 1346-1347, 1957.
306. HARROP, G. A. J., SOFFER, L. J., ELLSWORTH, R. AND TRESCHER, J. K.: Studies on the suprarenal cortex. III. Plasma electrolytes and electrolyte excretion during suprarenal insufficiency in the dog. J. exp. Med. 58: 17-38, 1933.
307. HART, F. D.: Corticosteroids in treatment of rheumatic disorders. Brit. med. J. 1: 493-496, 1960.
308. HAYANO, M. AND DORFMAN, R. I.: Studies on the inhibition of various enzymes by steroids. Ann. N. Y. Acad. Sci. 54: 608-618, 1951.
308a. HECHTER, O.: Concerning possible mechanisms of hormone action. Vitam. & Horm. 13: 293-346, 1955.
309. HECHTER, O., KROHN, L. AND HARRIS, J.: The effect of estrogen on the permeability of the uterine capillaries. Endocrinology 29: 386-392, 1941.

309a. HECHTER, O. AND LESTER, G.: Cell permeability and hormone action. Recent Progr. Hormone Res. 16: 139–179, 1960.

310. HECHTER, O. AND PINCUS, G.: Genesis of the adrenocortical secretion. Physiol. Rev. 34: 459–496, 1954.

310a. HENNE, A. L. AND PELLEY, R. L.: Acidity of trifluorinated alcohols and saponification rates of their acetates. J. Amer. chem. Soc. 74: 1426–1428, 1952.

310b. HELLMAN, L., BRADLOW, H. L., ZUMOFF, B., FUKUSHIMA, D. K. AND GALLAGHER, T. F.: Thyroid-androgen interrelations and the hypocholesteremic effect of androsterone. J. clin. Endocrin. 19: 936–948, 1959.

310c. HELLMAN, L., BRADLOW, H. L., ZUMOFF, B. AND GALLAGHER, T. F.: The influence of thyroid hormone on hydrocortisone production and metabolism. J. clin. Endocrin. 21: 1231–1247, 1961.

311. HENNEMAN, D. M. AND BUNKER, J. P.: The pattern of intermediary carbohydrate metabolism in Cushing's syndrome. Amer. J. Med. 23: 34–45, 1957.

312. HENNES, A. R., WAJCHENBERG, B. L., FAJANS, S. S. AND CONN, J. W.: The effect of adrenal steroids on blood levels of pyruvic and alpha-ketoglutaric acids in normal subjects. Metabolism 6: 339–345, 1957.

313. HERR, M. E., HOGG, J. A. AND LEVIN, R. H.: Synthesis of potent oral anabolic androgenic steroids. J. Amer. chem. Soc. 78: 500–501, 1956.

314. HERSHBERGER, L. G.: Effect of thyroparathyroidectomy on the anti-inflammatory activity of cortisol in the cotton granuloma test. Anat. Rec. 128: 563–564, 1957.

315. HERSHBERGER, L. G.: Effects of cortisol and phenylbutazone on experimental arthritis and serum polysaccharide/protein ratio in rats. Proc. Soc. exp. Biol., N. Y. 99: 55–57, 1958.

316. HERSHBERGER, L. G. AND CALHOUN, D. W.: A cotton pellet granuloma assay for locally administered anti-inflammatory drugs: 9 halo,21 desoxy-corticoids. Endocrinology 60: 153–160, 1957.

317. HERSHBERGER, L. G. AND HANSEN, L. M.: Effect of hydrocortisone on action and activity of anaphylotoxin *in vitro*. Proc. Soc. exp. Biol., N. Y. 98: 150–153, 1958.

318. HERZ, J. E., FRIED, J., GRABOWICH, P. AND SABO, E. F.: 21-Fluoro derivative of 9α-fluoro- and 1-dehydro-corticoids. J. Amer. chem. Soc. 78: 4812–4814, 1956.

319. HERZ, J. E., FRIED, J. AND SABO, E. F.: 12α-Halo derivatives of 11β-hydroxy-progesterone. J. Amer. chem. Soc. 78: 2017–2018, 1956.

320. HERTZ, R., TULLNER, W. AND RAFFELT, E.: Progestational activity of orally administered 17α-ethinyl-19-nortestosterone. Endocrinology 54: 228–230, 1954.

321. HERZOG, H. L., GENTLES, M. J. AND HERSHBERG, E. B.: 11-Oxygenated steroids. XVII. 1,4-Pregnadien-21-al-17α-ol-3,11,20-trione hydrate. J. org. Chem. 21: 688–689, 1956.

322. HERZOG, H. L., GENTLES, M. J., MARSHALL, H. M. AND HERSHBERG, E. B.: Synthesis of 17α-halo analogs of corticoids. 17α-Chloro- and 17α-fluoro-4-pregnen-21-ol-3,11,20-trione acetates. J. Amer. chem. Soc. 82: 3691–3696, 1960.

323. HERZOG, H. L., NOBILE, A., TOLKSDORF, S., CHARNEY, W., HERSHBERG, E. B., PERLMAN, P. L. AND PECHET, M. M.: New antiarthritic steroids. Science 121: 176, 1955.

324. HEUSSER, H., ENGEL, C. R. AND PLATTNER, P. A.: Über Steroide und Sexualhormone. Synthese des 17-Methyl-Progesterons B. Helv. chim. acta 33: 2237–2242, 1950.

325. HEUSSER, H., WAHBA, N. AND WINTERNITZ, F.: Über Steroide und Sexualhormone. (201. Mitteilung) 17aα-Methyl-D-homo-Testosteron, ein hochactives männliches Sexualhormon. Helv. chim. acta 37: 1052–1059, 1954.

326. HEWITT, H. B.: Sensitive method for assay of androgens based on their power to alter the reactions of the mouse kidney to chloroform. J. Endocrin. 14: 394–399, 1957.

327. HILL, S. R., JR., GOETZ, F. C., FOX, H. M., MURAWSKI, B. J., KRAKAUER, L. J., REIFENSTEIN, R. W., GRAY, S. J., REDDY, W. J., HEDBERG, S. E., ST. MARC, J. R. AND THORN, G. W.: Studies on adrenocortical and psychological response to stress in man. Arch. intern. Med. 97: 269–298, 1956.

328. HILLS, A. G., CHALTIERS, T. M., WEBSTER, G. D., JR. AND ROSENTHAL, O.: Adrenal cortical regulation of the distribution of water and electrolytes in the human body. J. clin. Invest. 32: 1236–1247, 1953.

329. HIRSCHMANN, H., DE COURCY, C., LEVY, R. P. AND MILLER, K. L.: Adrenal precursors of urinary 17-ketosteroids. J. biol. Chem. 235: PC 48–49, 1960.

330. HIRSCHMANN, R. F., BAILEY, G. A., POOS, G. I., WALKER, R. AND CHEMERDA, J. M.: A contribution to the chemistry of steroid 17,21-oxides. J. Amer. chem. Soc. 78: 4814, 1956.

330a. HIRSCHMANN, H., HIRSCHMANN, F. B. AND FARRELL, G. L.: Partial synthesis of 16α,21-diacetoxy-progesterone. J. Amer. chem. Soc. 75: 4862–4863, 1953.

331. HIRSCHMANN, R. F., MILLER, R., BEYLER, R. E., SARETT, L. H. AND TISHLER, M.: A new biologically potent steroid: 1-dehydro-9α-fluorohydrocortisone acetate. J. Amer. chem. Soc. 77: 3166–3167, 1955.

332. HODGES, J. R. AND VERNIKOS, J.: A comparison of the pituitary inhibitory effects of prednisone, prednisolone and hydrocortisone. Brit. J. Pharmacol. 13: 98–102, 1958.

333 and 334. HOGG, J. A., LINCOLN, F. H., JACKSON, R. W. AND SCHNEIDER, W. P.: The adrenal hormones and related compounds. III. Synthesis of 2-alkyl analogs. J. Amer. chem. Soc. 77: 6401–6402, 1955.

335. HOGG, J. A., LINCOLN, F. H., NATHAN, A. H., HANZE, A. R., SCHNEIDER, W. P., BEAL, P. F. AND KORMAN, J.: The adrenal hormones and related compounds. II. Synthesis of 1-dehydro analogs. J. Amer. chem. Soc. 77: 4438–4439, 1955.

336. HOLDEN, M. AND ADAMS, L. B.: Inhibitory effects of cortisone acetate and hydrocortisone on growth of fibroblasts. Proc. Soc. exp. Biol., N. Y. 95: 364–368, 1957.

337. HOLDEN, R. B.: Vascular reactions of the uterus of the immature rat. Endocrinology 25: 593–596, 1939.

338. HOLLANDER, J. L.: The use of intra-articular hydrocortisone, its analogs, and its higher esters in arthritis. Ann. N. Y. Acad. Sci. 61: 511–516, 1955.

339. Hollander, J. L., Brown, E. M., Jessar, R. A. and Brown, C. Y.: Hydrocortisone and cortisone injected into arthritic joints: comparative effects of and use of hydrocortisone as a local anti-arthritic agent. J. Amer. med. Ass. 147: 1620–1635, 1951.
340. Hollander, J. L, Brown, E. M., Jr., Jessar, R. A., Udell, L., Smulker, N. and Bowie, M. A.: Local anti-rheumatic effectiveness of higher esters and analogues of hydrocortisone. Ann. Rheum. Dis. 13: 297–301, 1954.
341. Holten, C., Lundbaek, K. and Staffeldt, I.: Cortisone and prednisone on intravenous glucose tolerance. Acta med. scand. 157: 257–262, 1957.
342. Holzbauer, M.: The effect of oestrogens on the secretory capacity of rat adrenal cortex *in vivo*. J. Physiol. 139: 306–315, 1957.
343. Howard, J. E. and Migeon, C. J.: Cushing's syndrome produced by normal replacement doses of cortisone in a patient with defective mechanism for steroid degradation. Amer. J. med. Sci. 235: 387–393, 1958.
344. Howes, E. L., Plotz, C. M., Blunt, J. W. and Ragan, C.: Retardation of normal healing by cortisone. Surgery 28: 177–180, 1950.
345. Hubener, H. J., Fukushima, D. K. and Gallagher, T. F.: Substrate specificity of enzymes reducing the 11- and 20-keto groups of steroids. J. biol. Chem. 220: 499–511, 1956.
346. Huffman, E. R., Wilson, G. M., Clark, G. M. and Smyth, C. J.: Acute renal effects of 9α-fluoro-hydrocortisone in human subjects with intact adrenals. J. Lab. clin. Med. 47: 747–751, 1956.
347. Huggins, C. and Jensen, E. V.: The significance of hydroxyl groups of steroids in promoting growth. J. exp. Med. 100: 241–246, 1954.
348. Huggins, C. and Jensen, E. V.: The depression of estrone-induced uterine growth by phenolic estrogens with oxygenated junctions at positions 6 or 16: the impeded oestrogens. J. exp. Med. 102: 335–346, 1955.
349. Huggins, C. and Jensen, E. V.: The depression of growth of the uterus, adrenals, and ovaries by fluorinated steroids in the pregnane series. J. exp. Med. 102: 347–360, 1955.
350. Huggins, C., Jensen, E. V. and Cleveland, A. S.: Chemical structure of steroids in relation to promotion of growth of the vagina and uterus of the hypophysectomised rat. J. exp. Med. 100: 225–240, 1954.
351. Huggins, C. and Mainzer, K.: Hormonal influences on mammary tumours of the rat. II. Retardation of growth of a transplanted fibroadenoma in intact female rats by steroids in the androstane series. J. exp. Med. 105: 485–500, 1957.
352. Hurley, J. V., Storey, E. and Ham, K. N.: The effects of aminoacetonitrile and cortisone on the healing of turpentine-induced abscesses in the rat. Brit. J. exp. Path. 39: 119–127, 1958.
352a. Hurlock, B. and Talalay, P.: Microsomal 3α- and 3β-hydroxysteroid dehydrogenases. Arch. Biochem. Biophys. 80: 468–470, 1959.
353. Idler, D. R., Ronald, A. P. and Schmidt, P. J.: Biochemical studies on sockeye salmon during spawning migration. VII. Steroid hormones in plasma. Canad. J. Biochem. Physiol. 37: 1227–1238, 1959.
354. Ingle, D. J.: Remarks in discussion, p. 107, 3rd Conf. Adrenal Cortex. Josiah Macy Jr. Foundation, 1951.
355. Ingle, D. J.: Some further studies on the relationship of adrenal cortical hormones to experimental diabetes. Diabetes 1: 345–350, 1952.
356. Ingle, D. J.: The role of the adrenal cortex in homeostasis. J. Endocrinol. 8: xxiii–xxxvii, 1952.
356a. Ingle, D. J. and Ingle, D. J.: The relationship of the adrenal glands to diseases of adaptation. 1st Int. Congr. Endocrin. Symposia, pp. 89–92. Copenhagen, 1960.
357. Ingle, D. J., Meeks, R. C. and Beary, D. F.: Time-response effect of cortisone upon liver glycogen in the rat. Proc. Soc. exp. Biol., N. Y. 84: 239–240, 1953.
358. Ingle, D. J., Nezamis, J. E. and Meeks, R. C.: Effect of adrenal cortical extract upon work output and glucose tolerance of adrenalectomised-eviscerate rats. Proc. Soc. exp. Biol., N. Y. 83: 537–539, 1953.
359. Ingle, D. J., Nezamis, J. E. and Morley, E. H.: The comparative value of cortisone, 17-hydroxycorticosterone and adrenal cortex extract given by continuous intravenous injection in sustaining the ability of the adrenalectomised rat to work. Endocrinology 50: 1–4, 1952.
360. Ingle, D. J., Nezamis, J. E. and Morley, E. H.: Effect of adrenal cortical extract, cortisone and hydrocortisone upon the work of the adrenalectomised-eviscerate rat. Endocrinology 53: 582–584, 1953.
361. Ingle, D. J., Nilson, H. W. and Kendall, E. C.: The effect of cortin on the concentrations of some constituents of the blood of adrenalectomised rats. Amer. J. Physiol. 118: 302–308, 1937.
362. Ingle, D. J., Prestrud, M. C. and Nezamis, J. E.: Effects of administering large doses of cortisone acetate to normal rats. Amer. J. Physiol. 166: 171–175, 1951.
363. Ingle, D. J., Sheppard, J., Evans, J. S. and Kuizenga, M. M.: A comparison of adrenal steroid diabetes and pancreatic diabetes in the rat. Endocrinology 37: 341–356, 1945.
364. Inhoffen, H. H., Logemann, W., Hohlweg, W. and Serini, A.: Untersuchungen in der Sexualhormon-Reihe. Ber. dtsch. chem. Ges. 71: 1024–1032, 1938.
365. Iriarte, J., Djerassi, C. and Ringold, H. J.: Steroids. CVII. $\Delta^{8(9)}$-19-nor steroids, a new class of potent anabolic agents. J. Amer. chem. Soc. 81: 436–438, 1959.
366. Iriarte, J. and Ringold, H. J.: Steroids. XCIV. Synthesis of 2-methyl- and 1,2-dimethyl-oestrogens. Tetrahedron 3: 28–36, 1958.
367. Iriarte, J., Ringold, H. J. and Djerassi, C.: Steroids. XCIX. Synthesis of Ring B oxygenated estrogens. J. Amer. chem. Soc. 80: 6105–6110, 1958.
368. Isselbacher, K. J. and Axelrod, J.: Enzymatic formation of corticosteroid glucuronides. J. Amer. chem. Soc. 77: 1070–1071, 1955.
369. Jackson, D. and Robson, J. M.: Local antagonism of steroidal effects by methyl cholanthrene. J. Endocrin. 14: 348–354, 1957.

370. JACOBSEN, T. N. AND JENSEN, E. V.: Preparation of 3β-fluoro-steroids. Chem. & Ind. 172–173, 1957.
371. JAILER, J. W. AND CHRISTY, N. P.: Endocrinology. Annu. Rev. Med. 8: 193–238, 1957.
371a. JASMIN, G., BOIS, P. AND MONGEAU, A.: Effect of cortisol and Norethandrolone on inflammation and tumor growth. Experientia 16: 212–213, 1960.
372. JENNINGS, M. A. AND FLOREY, H. W.: Effect of cortisone on inflammation and mucin regeneration in colon. Quart. J. exp. Physiol. 39: 271–277, 1954.
372a. JENSEN, E. V. AND JACOBSON, H. I.: Fate of steroid estrogens in target tissues. In: Biological Activities of Steroids in Relation to Cancer, ed. by G. Pincus and E. P. Vollmer, pp. 161–174. Academic Press, New York, 1960.
373. JENSEN, P. K.: Corticosterone inhibition of pyridine nucleotide oxidase from heart sarcosomes. Nature, Lond. 184: 451, 1959.
374. JOHNS, W. F.: Synthesis of 18,19-dinor-steroids. J. Amer. chem. Soc. 80: 6456–6457, 1958.
375. JOHNSON, B. B.: Bioassay of adrenal cortical steroids on the basis of electrolyte excretion by rats: effects of 11-deoxy- and 11-oxysteroids. Endocrinology 54: 196–208, 1954.
376. JOHNSON, W. S., LEMAIRE, H. AND PAPPO, R.: 18-Nor-D-homoandrostane-3,17a-dione. J. Amer. chem. Soc. 75: 4866–4867, 1953.
376a. JOHNSON, W. S., BANNISTER, B., PAPPO, R. AND PIKE, J. E.: Steroid total synthesis—hydrochrysene approach. X. Total synthesis of testosterone. J. Amer. chem. Soc. 78: 6354–6361, 1956.
376b. JOHNSON, W. S., DAVID, I. A., DEHM, H. C., HIGHET, R. J., WARNHOFF, E. W., WOOD, W. D. and JONES, E. T.: Configuration of the estrones. Total synthesis of the remaining stereoisomers. J. Amer. chem. Soc. 80: 661–679, 1958.
377. JONES, E. S.: An *in vitro* action of DOC, on red cell electrolytes. Experientia 14: 72, 1958.
378. JOST, A. AND GRANDOU, P.: Action opposée sur la surrénale du Rat, de l'hydrocortisone et de la 9-α-fluoro-11-β-hydroxy-17-α-méthyl-testostérone. C. R. Soc. Biol., Paris 152: 896–898, 1958.
379. JULIAN, P. L., COCHRANE, C. C., MAGNANI, A. AND KARPEL, W.: Sterols. XVI. Cortisone and analogs. Part 2. 17α,21-Dihydroxy-4-pregnene-3,12,20-trione. J. Amer. chem. Soc. 78: 3153–3158, 1956.
380. JULIAN, P. L., COLE, W., MEYER, E. W. AND REGAN, B. M.: Sterols. XV. Cortisone and analogs. Part 1. 16α-Hydroxy and 16α,17α-epoxy analogs of cortisone. J. Amer. chem. Soc. 77: 4601–4604, 1955.
381. KAGAWA, C. M.: Blocking urinary electrolyte effects of desoxycorticosterone with progesterone in rats. Proc. Soc. exp. Biol., N. Y. 99: 705–707, 1958.
382. KAGAWA, C. M. AND VAN ARMAN, C. G.: Sodium retaining activity of 19-nor-steroids in rats. Proc. Soc. exp. Biol., N. Y. 94: 444–448, 1957.
383. KAGAWA, C. M. AND VAN ARMAN, C. G.: Effects of cortisone and hydrocortisone on sodium excretion in adrenalectomised rats. Proc. Soc. exp. Biol., N. Y. 94: 683–686, 1957.
384. KAGAWA, C. M., CELLA, J. A. AND VAN ARMAN, C. G.: Action of new steroids in blocking effects of aldosterone and deoxy-corticosterone on salt. Science 126: 1015–1016, 1957.
385. KAGAWA, C. M. AND JACOBS, R. S., JR.: Action of testosterone in blocking urinary electrolyte effects of desoxy-corticosterone. Proc. Soc. exp. Biol., N. Y. 102: 521–523, 1959.
386. KAGAWA, C. M., STURTEVANT, F. M. AND VAN ARMAN, C. G.: Pharmacology of a new steroid that blocks salt activity of aldosterone and desoxycorticosterone. J. Pharmacol. 126: 123–130, 1959.
387. KÄGI, H. R.: Der Einfluss von Muskelarbeit auf die Blutkonzentration der Nebennierenrindenhormone. Helv. med. acta 22: 258–267, 1955.
388. KAHNT, F. W., NEHER, R. AND WETTSTEIN, A.: Über Steroide. (129. Mitteilung) Bildung von 18- and 19-Oxy-Cortexon durch Einwirkung von Nebennieren-Homogenaten auf Cortexon. Helv. chim. acta 38: 1237–1248, 1955.
389. KALANT, O. J. AND SELLERS, E. A.: Sex hormones on succinoxidase activity of adrenal gland of rat. Endocrinology 61: 264–270, 1957.
390. KALLISTRATOS, G. AND VOIGT, K. D.: Influence of cortisone glycinate and testosterone glycinate on adrenalectomised and control golden hamsters. Endocrinology 64: 231–239, 1959.
391. KALMAN, S. M.: The influence of estrogens on the electrolytes of the rat uterus. J. Pharmacol. 121: 252–257, 1957.
392. KALMAN, S. M. AND LOWENSTEIN, J. M.: The effect of estrogens on the uptake of albumin and electrolytes by the rat uterus. J. Pharmacol. 122: 163–167, 1958.
393. KAPPAS, A., SOYBEL, W., GLICKMAN, P. AND FUKUSHIMA, D. K.: Fever-producing steroids of endogenous origin in man. Arch. intern. Med. 105: 701–708, 1960.
394. KARL, H. J.: Untersuchungen über den Plasmagehalt und die Ausscheidung von 1-Dehydro-17-Hydroxycorticosteroiden nach Belastung mit Prednisolon beim Menschen. Klin. Wschr. 37: 495–500, 1959.
395. KARNOFSKY, D. A., RIDGWAY, L. P. AND PATTERSON, P. A.: Growth-inhibiting effect of cortisone acetate on the chick embryo. Endocrinology 48: 596–616, 1951.
396. KARUSH, F.: Heterogeneity of the binding sites of serum albumin. J. Amer. chem. Soc. 72: 2705–2713, 1950.
397. KASS, E. M., HECHTER, O., MACCHI, I. A. AND MOU, T. W.: Changes in patterns of secretion of corticosteroids in rabbits after prolonged treatment with ACTH. Proc. Soc. exp. Biol., N. Y. 85: 583–587, 1954.
398. KATZMAN, P., DOISY, E. A., JR., MATSCHINER, J. T. AND DOISY, E. A.: Steroid metabolism. Annu. Rev. Biochem. 28: 257–290, 1959.
399. KELEMEN, E.: Local inhibition of testicular extract induced oedema of rat's hind paw. Acta physiol. Acad. Sci. hung. 11: 121–124, 1957.
400. KELLEY, V. C.: Rationale for hormone therapy in rheumatic fever. Ann. N. Y. Acad. Sci. 61: 369–376, 1955.
401. KERPPOLA, W.: Inhibition of phosphorylase with cortisone and its activation with adrenaline in the rabbit. Endocrinology 51: 192–202, 1952.
402. KERR, D. N. S., READ, A. E., HASLAM, R. M. AND SHERLOCK, S.: The use of a steroidal spirolactone in the treatment of ascites in hepatic cirrhosis. Lancet 2: 1084–1087, 1958.

403. Kessler, E., Nelson, S. S., Elder, N. R., Rosano, C. L. and Nelson, W. P., 3rd: Effect of single intravenous dose of hydrocortisone on erythrocyte electrolyte exchange. J. appl. Physiol. **10:** 93–97, 1957.
404. Keyes, G. H. and Kelley, V. C.: Glycogenic effect of adrenal cortical extract. Amer. J. Physiol. **158:** 351–357, 1949.
405. Kincl, F. A.: Steroide. CXLI. Steroid-Thiole-(3). Chem. Ber. **93:** 1043–1046, 1960.
406. Kincl, F. A. and García, M.: Steroide. CXIV. 16β-Methyl-Östran-Derivate. Chem. Ber. **92:** 595–600, 1959.
407. Kincl, F. A., Ringold, H. J. and Rosenkranz, G.: Steroids. LXXXVII. Preparation of some estrone ethers. J. org. Chem. **22:** 1127–1128, 1957.
408. Kirk, D. N., Patel, D. K. and Petrow, V.: Modified steroid hormones. Part III. Some 4-chloro-3-oxo-Δ^4-derivatives. J. chem. Soc. **1956:** 1184–1186.
409. Kirk, D. N., Patel, D. K. and Petrow, V.: Some steroidal 1:4-diene-3:11-diones and 1:4:6-triene-3:11-diones. J. chem. Soc. **1957:** 1046–1054.
410. Kirk, D. N. and Petrow, V.: Modified steroid hormones. Part XX. The dienone-phenol rearrangement of some 11-oxygenated $\Delta^{1,4,6}$-steroidal 3-ketones. J. chem. Soc. **1960:** 4664–4668.
411. Kissman, H. M., Small, A. M. and Weiss, M. J.: Fluorinated steroids. II. The reaction of perchloryl fluoride with alkoxalated steroids. Synthesis of certain 2α-fluoro and 21,21-difluoro steroids. J. Amer. chem. Soc. **82:** 2312–2317, 1960.
412. Kissman, H. M., Small, A. M. and Weiss, M. J.: Fluorinated steroids. I. The synthesis of 2α-fluorohydrocortisone. J. Amer. chem. Soc. **81:** 1262–1263, 1959.
413. Kistner, R. W.: The use of the new progestins in the treatment of endometriosis. Amer. J. Obstet. Gynec. **75:** 264–278, 1958.
414. Kleeman, C. R., Klopowitz, J. and Mazwell, M. H.: Acute and chronic effects of two new adrenal analogues: Δ^1-6-methyl hydrocortisone (Medrol) and Δ^1-6-methyl-9a fluoro-21-desoxyhydrocortisone. Clin. Res. **6:** 54–55, 1958.
415. Klotz, I. M.: The nature of some ion-protein complexes. Cold Spr. Harb. Symp. quant. Biol. **14:** 97–112, 1950.
416. Klotz, I. M., Walker, F. M. and Pivan, R. B.: The binding of organic ions by proteins. J. Amer. chem. Soc. **68:** 1486–1490, 1946.
416a. Klyne, W.: Some aspects of the stereochemistry of C-20. Ciba Foundation Colloquia on Endocrinology, ed. by G. E. W. Wolstenholme and M. P. Cameron, vol. 7, pp. 127–138. Churchill, London, **1953.**
417. Knowlton, A. I. and Loeb, E. N.: Depletion of carcass potassium in rats made hypertensive with deoxycorticosterone acetate (DOCA) and with cortisone. J. clin. Invest. **36:** 1295–1300, 1957.
418. Knowlton, A. I., Loeb, E. N. and Stoerk, H. C.: Effect of synthetic analogues of hydrocortisone on the blood pressure of adrenalectomized rats on sodium restriction. Endocrinology **60:** 768–777, 1957.
419. Knox, L. H., Zderic, J. A., Ruelas, J. P., Djerassi, C. and Ringold, H. J.: Steroids. CXXXIV. Derivatives of 17α-ethynyltestosterone and 17α-ethynyl-19-nortestosterone. J. Amer. chem. Soc. **82:** 1230–1235, 1960.
420. Knox, W. E.: Two mechanisms which increase *in vivo* the liver tryptophan peroxidase activity: specific enzyme adaptation and stimulation of the pituitary-adrenal system. Brit. J. exp. Path. **32:** 462–469, 1951.
421. Knox, W. E. and Auerbach, V. H.: The hormonal control of tryptophan peroxidase in the rat. J. biol. Chem. **214:** 307–313, 1955.
422. Knox, W. E., Auerbach, V. H. and Lin, E. C. C.: Enzymatic and metabolic adaptations in animals. Physiol. Rev. **36:** 164–254, 1956.
422a. Koch, W., Heim, E. and Eschweiler, J.: Der Einfluss der Geschlechtshormone auf die Muskulatur. Acta endocr., Copenhagen **16:** 369–376, 1954.
423. Kochakian, C. D.: Renotrophic-androgenic and somatrophic properties of further steroids. Amer. J. Physiol. **158:** 51–56, 1949.
424. Kochakian, C. D.: Renotrophic-androgenic properties of orally administered androgens. Proc. Soc. exp. Biol., N. Y. **80:** 386–388, 1952.
425. Koepf, G. F., Horn, H. W., Gemmill, C. L. and Thorn, G. W.: The effect of adrenal cortical hormones on the synthesis of carbohydrate in liver slices. Amer. J. Physiol. **135:** 175–186, 1941.
426. Kramár, J., Levine, V. E., Meyers, V. W. and Sass, R. N.: Capillary resistance and the corticoids. Amer. J. Physiol. **192:** 603–608, 1958.
427. Kramár, J., Meyers, W. V. and Wilhelmj, C. M., Jr.: Capillary stress response and species. Proc. Soc. exp. Biol., N. Y. **89:** 528–533, 1955.
428. Kramár, J., Simay-Kramár, M. and Carnazzo, A. J.: Reduction of capillary resistance and adrenal corticosteroids. Stress-induced changes in the sensitivity of capillaries in corticosteroids. J. appl. Physiol. **13:** 257–262, 1958.
429. Kramár, J., Simay-Kramár, M. and Levine, V. E.: Correlation between chemical constitution and capillary activity of adrenal cortical hormones. Proc. Soc. exp. Biol., N. Y. **92:** 282–285, 1956.
430. Kunz, H. A. and Sulser, E.: Beziehung zwischen Nebenniere und aktivem Kationentransport. Experientia **14:** 278–279, 1958.
431. Landau, R. L., Bergenstal, D. M., Lugibihl, K. and Kascht, M. E.: The metabolic effects of progesterone in man. J. clin. Endocrin. **15:** 1194–1215, 1955.
432. Layton, L. L.: Effect of cortisone upon chondroitin sulphate synthesis by animal tissues. Proc. Soc. exp. Biol., N. Y. **76:** 596–598, 1951.
433. Leanza, W. J., Conbere, J. P., Rogers, E. F. and Pfister, K., 3rd: Aldehydes derived from cortisone and hydrocortisone. J. Amer. chem. Soc. **76:** 1691–1694, 1954.
434. Leathem, J. H. and Wolf, R. C.: Life maintaining action of 9-alpha chlorohydrocortisone acetate in adrenalectomized rat. Proc. Soc. exp. Biol., N. Y. **86:** 724–725, 1954.

435. LEFCOE, N. M.: Effect of hydrocortisone hemisuccinate on tracheal smooth muscle of the guinea pig and cat. J. Allergy **27**: 352–358, 1956.
436. LENHARD, R. H. AND BERNSTEIN, S.: The synthesis of 9α-substituted-Δ^4-androstene-11β-ol-3,17-diones. J. Amer. chem. Soc. **77**: 6665–6666, 1955.
436a. LENHARD, R. H. AND BERNSTEIN, S.: The synthesis of 7-keto-desoxycorticosterone. J. Amer. chem. Soc. **78**: 989–992, 1956.
437. LEONARD, S. L.: The effect of hormones on phosphorylase activity in skeletal muscle. Endocrinology **60**: 619–624, 1957.
438. LEOPOLD, I. H., PURNELL, J. E., CANNON, E. J., STEINMETZ, C. G. AND MCDONALD, P. R.: Local and systemic cortisone in ocular disease. Amer. J. Ophth. **34**: 361–371, 1957.
439. LERMAN, L. H.: Viadril: a new steroid anaesthetic. Brit. med. J. **2**: 129–132, 1956.
440. LETTRE, H.: Some investigations on cell behaviour under various conditions: a review. Cancer Res. **12**: 847–860, 1952.
441. LIDDLE, G. W.: Sodium diuresis induced by steroidal antagonists of aldosterone. Science **126**: 1016–1018, 1957.
442. LIDDLE, G. W.: Aldosterone antagonists. Arch. intern. Med. **102**: 998–1004, 1958.
443. LIDDLE, G. W.: Effects of anti-inflammatory steroids on electrolyte metabolism. Ann. N. Y. Acad. Sci. **82**: 854–867, 1959.
444. LIDDLE, G. W., PECHET, M. M. AND BARTTER, F. C.: Enhancement of biological activities of corticosteroids by substitution of halogen atoms in 9α position. Science **120**: 496–497, 1954.
445. LIDDLE, G. W. AND RICHARD, J. E.: 2-Methylhydrocortisones: a new series of steroids with enhanced potency and prolonged action. Science **123**: 324–325, 1956.
446. LIDDLE, G. W., RICHARD, J. E. AND TOMKINS, G. M.: Studies of structure-function relationships of steroids: the 2-methyl corticosteroids. Metabolism **5**: 384–394, 1956.
447. LIEBERMAN, S., FUKUSHIMA, D. K. AND DOBRINER, K.: Studies in steroid metabolism. III. Identification and characterization of additional ketosteroids isolated from urine of healthy and diseased persons. J. biol. Chem. **182**: 299–316, 1950.
448. LIEBERMAN, S. AND TEICH, S.: Recent trends in the biochemistry of the steroid hormones. Pharmacol. Rev. **5**: 285–380, 1953.
449. LIN, E. C. C. AND KNOX, W. E.: Specificity of the adaptive response of tyrosine-α-ketoglutarate transaminase in the rat. J. biol. Chem. **233**: 1186–1189, 1958.
450. LINDNER, H. R.: Androgens in spermatic vein blood of farm animals. J. Endocrin. **20**: v-vi, 1960.
451. LITTELL, R. AND BERNSTEIN, S.: Steroidal cyclic ketals. XVIII. The preparation of 9α-hydroxyhydrocortisone. J. Amer. chem. Soc. **78**: 984–989, 1956.
452. LOEB, R. F., ATCHLEY, D. W., BENEDICT, E. M. AND LELAND, J.: Electrolyte balance studies in adrenalectomised dogs with particular reference to the excretion of sodium. J. exp. Med. **57**: 775–792, 1933.
453. LONG, C. N. H., KATZIN, B. AND FRY, E. G.: The adrenal cortex and carbohydrate metabolism. Endocrinology **26**: 309–344, 1940.
453a. LONG, C. N. H., SMITH, O. K. AND FRY, E. G.: Actions of cortisol on carbohydrate and protein metabolism. In: Ciba Foundation Study Group No. 6, ed. by G. E. W. Wolstenholme and M. O'Connor, pp. 4–24. Churchill, London, 1960.
454. LÖVGREN, O.: The effect of cortisone and ACTH treatment on certain carbohydrate metabolites. Acta endocr., Copenhagen **9**: 421–427, 1952.
455. LOWE, C. U. AND LEHNINGER, A. L.: Oxidation and phosphorylation in liver mitochondria lacking "polymerized" ribonucleic acid. J. biophys. biochem. Cytol. **1**: 89–92, 1955.
456. LOWE, C. U. AND WILLIAMS, W. L.: Effect of cortisone administration on intracellular composition of rat liver. Proc. Soc. exp. Biol., N. Y. **84**: 70–74, 1953.
457. LUETSCHER, J. A., JR. AND LIEBERMAN, A. H.: Aldosterone. Arch. intern. Med. **102**: 314–330, 1958.
458. LUFT, R., SJÖGREN, B., IKKOS, D., LJUNGGREN, H. AND TARUKOSKI, H.: Clinical studies on electrolyte and fluid metabolism. Recent Progr. Hormone Res. **10**: 425–470, 1954.
459. MCBEE, E. T., MARZLUFF, W. F. AND PIERCE, O. R.: The ionization constants of some fluorine-containing alcohols. J. Amer. chem. Soc. **74**: 444–446, 1952.
460. MCGUCKIN, W. F. AND MASON, H. L.: Adrenal hormone analogs: 16α, 17α-epoxy-11-dehydrocorticosterone acetate and Δ^{16}-11-dehydrocorticosterone acetate. J. Amer. chem. Soc. **77**: 1822–1824, 1955.
461. MCGUIRE, J. S. AND TOMKINS, G. M.: The multiplicity and specificity of Δ^4-3-ketosteroid dehydrogenases (5α). Arch. Biochem. Biophys. **82**: 476–477, 1959.
462. MCGUIRE, J. S. AND TOMKINS, G. M.: The effects of thyroxin administration on the enzymic reduction of Δ^4-3-ketosteroids. J. biol. Chem. **234**: 791–794, 1959.
463. MCPHAIL, M. K.: The assay of progestin. J. Physiol. **83**: 145–156, 1934.
464. MADJEREK, Z., DE VISSER, J., VANDEN VIES, J. AND OVERBEEK, G. A.: Allylestrenol, a pregnancy-maintaining oral gestagen. Dtsch. Ges. Endocrin. Preliminary communication, April 28, 1959.
464a. MAFFII, G., FONTANELLA, L., SCHIATTI, P. AND TESTA, E.: Herstellung und biologische Wirkung des 16α-Methyl-Desoxycorticosteron-Acetats. Experientia **16**: 422–423, 1960.
465. MAGERLEIN, B. J. AND HOGG, J. A.: Preparation and reactions of 11-substituted 1,3,5(10)-estratrienes. I. 11-Oxygenated estriones and estradiols. J. Amer. chem. Soc. **80**: 2220–2225, 1958.
466. MAGERLEIN, B. J. AND HOGG, J. A.: Preparation and reactions of 11-substituted 1,3,5(10)-estratrienes. II. Synthesis of 19-norhydrocortisone. J. Amer. chem. Soc. **80**: 2226–2229, 1958.
467. MAGERLEIN, B. J. AND HOGG, J. A.: 11-Oxygenated 17α-acetoxy-9α-fluoro-6α-methyl-1,4-pregnadiene-3,20-diones. J. org. Chem. **25**: 1675–1676, 1960.

468. MAGRATH, D., PETROW, V. AND ROYER, R.: The preparation of 17α-propargyltestosterone. J. chem. Soc. 1951: 845–846.
469. MAHESH, V. B. AND ULRICH, F.: Distribution of enzyme systems responsible for steroid metabolism in different tissues and subcellular fractions. Nature, Lond. 184: 1147–1148, 1959.
469a. MAHESH, V. B. AND ULRICH, F.: Metabolism of cortisol and cortisone by various tissues and subcellular particles. J. biol. Chem. 235: 356–360, 1960.
470. MANCERA, O. AND RINGOLD, H. J.: Steroids. CXV. The synthesis of halogenated steroid hormones, 4-chloro-19-nor hormone analogs. Canad. J. Chem. 37: 1785–1788, 1959.
471. MARCUS, P. I. AND TALALAY, P.: On the molecular specificity of steroid-enzyme combinations. The kinetics of β-hydroxysteroid dehydrogenase. Proc. Roy. Soc., ser. B 144: 116–132, 1955.
472. MARCUS, P. I. AND TALALAY, P.: Induction and purification of a β-hydroxysteroid dehydrogenase. J. biol. Chem. 218: 661–674, 1956.
473. MARSHALL, C. W., RALLS, J. W., SAUNDERS, F. J. AND RIEGEL, B.: 11-Oxygenated ethynyl-, vinyl-, and ethyltestosterones. J. biol. Chem. 228: 339–348, 1957.
474. MARSHALL, C. W., RAY, R. E., LAOS, I. AND RIEGEL, B.: 7-Keto steroids. I. Steroidal 3-hydroxy-3,5-dien-7-ones. J. Amer. chem. Soc. 79: 6303, 1957.
475. MARSHALL, C. W., RAY, R. E., LAOS, I. AND RIEGEL, B.: 7-Keto steroids. II. Steroidal 3-beta-hydroxy-Δ^5-7-ones and $\Delta^{3,5}$-7-ones. J. Amer. chem. Soc. 79: 6308, 1957.
476. MARSHALL, D. J. AND GAUDRY, R.: 17α-Halogenated progesterones: orally-active progestins. Canad. J. Chem. 38: 1495–1504, 1960.
477. MASON, J. W.: Visceral functions of the nervous system. Annu. Rev. Physiol. 21: 353–380, 1959.
478. MASON, J. W.: Psychological influences on the pituitary-adrenal cortical system. Recent Progr. Hormone Res. 15: 345–378, 1959.
479. MASSON, G. M. C., KORITZ, S. B. AND PERON, F. G.: Corticosteroid formation in regenerating rat adrenals. Endocrinology 62: 229–233, 1958.
480. MATTOX, V. R., MASON, H. L., ALBERT, A. AND CODE, C. F.: Properties of a sodium-retaining principle from beef adrenal extract. J. Amer. chem. Soc. 75: 4869–4870, 1953.
481. MAULI, R., RINGOLD, H. J. AND DJERASSI, C.: Steroids. CXLV. 2-Methylandrostane derivatives. Demonstration of boat form in the bromination of 2α-methyl-androstan-17β-ol-3-one. J. Amer. chem. Soc. 82: 5494–5500, 1960.
482. Joint Committee of the Medical Research Council and Nuffield Foundation on clinical trials of cortisone, ACTH, and other therapeutic measures in chronic rheumatic diseases. A comparison of prednisolone with aspirin or other analgesics in the treatment of rheumatoid arthritis. Ann. Rheum. Dis. 18: 173–187, 1959.
483. MEHLER, A. R., MCDANIEL, E. G. AND HUNDLEY, J. M.: Changes in the enzymatic compositions of liver. II. Influence of hormones on picolinic carboxylase and tryptophan peroxidase. J. biol. Chem. 232: 331–335, 1958.
484. MELBY, J. C., EGDAHL, R. H., STORY, J. L. AND SPINK, W. W.: Production and catabolism of cortisol following the administration of thyroxine analogs. Endocrinology 67: 389–393, 1960.
485. MENDELSOHN, M. L. AND PEARSON, O. F.: Alterations in water and salt metabolism after bilateral adrenalectomy in man. J. clin. Endocrin. 15: 409–423, 1955.
486. MENKIN, V.: Direct effect of corticotropin (ACTH) on capillary permeability in inflammation. Amer. J. Physiol. 189: 98–104, 1957.
487. MEYSTRE, C., VISCHER, E. AND WETTSTEIN, A.: Mikrobiologische Hydroxylierungen von Cortexon in der 7α-, 15α- oder 15β-Stellung. Helv. chim. acta 38: 381–393, 1955.
488. MIESCHER, K. AND KLARER, W.: Über Steroide. (22. Mitteilung) 17-Epimere Methyl-Androstendiole und Methyltestosterone. Helv. chim. acta 22: 962–969, 1939.
489. MIESCHER, K., HUNZIKER, F. AND WETTSTEIN, A.: Homologe der Keimdrüsenhormone. II. 20-Nor-Progesteron. Helv. chim. acta 23: 400–404, 1940.
490. MIGEON, C. J., BERTRAND, J. AND WALL, P. E.: Physiological disposition of [4-^{14}C] cortisol during late pregnancy. J. clin. Invest. 36: 1350–1362, 1957.
491. MIGEON, C. J., FRENCH, A. B., SAMUELS, L. T. AND BOWERS, J. Z.: Plasma 17-hydroxycorticosteroid levels and leucocyte values in the rhesus monkey, including normal variation and the effect of ACTH. Amer. J. Physiol. 182: 462–468, 1955.
492. MILLER, A. M.: Liver glycogen response to adrenal cortical extract of diabetic and non-diabetic rats. Proc. Soc. exp. Biol., N. Y. 72: 635–636, 1949.
493. MILLER, L. L. AND AXELROD, L. R.: Cortisone metabolism in the perfused normal and experimental cirrhotic rat liver. Metabolism 3: 438–448, 1954.
494. MILLS, J. S., BARRERA, J., OLIVARES, E. AND GARCIA, E.: Steroids. CL. 10β-Halo steroids. J. Amer. chem. Soc. 82: 5882–5889, 1960.
495. MILLS, J. S., BOWERS, A., CAMPILLO, C. C., DJERASSI, C. AND RINGOLD, H. J.: Steroids. CXVII. 6α-Fluoro-16α-hydroxy cortical hormones. J. Amer. chem. Soc. 81: 1264–1265, 1959.
496. MILLS, J. S., BOWERS, A., DJERASSI, C. AND RINGOLD, H. J.: Steroids. CXXXVII. Synthesis of a new class of potent cortical hormones. 6α,9α-Difluoro-16α-hydroxyprednisolone and its acetonide. J. Amer. chem. Soc. 82: 3399–3404, 1960.
497. MILLS, J. S., CANDIANI, O. AND DJERASSI, C.: Steroids. CXLIV. Synthesis of some 6α,17α-dihaloprogesterones. J. org. Chem. 25: 1056–1058, 1960.
498. MILLS, J. N. AND THOMAS, S.: The influence of adrenal corticoids on phosphate and glucose exchange in muscle and liver in man. J. Physiol. 148: 227–239, 1959.

499. MILLS, J. N., THOMAS, S. AND WILLIAMSON, K. S.: The acute effect of hydrocortisone, deoxycorticosterone and aldosterone upon the excretion of sodium, potassium and acid by the human kidney. J. Physiol. 151: 312-331, 1960.
500. MIRAMONTES, L., ROSENKRANZ, G. AND DJERASSI, C.: Steroids. XXII. The synthesis of 19-norprogesterone. J. Amer. chem. Soc. 73: 3540-3541, 1951.
501. MOKRASCH, L. C., DAVIDSON, W. D. AND MCGILVERY, R. W.: The response to glucogenic stress of fructose-1,6-diphosphatase in rabbit liver. J. biol. Chem. 222: 179-184, 1956.
501a. MORRIS, C. J. O. R. AND WILLIAMS, D. C.: The polarographic estimation of steroid hormones. 6. Determination of individual adrenocortical hormones in human peripheral blood. Biochem. J. 54: 470-475, 1953.
502. MORRIS-OWEN, R. M. AND TRUELOVE, S. C.: Treatment of hay-fever with local hydrocortisone hemisuccinate sodium. Brit. med. J. 1: 969-972, 1958.
503. MUELLER, G. P., JOHNS, W. F., COOK, D. L. AND EDGREN, R. A.: 16α-Chloro- and 16α-iodoestrone methyl ether, new and potent lipid-shifting agents. J. Amer. chem. Soc. 80: 1769-1770, 1958.
503a. MUELLER, G. C.: Biological parameters of estrogen action. In: Biological Activities of Steroids in Relation to Cancer, ed. by G. Pincus and E. P. Vollmer, pp. 129-142. Academic Press, New York, 1960.
504. MUNCK, A.: The interfacial activity of steroid hormones and synthetic oestrogens. Biochim. biophys. Acta 24: 507-514, 1957.
505. MUNCK, A., SCOTT, J. F. AND ENGEL, L. L.: The interaction of steroid hormones and coenzyme components. Biochim. biophys. Acta 26: 397-407, 1957.
506. MUNRO, D. S.: Effect of cortisone on sodium metabolism in Simmonds' disease. J. clin. Endocrin. 16: 1089-1095, 1956.
507. MUNSON, P.: Quoted in: Steroids, by L. F. Fieser and M. Fieser, p. 521. Reinhold, New York, 1959.
508. MYERS, T. C., PRATT, R. J., MORGAN, R. L., O'DONNELL, J. AND JENSEN, E. V.: Hormone analogs. I. Preparation of intermediate ketones for the synthesis of stilbestrol analogs. J. Amer. chem. Soc. 77: 5655-5657, 1955.
508a. MORGAN, R. L., TANNHAUSER, P., PRATT, R. J., MYERS, T. C. AND JENSEN, E. V.: II. The preparation of stilbestrol analogs. J. Amer. chem. Soc. 77: 5658-5660, 1955.
508b. PRATT, R. J. AND JENSEN, E. V.: III. The preparation of hexoestrol analogs. J. Amer. chem. Soc. 78: 4430-4433, 1956.
509. NABARRO, J. D. N., STEWART, J. S. AND WALKER, G.: Clinical and metabolic effects of prednisone. Lancet (Nov. 12), 993-998, 1955.
510. NABARRO, J. D. N. AND WALKER, G.: Treatment of adrenal cortical insufficiency. Brit. med. J. 2: 17-19, 1957.
511. NADEL, E. M., YOUNG, B. G. AND HILGAR, A. G.: Strain differences in urinary hydroxycorticosteroids in guinea pigs. Amer. J. Physiol. 196: 273-275, 1959.
512. NAHAS, G. G.: Effect of hydrocortisone on acidotic failure of the isolated heart. Circulation Res. 5: 489-492, 1957.
513. NAKAO, T., HIRAGA, K., SAITO, T. AND MURAYAMA, Y.: Pharmacological activities of "Pregnane-3β,5α,6β,-16β,3,20α,-pentaol" (POL) that blocks electrolyte activity of aldosterone. Kekai med. J. 6: 1-6, 1957.
514. NATHAN, A. H., BABCOCK, J. C. AND HOGG, J. A.: The adrenal hormones and related compounds. VI. Fluorinated derivatives of testosterone and progesterone. J. Amer. chem. Soc. 82: 1436-1437, 1960.
515. NEHER, R.: Blood electrolytes under the influence of cortical hormones. Clin. Chem. 3: 344-370, 1957.
516. NEHER, R. AND WETTSTEIN, A.: Isolierung und Konstitutionsermittlung weiterer Pregnanverbindungen aus Nebennieren. Helv. chim. acta 39: 2062-2088, 1956.
517. NELSON, D. H. AND AUGUST, T. H.: Abnormal response of oedematous patients to aldosterone or deoxycortone. Lancet 2: 883-885, 1959.
518. NELSON, N. A. AND GARLAND, R. B.: Steroidal hormone analogs. I. Synthesis of 18,19-dinorprogesterone and 14-hydroxy-18,19-dinorprogesterone. J. Amer. chem. Soc. 79: 6313-6320, 1957.
519. NEUMAN, F., MANCERA, O., ROSENKRANZ, G. AND SONDHEIMER, F.: Steroids. LXXII. 16-Methylene-testosterone. J. Amer. chem. Soc. 77: 5676-5678, 1955.
520. NICHOLS, G. AND NICHOLS, N.: The role of bone in sodium metabolism. Metabolism 5: 438-446, 1950.
521. NICHOLSON, T. F.: A comparison of the effects of proximal and distal tubular damage on the action of desoxycorticosterone and aldosterone. Canad. J. Biochem. Physiol. 35: 641-644, 1957.
522. NICOL, T. AND SNELL, R. S.: Effect of deoxycorticosterone on the reticulo-endothelial system. Nature, Lond. 175: 995-996, 1955.
523. NIEDERL, J. B. AND VOGEL, H. J.: Estracatechol. J. Amer. chem. Soc. 71: 2566-2568, 1949.
524. NOALL, M. W., RIGGS, T. R., WALKER, L. M. AND CHRISTENSEN, H. N.: Endocrine control of amino acid transfer. Distribution of an unmetabolizable amino acid. Science 126: 1002-1005, 1957.
525. NOBILE, A., CHARNEY, W., PERLMAN, P. L., HERZOG, H. L., PAYNE, C. C., TULLEY, M. E., JEVNIK, M. A. AND HERSHBERG, E. B.: Microbiological transformation of steroids. 1.$\Delta^{1,4}$-Diene-3-ketosteroids. J. Amer. chem. Soc. 77: 4184, 1955.
525a. NOVELLO, F. C. AND CHRISTY, M. E.: Preparation of 3-(6-methoxynaphthyl-2)-2-cyclohexen-1-one and related compounds. J. Amer. chem. Soc. 75: 5431-5432, 1953.
526. NYDEN, S. J. AND WILLIAMS-ASHMAN, H. G.: Influence of androgens on synthetic reactions in ventral prostate tissue. Amer. J. Physiol. 172: 588-600, 1953.
527. OERTEL, G. W. AND EIK-NES, K. B.: Isolation and identification of testosterone in systemic blood from normal human male adults. Proc. Soc. exp. Biol., N. Y. 102: 553-556, 1959.
528. OLIVETO, E. P.: Synthesis of 16α- and 16β-methyl corticoids. Ann. N. Y. Acad. Sci. 82: 809-820, 1959.
529. OLIVETO, E. P., RAUSSER, R., GEROLD, C., HERSHBERG, E. B., EISLER, M., NERI, R. AND PERLMAN, P. L.:

Attempts to prepare new progestational agents: synthesis and biological activity of 11β-acyloxyprogesterones. J. org. Chem. **23**: 121–123, 1958.

530. Oliveto, E. P., Rausser, R., Nussbaum, A. L., Gebert, W., Hershberg, E. B., Tolksdorf, S., Eisler, M., Perlman, P. L. and Pechet, M. M.: 16-Alkylated corticoids. I. 16α-Methyl-prednisone and 16β-methyl-prednisolone. J. Amer. chem. Soc. **80**: 4428, 1958.
531. Oliveto, E. P., Rausser, R., Weber, L., Nussbaum, A. L., Gebert, W., Coniglio, C. T., Hershberg, E. B., Tolksdorf, S., Eisler, M., Perlman, P. L. and Pechet, M. M.: 16-Alkylated corticoids. II. 9α-Fluoro-16α-methyl prednisolone 21-acetate. J. Amer. chem. Soc. **80**: 4431, 1958.
532. Overbeek, G. A. and de Visser, J.: A new substance with progestational activity. II. Pharmacological properties. Acta endocr., Copenhagen **22**: 318–329, 1956.
533. Overell, B. G., Condon, S. E. and Petrow, V.: The effect of hormones and their analogues upon the uptake of glucose by mouse skin *in vitro*. J. Pharm., Lond. **12**: 150–153, 1959.
534. Oyakawa, E. K. and Levedahl, B. H.: Testosterone binding to bovine and human serum albumin: the role of tyrosine groups. Arch. Biochem. Biophys. **74**: 17–23, 1958.
534a. Pabst, M. L., Sheppard, R. and Kuizenga, M. H.: Comparison of liver glycogen deposition and work performance tests for the bioassay of adrenal cortex hormones. Endocrinology **41**: 55–65, 1947.
535. Patrick, R. S.: The influence of cortisone and ACTH on experimental zonal necrosis of the liver. J. Path. Bact. **70**: 377–385, 1955.
536. Pearlman, W. H.: [16-^{3}H] Progesterone metabolism in advanced pregnancy and in oophorectomized-hysterectomized women. Biochem. J. **67**: 1–5, 1957.
537. Pechet, M. M., Hesse, R. H. and Kohler, H.: The metabolism of aldosterone: isolation and characterization of two new metabolites. J. Amer. chem. Soc. **82**: 5251–5263, 1960.
538. Perelman, M., Farkas, E., Fornefeld, E. J., Kraay, R. J. and Rapala, R. T.: A new class of active steroids: the 19-nor-$\Delta^{4,9}$-3-ketosteroids. J. Amer. chem. Soc. **82**: 2402–2403, 1960.
539. Perlman, D., Dutcher, J. D., Fried, J. and Titus, E. O.: 9α-Hydroxyprogesterone. U. S. Pat. 2,840,578. June 24, 1958.
540. Pesch, L. A. and Clark, J. H.: Organ specific effects of cortisone on protein synthesis in protein depleted rats. Proc. Soc. exp. Biol., N. Y. **91**: 510–512, 1956.
541. Peterson, R. E.: The influence of the thyroid on adrenal cortical function. J. clin. Invest. **37**: 736–743, 1958.
542. Peterson, R. E.: Metabolism of adrenocorticosteroids in man. Ann. N. Y. Acad. Sci. **82**: 846–853, 1959.
543. Peterson, R. E., Pierce, C. E., Wyngaarden, J. B., Bunim, J. J. and Brodie, B. B.: The physiological disposition and metabolic fate of cortisone in man. J. clin. Invest. **36**: 1301–1312, 1957.
544. Peterson, R. E. and Wyngaarden, J. B.: The miscible pool and turnover rate of hydrocortisone. J. clin. Invest. **34**: 957, 1955.
545. Peterson, R. E., Wyngaarden, J. B., Guerra, S. L., Brodie, B. B. and Bunim, J. J.: The physiological disposition and metabolic fate of hydrocortisone in man. J. clin. Invest. **34**: 1779–1794, 1955.
546. Petrow, V. and Williamson, D. M.: Modified steroid hormones. XI. 16α-Methyldeoxycorticosterone acetate. J. chem. Soc. **1959**: 3595–3597.
547. Phillips, J. G.: Adrenocorticosteroids in fish. J. Endocrin. **18**: xxxvii–xxxix, 1959.
548. Phillips, J. G., Holmes, W. N. and Bondy, P. K.: Adrenocorticosteroids in salmon plasma (*Oncorhynchus nerka*). Endocrinology **65**: 811–818, 1959.
549. Phillips, J. G. and Mulrow, P. J.: Corticosteroid production *in vitro* by the interrenal tissue of Killifish, *Fundulus heteroclitus* (Linn). Proc. Soc. exp. Biol., N. Y. **101**: 262–264, 1959.
550. Pincus, G.: Some basic hormone problems. J. clin. Endocrin. **12**: 1187–1196, 1952.
551. Pincus, G., Chang, M. C., Hafez, E. S. E., Zarrow, M. X. and Merrill, A.: Effects of certain 19-nor steroids on reproductive processes in animals. Science **124**: 890–891, 1956.
552 and 553. Pincus, G., Chang, M. C., Zarrow, M. X., Hafez, E. S. E. and Merrill, A.: Studies of the biological activity of certain 19-nor steroids in female animals. Endocrinology **59**: 695–707, 1956.
554. Pincus, G. and Hopkins, T. F.: The effects of various estrogens and steroid substances on sex differentiation in the fowl. Endocrinology **62**: 112–118, 1958.
555. Plattner, P. A., Heusser, H. and Herzig, P. T.: Die Synthese von 17-Methyl-Progesteron. Helv. chim. acta **32**: 270–275, 1949.
556. Plattner, P. A., Heusser, H. and Segre, A.: Über Steroide und Sexualhormone. 148. Mitt. Synthese des 14-Allo-17-iso-Progesterons. Helv. chim. acta **31**: 249–256, 1948.
557. Plattner, P. A. and Schreck, W.: Herstellung eines Homolgen des Progesterons. Helv. chim. acta **24**: 472–477, 1941.
558. Poos, G. I., Lukes, R. M., Arth, G. E. and Sarett, L. H.: Approaches to the total synthesis of adrenal steroids. XI. Dehydrocorticosterone and cortisone. J. Amer. chem. Soc. **76**: 5031–5034, 1954.
559. Porter, C. C. and Silber, R. H.: The absorption and glycogenic activity of cortisone and hydrocortisone after parenteral administration to rats. Endocrinology **53**: 73–78, 1953.
560. Prelog, V., Ruzicka, L. and Wieland, P.: Steroide und Sexualhormone (111. Mitteilung). Über ein neues Stereoisomeres des Oestriols. Helv. chim. acta **28**: 250–256, 1945.
560a. Prelog, V.: The steric course of some microbiological and enzymic reductions of ketones, Ciba Foundation Study Group No. 2, ed. by G. E. W. Wolstenholme and C. M. O'Connor, pp. 79–90. Churchill, London, 1959.
561. Preston, T. R. and Gee, I.: The influence of the route of administration on the effect of hexoestrol on fattening wether lambs. Brit. J. Nutr. **12**: 158–163, 1958.
561a. Ramey, E., Goldstein, M. S. and Levine, R.: The mechanism of muscular fatigue in adrenalectomised animals. Amer. J. Physiol. **162**: 10–16, 1950.

562. RANNEY, R. E.: Effect of Δ^4-cholesterone on cholesterol and phospholipid metabolism in the chick. Proc. Soc. exp. Biol., N. Y. **99:** 462–466, 1958.
563. RANNEY, R. E. AND DRILL, V. A.: The ability of 17-ethyl-19-nortestosterone to block ethionine-induced fatty liver in rats. Endocrinology **61:** 476–477, 1957.
564. RANNEY, R. E. AND WEISS, S. E.: The effect of natural oestrogens and a synthetic steroid on the phospholipid metabolism of the chick. Endocrinology **62:** 828–832, 1958.
565. REICH, H. AND REICHSTEIN, T.: Über Bestandteile der Nebennierenrinde und verwandte Stoffe (27. Mitteilung). Δ^4-3-Keto-Androstenyl-Glyoxal-17 und verwandte Stoffe. Helv. chim. acta **22:** 1124–1138, 1939.
566. REIMANN, H., OLIVETO, E. P., NERI, R., EISLER, M. AND PERLMAN, P.: Halogenated progesterones. I. 9α,11β-Dihaloprogesterones. J. Amer. chem. Soc. **82:** 2308–2311, 1960.
567. REISS, E. AND KIPNIS, D.: The mechanism of action of growth hormone and hydrocortisone on protein synthesis in striated muscles. J. Lab. clin. Med. **54:** 937–938, 1959.
568. RENOLD, A. E., ASHMORE, J. AND HASTINGS, A. B.: Regulation of carbohydrate metabolism in isolated tissues. Vitam. & Horm. **14:** 139–185, 1956.
569. RENOLD, A. E. AND HASTINGS, A. B.: Modifications du métabolisme des hydrates de carbone dans le foie d'animaux diabétiques et dans le foie d'animaux traités à la cortisone. Acta endocr., Copenhagen **14:** 47–51, 1953.
570. RENOLD, A. E., HAYDAR, N. A., REDDY, W. J., GOLDFIEN, A., ST. MARC, J. R. AND LAIDLAW, J. C.: Biological effects of fluorinated derivatives of hydrocortisone and progesterone in man. Ann. N. Y. Acad. Sci. **61:** 582–590, 1955.
571. RICHTER, R.: The effect of 17α-ethyl-19-*nor*testosterone on the estrus cycle. Experientia **14:** 219, 1958.
572. RINGLER, I., BORTLE, L., HEYDER, E., MONTEFORTE, A., PERRINE, J. AND ROSS, E.: Adrenal corticoid activities of Δ^1,9α-fluoro-hydrocortisone 16α,17α isopropylidenedioxy. Proc. Soc. exp. Biol., N. Y. **102:** 628–631, 1959.
573. RINGOLD, H. J.: In Discussion of MCGINTY, D. A. AND DJERASSI, C.: Some chemical and biological properties of 19-nor-17α-ethynyltestosterone. Ann. N. Y. Acad. Sci. **71:** 500–515, 1958.
574. RINGOLD, H. J.: In Discussion of DRILL, V. A. AND RIEGEL, B.: Structural and hormonal activity of some new steroids. Recent Progr. Hormone Res. **14:** 29–76, 1958.
574a. RINGOLD, H. J.: β-Homo-androstane derivatives. J. Amer. chem. Soc. **82:** 961–963, 1960.
574b. RINGOLD, H. J.: Modification of hormonal activity by stereochemical and electronic alteration of the steroid molecule. In: Mechanism of Action of Steroid Hormones, ed. by C. A. Villee and L. L. Engel, pp. 200–232. Pergamon Press, London, 1961.
575. RINGOLD, H. J., BATRES, E., BOWERS, A., EDWARDS, J. AND ZDERIC, J.: Steroids. CXXVII. 6-Halo progestational agents. J. Amer. chem. Soc. **81:** 3485–3486, 1959.
576. RINGOLD, H. J., BATRES, E., MALPERN, O. AND NECOECHEA, E.: Steroids. CV. 2-Methyl and 2-hydroxy-methylene-androstane derivatives. J. Amer. chem. Soc. **81:** 427–432, 1959.
577. RINGOLD, H. J., BATRES, E. AND ROSENKRANZ, G.: Steroids. LXXXIV. Synthesis of 6-methyl hormone analogs. J. org. Chem. **22:** 99–100, 1957.
578. RINGOLD, H. J., MANCERA, O., DJERASSI, C,. BOWERS, A., BATRES, E., MARTINEZ, H., NECOECHEA, E., EDWARDS, J., VELASCO, M., CAMPILLO, C. C. AND DORFMAN, R. I.: A new class of potent cortical hormones. 6α-Chloro corticoids. J. Amer. chem. Soc. **80:** 6464, 1958.
579. RINGOLD, H. J. AND ROSENKRANZ, G.: Steroids. LXXXIII. Synthesis of 2-methyl and 2,2-dimethyl hormone analogs. J. org. Chem. **21:** 1333–1334, 1956.
580. RINGOLD, H. J. AND ROSENKRANZ, G.: Steroids. LXXXV. Synthesis of 4-methyl and 4,4-dimethyl hormones. J. org. Chem. **22:** 602–605, 1957.
581. RINGOLD, H. J., ROSENKRANZ, G. AND SONDHEIMER, F.: Steroids. LXXX. 1-Methyl-19-nortestosterone and 1-methyl-17α-ethinyl-19-nortestosterone. J. Amer. chem. Soc. **78:** 2477–2479, 1956.
582. RINGOLD, H. J., RUELAS, J. P., BATRES, E. AND DJERASSI, C.: Steroids. CXVIII. 6-Methyl derivatives of 17α-hydroxyprogesterone and of Reichstein's Substance "S." J. Amer. chem. Soc. **81:** 3712–3716, 1959.
583. ROBERT, A. AND NEZAMIS, J. E.: The granuloma pouch as a routine assay for antiphlogistic compounds. Acta endocr., Copenhagen **25:** 105–112, 1957.
584. ROBERTS, K. B., FLOREY, H. W. AND JOKLIK, W. K.: The influence of cortisone on cell division. Quart. J. exp. Physiol. **37:** 239–257, 1952.
585. ROBERTS, K. E. AND PITTS, R. F.: The influence of cortisone on renal function and electrolyte excretion in the adrenalectomised dog. Endocrinology **50:** 51–60, 1952.
586. ROBERTS, S.: The influence of the adrenal cortex on the mobilization of tissue protein. J. biol. Chem. **200:** 77–88, 1953.
587. ROBERTS, S. AND SZEGO, C. M.: Steroid interaction in the metabolism of reproductive target organs. Physiol. Rev. **33:** 593–629, 1953.
588. ROBERTS, S. AND SZEGO, C. M.: Biochemistry of the steroid hormones. Annu. Rev. Biochem. **24:** 543–596, 1955.
589. ROBERTSON, O. M.: Adrenal hypercorticalism in spawning Pacific salmon. Science **128:** 1148–1149, 1958.
590. ROBINSON, C. H., BRUCE, N. F., OLIVETO, E. P., TOLKSDORF, S., STEINBERG, M. AND PERLMAN, P. L.: 16,16-Difluoroestrone derivatives. A new series of cholesterol lowering agents. J. Amer. chem. Soc. **82:** 5256–5257, 1960.
591. ROBINSON, C. H., FINCKENOR, L., OLIVETO, E. P. AND GOULD, D. H.: A new class of potent anti-inflammatory agents; synthesis of 9α,11β-dihalocorticosteroids. J. Amer. chem. Soc. **81:** 2191–2195, 1959.
592. ROBINSON, C. H., FINCKENOR, L. E., TIBERI, R., EISLER, M., NERI, R., WATNICK, A., PERLMAN, P. L., HOLROYD, P., CHARNEY, W. AND OLIVETO, E. P.: New anabolic agents: 9α,11β-dihalogenoandrostane derivatives. J. Amer. chem. Soc. **82:** 4611–4615, 1960.

593. Rodrigues, R. R., Marques, M., Conceiçao, J. M. and Letti, N.: Action diabétogène des corticoïdes synthétiques. C. R. Soc. Biol., Paris 150: 1281–1282, 1956.
594. Roemmelt, J. C., Sartorius, O. W. and Pitts, R. F.: Excretion and reabsorption of sodium and water in the adrenalectomized dog. Amer. J. Physiol. 159: 124–136, 1949.
595. Rogers, E. F., Leanza, W. J., Conbere, J. P. and Pfister, K., III: Aldehydes derived from cortisone and hydrocortisone. J. Amer. chem. Soc. 74: 2947, 1952.
596. Rolf, D., Armstrong, C. M., Steiger, B. W., Audia, M. and White, H. C.: Comparison of external loss of sodium with loss from sucrose space in adrenalectomised rats. Amer. J. Physiol. 196: 1109–1111, 1959.
597. Rose, H. G., Robertson, M. C. and Schwartz, T. B.: Hormonal and metabolic influences on intracellular peptidase activity. Amer. J. Physiol. 197: 1063–1069, 1959.
598. Rosen, F., Roberts, N. R., Budnick, L. E. and Nichol, C. A.: An enzymatic basis for the gluconeogenic action of hydrocortisone. Science 127: 287–288, 1958.
599. Rosen, F., Roberts, N. R., Budnick, L. E. and Nichol, C. A.: Corticosteroids and transaminase activity: the specificity of the glutamic-pyruvic transaminase response. Endocrinology 65: 256–264, 1959.
600. Rosen, F., Roberts, N. R. and Nichol, C. A.: Glucocorticosteroids and transaminase activity. J. biol. Chem. 234: 476–480, 1959.
601. Rosenkranz, G., Pataki, J., Kaufmann, S., Berlin, J. and Djerassi, C.: Steroids. V. The synthesis of adrenal corticosteroids and analogs from allopregnan-3β-ol-20-one. J. Amer. chem. Soc. 72: 4081–4085, 1950.
602. Ross, E. J.: The "permeability" hypothesis of the action of insulin. Medicine 35: 355–388, 1956.
603. Rubin, B. and Salter, W. T.: The effects of progesterone and some related steroids on the contraction of isolated myocardium. J. Pharmacol. 105: 443–449, 1952.
604. Ruelas, J. P., Iriarte, J., Kincl, F. and Djerassi, C.: Steroids. XCVIII. Synthesis of some 10β-hydroxy-19-norsteroids. J. org. Chem. 23: 1744–1747, 1958.
605. Ruzicka, L. and Kägi, H.: Sexualhormone. XXVII. Über 17-cis- und 17-trans-isomere Diole und Oxyketone des Androstans und Androstens. Helv. chim. acta 20: 1557–1564, 1937.
606. Ruzicka, L., Goldberg, M. W. and Rosenberg, H. R.: Sexualhormone. X. Herstellung des 17-Methyl-Testosterons und anderer Androsten- und Androstanderivate. Zusammenhänge zwischen chemischer Konstitution und männlicher Hormonwirkung. Helv. chim. acta 18: 1487–1498, 1935.
607. Ruzicka, L. and Wettstein, A.: Sexualhormone. XVI. Über einige Ester des Testosterons und der Androsteronreihe. Helv. chim. acta 19: 1141–1146, 1936.
607a. Ryan, K. J.: Biological aromatization of steroids. J. biol. Chem. 234: 268–272, 1959.
608. Sakiz, E.: Sur les rapports fonctionnels de la cortisone et de la progestérone. C. R. Soc. Biol., Paris 151: 56–57, 1957.
609. Sandberg, A. A., Eik-Nes, K., Nelson, D. H. and Tyler, F. H.: Levels of 17-hydroxy-corticosteroids in body fluids. J. Lab. clin. Med. 43: 874–879, 1954.
610. Sandberg, A. A., Nelson, D. H., Palmer, J., Samuels, L. T. and Tyler, F. H.: The effects of epinephrine on the metabolism of 17-hydroxycorticoids in the human. J. clin. Endocrin. 13: 629–647, 1953.
611. Sandberg, A. A. and Slaunwhite, W. R., Jr.: Studies on phenolic steroids in human subjects. II. The metabolic fate and hepato-biliary-enteric circulation of C^{14}-estrone and C^{14}-estradiol in women. J. clin. Invest. 36: 1266–1278, 1957.
612. Sandberg, A. A. and Slaunwhite, W. R., Jr.: The binding of steroids and steroid conjugates to human plasma proteins. Recent Progr. Hormone Res. 13: 209–260, 1957.
613. Sandberg, A. A. and Slaunwhite, W. R., Jr.: Differences in metabolism of prednisolone-C^{14} and cortisol-C^{14}. J. clin. Endocrin. 17: 1040–1050, 1957.
614. Sandoval, A., Miramontes, L., Rosenkranz, G., Djerassi, C. and Sondheimer, F.: Steroids. XLIX. 19-Nor-desoxycorticosterone, a potent mineralocorticoid hormone. J. Amer. chem. Soc. 75: 4117–4118, 1953.
615. Sandoval, A., Thomas, G. H., Djerassi, C., Rosenkranz, G. and Sondheimer, F.: Steroids. LXI. Synthesis of 19-nor-desoxy-corticosterone, a potent mineralocorticoid hormone. J. Amer. chem. Soc. 77: 148–151, 1955.
616. Sarett, L. H.: Some aspects of the evolution of anti-inflammatory steroids. Ann. N. Y. Acad. Sci. 82: 802–808, 1959.
616a. Sartorius, O. W., Calhoon, D. and Pitts, R. F.: The capacity of the adrenalectomised rat to secrete hydrogen and ammonium ions. Endocrinology 51: 444–450, 1952.
617. Saunders, F. J.: Survival of adrenalectomised mice after several types of stress and the effects of various steroids. Endocrinology 58: 412–419, 1956.
618. Saunders, F. J.: The duration of action of several androgenic-anabolic steroids. Acta endocr., Copenhagen 26: 345–352, 1957.
619. Saunders, F. J.: The effects of estrone on the response to testosterone propionate in castrated male rats. Endocrinology 63: 498–500, 1958.
620. Saunders, F. J.: The effects of several steroids on fecundity in female rats. Endocrinology 63: 561–565, 1958.
621. Saunders, F. J.: Effects of several steroids on mating behaviour, ovulation and pregnancy in female rats. Endocrinology 63: 566–569, 1958.
622. Saunders, F. J., Colton, F. B. and Drill, V. A.: Progesterone-like activity of a series of 19-nortestosterones. Proc. Soc. exp. Biol., N. Y. 94: 717–720, 1957.
623. Saunders, F. J. and Drill, V. A.: The myotrophic and androgenic effects of 17-ethyl-19-nortestosterone and related compounds. Endocrinology 58: 567–572, 1956.
624. Saunders, F. J. and Drill, V. A.: Comparative androgenic and anabolic effects of several steroids. Proc. Soc. exp. Biol., N. Y. 94: 646–649, 1957.

625. SAUNDERS, F. J. AND DRILL, V. A.: Some biological activities of 17-ethynyl and 17-alkyl derivatives of 17-hydroxy-estrenones. Ann. N. Y. Acad. Sci. 71: 516–530, 1958.
626. SAUNDERS, F. J. AND ELTON, R. L.: Progestational action of some newer steroids with special reference to maintenance of pregnancy. In: Recent Progress in the Endocrinology of Reproduction, ed. by C. W. Lloyd, p. 227. Academic Press, N. Y., 1959.
627. SAVAGE, O., DAVIS, P., CHAPMAN, L., POPERT, A. J., ROBERTSON, J. D. AND COPEMAN, W. S. C.: The clinical course and corticosteroid excretion of patients with rheumatoid arthritis during long-term treatment with corticotropin. Brit. med. J. 2: 1257–1262, 1957.
628. SAVAGE, O., DAVIS, P. S., CHAPMAN, L., WICKINGS, J., ROBERTSON, J. D. AND COPEMAN, W. S. C.: Corticotrophin (ACTH) in rheumatoid arthritis. Ann. Rheum. Dis. 18: 100–110, 1959.
629. SAYERS, G.: The adrenal cortex and homeostasis. Physiol. Rev. 30: 241–320, 1950.
630. SCHATZMANN, H. J.: Kompetitiver Antagonismus zwischen g-Strophanthin und Corticosteron an isolierten Streifen von Rattenaorten. Experientia 15: 73–74, 1959.
631. SCHELLMAN, J. A.: The application of the Bjerrum ion association theory to the binding of anions by proteins. J. phys. Chem. 57: 472–475, 1953.
632. SCHELLMAN, J. A., LUMRY, R. AND SAMUELS, L. T.: The binding of uncharged molecules to proteins. II. Testosterone and bovine serum albumin. J. Amer. chem. Soc. 76: 2808–2813, 1954.
633. SCHLAGEL, C. A. AND NORTHAM, J. I.: Comparative anti-inflammatory efficacy of topically applied steroids on human skin. Proc. Soc. exp. Biol., N. Y. 101: 629–632, 1959.
633a. SCHMIDLIN, J., ANNER, G., BILLETER, J.-R. AND WETTSTEIN, A.: Über Synthesen in der Aldosteron-Reihe. I. Totalsynthese des racemischen Aldosterons. Experientia 11: 365–368, 1955.
634. SCHNEIDER, J. J.: Enzymatic reduction of Compound E-21-aldehyde to Compound E. J. Amer. chem. Soc. 75: 2024, 1953.
635. SCHNEIDER, W. P. AND MURRAY, H. C.: 6α,16α-Dimethyl analogues of cortical hormones. Chem. & Ind. 1163–1164, 1960.
636. SCHOFIELD, B. M.: Influence of ovarian hormones on myometrial behaviour in the intact rabbit. J. Physiol. 129: 289–304, 1955.
637. SCHÖLER, H. F. L.: Biological properties of 9,10-isomeric steroids. Acta endocr., Copenhagen 35: 188–196, 1960.
638. SCHREIBER, S. S.: Potassium and sodium exchange in the working frog heart. Effects of overwork on external concentrations of potassium and ouabain. Amer. J. Physiol. 185: 337–347, 1956.
639. SCHRIEFERS, H., KORUS, W. AND DIRSCHERL, W.: Vergleichende Untersuchungen über den Stoffwechsel von Cortison und anderen antirheumatisch wirksamen Steroiden in Rattenleberschnitten. Acta endocr., Copenhagen 26: 331–344, 1957.
640. SCHWARTZ, T. B., ROBERTSON, M. C. AND HOLMES, L. F.: Adrenocortical influences on dipeptidase activity of surviving rat diaphragm. Endocrinology 58: 453–460, 1956.
641. SCHWENK, E. AND STAHL-ODERWALD, E.: Komplexbildung von Steroidhormonen mit Säureamiden. Hoppe-Seyl. Z. 303: 224–229, 1956.
642. SCOTT, J. F. AND ENGEL, L. L.: Molecular interaction between purines and steroids. Biochim. biophys. Acta 23: 665–667, 1957.
643. SEBEK, O. K. AND MICHAELS, R. M.: Interconversion of 17-keto and 17β-hydroxyl groups in steroids by protozoa. Nature, Lond. 179: 210–211, 1957.
644. SECKEL, H. P. G.: The influence of various physiological substances on the glycogenolysis of surviving rat liver: the influence of cortical hormone added *in vitro*. Endocrinology 26: 97–101, 1940.
645. SEITLIFF, J. A., BASS, A. D. AND CHEATHAM, C.: Cortisone. A possible *in vitro* action. Fed. Proc. 17: 411, 1958.
646. SELITTO, J. J. AND RANDALL, L. O.: Screening of anti-inflammatory agents in rats. Fed. Proc. 13: 403–404, 1954.
647. SELYE, H.: Simultaneous relative hyper- and hypo-corticoidism in different regions of the same individual. Endocrinology 55: 105–111, 1954.
648. SELYE, H.: Experimental model illustrating pathogenesis of diseases of adaptation. J. clin. Endocrin. 14: 997–1005, 1954.
649. SERDIUKOVA, O. E. AND IOSFINA, E. Z.: Reaction of the adrenal cortex to inflammatory processes in the organism. Probl. Endokr. Gormonoterap. 1: 97–104, 1955.
650. SEVITT, S.: Splenic eosinopenia and adrenocortical hyper-activity. J. Path. Bact. 70: 65–80, 1955.
651. SEXTON, A. W.: Factors influencing the passage of water and electrolytes across the goldfish gills. Univ. Missouri Diss. Abstr. 15: 2270, 1955.
652. SHARE, L.: Depletion and reaccumulation of mitochondrial sodium and potassium: effect of adrenalectomy. Amer. J. Physiol. 194: 47–52, 1958.
653. SHEFFNER, A. L. AND BERGEIM, O.: Effects of adrencorticotropic hormone (ACTH) upon free amino acid levels of plasma and tissues. Arch. Biochem. Biophys. 49: 327–334, 1952.
654. SHEPPARD, R. H., MORSE, W. I., RENOLD, A. E. AND THORN, G. W.: Studies on aldosterone in man. J. clin. Endocrin. 15: 853–854, 1955.
655. SHEWELL, J.: The activity of different steroids in producing thymic involution. Brit. J. Pharmacol. 12: 133–139, 1957.
656. SHOPPEE, C. W.: Über Bestandteile der Nebennierenrinde und verwandte Stoffe. 40. Mitt. 17-Iso-Desoxy-Corticosteron. Helv. chim. acta 23: 925–934, 1940.
657. SHULL, G. M., KITA, D. A. AND DAVISSON, J.: U. S. Pat. 2, 745, 784. W. Chas. Pfizer Co., 1956.
658. SIEGEL, B. V., SMITH, M. J. AND GERSTL, B.: Effects of cortisone on the developing chick embryo. Arch. Path. 63: 562–570, 1957.

659. SILBER, R. H.: Estimation of hydrocortisone secretion. Method of calculation from urinary-excretion data. Clin. Chem. **1**: 234–240, 1955.
660. SILBER, R. H.: The biology of anti-inflammatory steroids. Ann. N. Y. Acad. Sci. **82**: 821–828, 1959.
661. SILBER, R. H. AND MORGAN, E. R.: Absorption and excretion of 17,21-dihydroxy-20-ketosteroids in dogs. Clin. Chem. **2**: 170–174, 1956.
662. SIMONS, H. E. AND WILEY, D. W.: Fluoroketones. I. J. Amer. chem. Soc. **82**: 2288–2296, 1960.
663. SIMPSON, S. A. AND TAIT, J. F.: A quantitative method for the bioassay of the effect of adrenal cortical steroids on mineral metabolism. Endocrinology **50**: 150–161, 1952.
664. SIMPSON, S. A., TAIT, J. F. AND BUSH, I. E.: Secretion of a salt-retaining hormone by the mammalian adrenal cortex. Lancet (Aug. 2), 226–227, 1952.
665. SINGER, B. AND STACK-DUNNE, M.P.: The secretion of aldosterone and corticosterone by the rat adrenal. J. Endocrin. **12**: 130–145, 1955.
666. SANDBERG, A. A. AND SLAUNWHITE, W. R.: Transcortin, a corticosteroid-binding protein. J. clin. Invest. **38**: 384–391, 1959.
667. SMITH, L. L. AND MARX, M.: 16α-Hydroxy steroids. VIII. 16α,17α-Cyclic orthoesters of triamcinolone. J. Amer. chem. Soc. **82**: 4625–4629, 1960.
668. SMITH, L. L., MARX, M., GARBARINI, J. J., FOELL, T., ORIGONI, V. E. AND GOODMAN, J. J.: 16α-Hydroxy steroids. VII. The isomerization of triamcinolone. J. Amer. chem. Soc. **82**: 4616–4625, 1960.
669. SOLLMAN, P. B., ELTON, R. L. AND DODSON, R. M.: 6-Methyl-17α-acetoxy-21-fluoro-4,6-pregnadiene-3,20-dione. A new orally active progestin. J. Amer. chem. Soc. **81**: 4435–4436, 1959.
670. SOLOMON, A. K., GILL, T. J., III AND GOLD, G. L.: The kinetics of cardiac glycoside inhibition of potassium transport in human erythrocytes. J. gen. Physiol. **40**: 327–350, 1956.
670a. SOLOMON, N. AND SAYERS, G.: In: The Human Adrenal Cortex, ed. by A. R. Currie, T. Symington and J. K. Grant, pp. 314–324. E. & S. Livingstone, Edinburgh, 1961.
671. SONDHEIMER, F., KAUFMANN, S., ROMO, J., MARTINEZ, H. AND ROSENKRANZ, G.: Steroids. XLVII. Synthesis of steroidal hormone analogs hydroxylated at C-2[2]. J. Amer. chem. Soc. **75**: 4712–4715, 1953.
672. SONDHEIMER, F., VELASCO, M. AND ROSENKRANZ, G.: Steroids. LXXI. Synthesis of 1-dehydro-progesterone and 1-dehydro-17-ethinyl-testosterone. J. Amer. chem. Soc. **77**: 5673–5676, 1955.
673. SPAZIANI, E. AND SZEGO, C. M.: The influence of estradiol and cortisol on uterine histamine of the ovariectomised rat. Endocrinology **63**: 669–678, 1958.
674. SPAZIANI, E. AND SZEGO, C. M.: Further evidence for mediation by histamine of oestrogenic stimulation of the rat uterus. Endocrinology **64**: 713–723, 1959.
675. SPAZIANI, E. AND SZEGO, C. M.: Early effects of oestradiol and cortisol on water and electrolyte shifts in the uterus of the immature rat. Amer. J. Physiol. **197**: 355–359, 1959.
676. SPECTOR, W. G. AND STOREY, E.: A factor in the oestrogen-treated uterus responsible for leucocyte emigration. J. Path. Bact. **75**: 387–398, 1958.
677. SPECTOR, W. G. AND WILLOUGHBY, D. A.: The demonstration of the role of mediators in turpentine pleurisy in rats by experimental suppression of the inflammatory changes. J. Path. Bact. **77**: 1–17, 1959.
678. SPECTOR, W. G. AND WILLOUGHBY, D. A.: The suppression by antiesterases of increased capillary permeability in acute inflammation. J. Path. Bact. **79**: 21–35, 1960.
679. SPEIERS, R. S., SIMPSON, S. A. AND TAIT, J. F.: Certain biological activities of crystalline electrocortin. Endocrinology **55**: 233–235, 1954.
680. SPERO, G. B., THOMPSON, J. L., LINCOLN, F. H., SCHNEIDER, W. P. AND HOGG, J. A.: Adrenal hormones and related compounds. V. Fluorinated 6-methyl steroids. J. Amer. chem. Soc. **79**: 1515–1516, 1957.
681. SPERO, G. B., THOMPSON, J. L., MAGERLEIN, B. J., HANZE, A. R., MURRAY, H. C., SEBEK, O. K. AND HOGG, J. A.: Adrenal hormones and related compounds. IV. 6-Methyl steroids. J. Amer. chem. Soc. **78**: 6213–6214, 1956.
682. STAFFORD, R. O., BARNES, L. E., BOWMAN, B. J. AND MEINZINGER, M. M.: Glucocorticoid and mineralocorticoid activities of Δ[1]-fluorohydrocortisone. Proc. Soc. exp. Biol., N. Y. **89**: 371–374, 1955.
683. STAFFORD, R. O., ROBERT, A., LYSTER, S. C., SCHMIDT, F. L. AND DULIN, W. E.: Fluorometholone, a preferentially anti-inflammatory corticoid. Proc. Soc. exp. Biol., N. Y. **101**: 653–655, 1959.
684. STEENBURG, R. W. AND GANONG, W. F.: Observations on the influence of extra-adrenal factors on circulating 17-hydroxycorticoids in the surgically stressed adrenalectomised animal. Surgery **38**: 92–104, 1955.
684a. STEINBERG, N. G., HIRSCHMANN, R. AND CHEMERDA, J. M.: Partial synthesis of 4-methylhydrocortisone acetate. A suggested route to hydrocortisone-[14]C and related compounds. Chem. & Ind. 975–976, 1958.
685. STEPHENSON, N. R.: The relative potency of adrenal corticoids by the thymus involution method. Canad. J. Biochem. Physiol. **34**: 253–258, 1956.
686. STERN, T. N., COLE, V. V., BASS, A. C. AND OVERMAN, R. R.: Dynamic aspects of sodium metabolism in experimental adrenal insufficiency using radioactive sodium. Amer. J. Physiol. **164**: 437–449, 1951.
687. STETTEN, D., JR.: Relationship of hormone dosage to physiological response. Science **124**: 365–366, 1956.
688. STORK, G., KHASTGIR, H. N. AND SOLO, A. J.: Synthesis of 17,18-bisnorsteroids. J. Amer. chem. Soc. **80**: 6457–6458, 1958.
689. STURTEVANT, F. M.: Response of metacorticoid hypertension to bistrium, apresoline, veriloid and serpentine. Proc. Soc. exp. Biol., N. Y. **84**: 101–102, 1953.
690. STURTEVANT, F. M.: Effect of androstane derivative on metacorticoid hypertension. Proc. Soc. exp. Biol., N. Y. **84**: 262–263, 1953.
691. STURTEVANT, F. M.: The effect of two steroid analogs on organ weights of intact and hypophysectomised rats. Endocrinology **56**: 256–260, 1955.

692. STURTEVANT, F. M.: Hypotensive activity of natural and synthetic estrogens in metacorticoid hypertensive rats. Proc. Soc. exp. Biol., N. Y. **93**: 550–551, 1956.
693. STURTEVANT, F. M.: Pharmacology of a new hypotensive steroid: 17α-propyl-4,5β-dihydro-19-nortestosterone. J. Pharmacol. **121**: 369–377, 1957.
694. STURTEVANT, F. M.: The biology of metacorticoid hypertension. Ann. intern. Med. **49**: 1281–1293, 1958.
695. STURTEVANT, F. M.: Statistical relationships among metabolic parameters in steroid diabetic guinea pigs. Endocrinology **63**: 151–154, 1958.
696. STURTEVANT, F. M.: Effect of a hypotensive steroid on the action of desoxycorticosterone in the rat. J. Lab. clin. Med. **52**: 899–901, 1958.
697. STURTEVANT, F. M.: Anti-hypertensive action of 17-imidazolyl-steroids in the rat. Proc. Soc. exp. Biol., N. Y. **97**: 619–621, 1958.
698. STURTEVANT, F. M.: Antihypertensive effects of an aldosterone antagonist. Science **127**: 1393–1394, 1958.
699. STURTEVANT, F. M.: Prevention of adrenal-regeneration hypertension by an aldosterone-blocker. Endocrinology **64**: 299–300, 1959.
700. STURTEVANT, F. M. AND HANSEN, N.: Increased sensitivity to salt in rats pretreated with deoxycortisone. Amer. J. med. Sci. **229**: 188–190, 1955.
701. STURTEVANT, F. M. AND RADCLIFFE, M. J.: Cardiovascular reactivity to norepinephrine in metacorticoid hypertensive rats and the effect of pretreatment with 17α-propyl-4,5β-dihydro-19-nortestosterone. Arch. int. Pharmacodyn. **118**: 70–73, 1959.
702. SULSER, F., KUNZ, H. A., GANTENBEIN, R. AND WILBRANDT, W.: Zur Frage einer antagonistischen Wirkung zwischen Herzglykosiden und Corticosteroiden auf die Electrolytausscheidung der Niere. Arch. exp. Path. Pharmak. **235**: 400-411, 1959.
703. SWINGLE, W. W., BAKER, C., EISLER, M., LEBRIE, S. J. AND BRANNICK, L.: Maintenance of adrenalectomized dogs with 9 alpha halo adrenal and other steroids. Endocrinology **57**: 220–230, 1955.
704. SWINGLE, W. W., BARLOW, G., COLLINS, E., FEDOR, E. J., WELCH, W. J. AND RAMPONA, J. M.: Serum potassium and electrocardiographic changes in adrenalectomised dogs maintained on cortisone acetate. Endocrinology **51**: 353–361, 1952.
705. SWINGLE, W. W., BRANNICK, L. J. AND PARLOW, A. F.: High potency of 2-methyl-9α-fluorohydrocortisone for life maintenance and revival from insufficiency of adrenalectomized dogs. Endocrinology **60**: 658–663, 1957.
706. SWINGLE, W. W., BRANNICK, L. J. AND PARLOW, A. F.: Effect of 2-methyl-9-alpha-fluorohydrocortisone on internal distribution of fluid and electrolytes of fasted adrenalectomised dogs. Proc. Soc. exp. Biol., N. Y. **94**: 156–159, 1957.
707. SWINGLE, W. W., BRANNICK, L. J. AND PARLOW, A. F.: Extrarenal effects of adrenal steroids on the distribution of sodium, chloride, and water. Schweiz. med. Wschr. **87**: suppl. 14, 403–406, 1957.
708. SWINGLE, W. W., BRANNICK, L. J., PARLOW, A. F., BAKER, C. AND LEBRIE, S. J.: Life maintenance and mineralocorticoid activity of some new derivatives of cortisone, hydrocortisone and DOC. Endocrinology **59**: 226–232, 1956.
709. SWINGLE, W. W., DAVANZO, J. P., CROSSFIELD, H. C., GLENISTER, D., OSBORNE, M. AND WAGLE, G.: Effect of various adrenal steroids on internal fluid and electrolyte shifts of fasted adrenalectomised dogs. Proc. Soc. exp. Biol., N. Y. **98**: 75–78, 1958.
710. SWINGLE, W. W., DAVANZO, J. P., GLENISTER, D., CROSSFIELD, H. C. AND WAGLE, G.: Role of gluco- and mineralocorticoids in salt and water metabolism of adrenalectomised dogs. Amer. J. Physiol. **196**: 283–284, 1959.
711. SWINGLE, W. W., EISLER, M., BAKER, C., LEBRIE, S. J. AND BRANNICK, L.: Prevention of eosinopenia in adrenalectomized dogs by an antihistaminic drug. Amer. J. Physiol. **182**: 256–262, 1955.
712. SWINGLE, W. W., PFIFFNER, J. J., VARS, H. M. AND PARKINS, W. M.: The effect of fluid deprivation and fluid intake upon the revival of dogs from adrenal insufficiency. Amer. J. Physiol. **108**: 144–150, 1934.
713. SWINGLE, W. W., PFIFFNER, J. J., VARS, H. M. AND PARKINS, W. M.: The relation between blood pressure, blood urea N_2 and fluid balance of the adrenalectomized dog. Amer. J. Physiol. **108**: 428–437, 1934.
714. SWINGLE, W. W., PFIFFNER, J. J., VARS, H. M. AND PARKINS, W. M.: Effect of hemorrhage on the normal and adrenalectomized dog. Amer. J. Phyeiol. **107**: 259–274, 1934.
715. SWINGLE, W. W., PFIFFNER, J. J., VARS, H. M. AND PARKINS, W. M.: The influence of adrenal cortical hormone upon electrolyte and fluid distribution in adrenalectomized dogs maintained on a sodium and chloride free diet. Amer. J. Physiol. **119**: 684–691, 1937.
716. SWYER, G. I. M.: The oestrogens. Brit. med. J. **1**: 1029–1031, 1959.
717. SYDNOR, K. L.: Blood adrenocorticotrophin in the stressed adrenalectomised cat after intravenous injection of hydrocortisone. Endocrinology **56**: 204–208, 1955.
718. SZIRMAI, J. A.: Connective tissue of cock comb. III. Mast cells and effect of testosterone. Acta endocr., Copenhagen **25**: 225–237, 1957.
719. TALAAT, M., HABIB, Y. A. AND HABIB, M.: Testosterone on the carbohydrate metabolism in normal subject. Arch. int. Pharmacodyn. **111**: 215–226, 1957.
720. TALALAY, P.: The specificity of steroid-enzyme interactions. Cancer, N. Y. **10**: 738–740, 1957.
721. TALALAY, P.: Enzymatic mechanisms in steroid metabolism. Physiol. Rev. **37**: 362–389, 1957.
722. TALALAY, P.: Molecular specificity in the enzymatic oxidation of steroids. Rec. chem. Progr. **18**: 31–49, 1957.
723. TALALAY, P., LOEWUS, F. A. AND VENNESLAND, B.: The enzymatic transfer of hydrogen. IV. The reaction catalysed by a β-hydroxy-steroid dehydrogenase. J. biol. Chem. **212**: 801–809, 1955.
724. TALALAY, P. AND WILLIAMS-ASHMAN, H. G.: Activation of hydrogen transfer between pyridine nucleotides by steroid hormones. Proc. nat. Acad. Sci., Wash. **44**: 15–26, 1958.

724a. TALALAY, P. AND WILLIAMS-ASHMAN, H. G.: Participation of steroid hormones in the enzymatic transfer of hydrogen. Recent Progr. Hormone Res. **16:** 1–47, 1960.

725. TANNHAUSER, P., PRATT, R. J. AND JENSEN, E. V.: The preparation of 21-fluorosteroids. J. Amer. chem. Soc. **78:** 2658–2659, 1956.

726. TANZ, R. D., CLARK, G. M. AND WHITEHEAD, R. W.: Influence of 9-α-fluorohydrocortisone on contractility and Na content of isolated cat papillary muscle. Proc. Soc. exp. Biol., N. Y. **92:** 167–169, 1956.

727. TAUB, D., HOFFSOMMER, R. D., SLATES, H. L., KUO, C. H. AND WENDLER, N. L.: 16β-Methyl cortical steroids. J. Amer. chem. Soc. **82:** 4012–4026, 1960.

728. TAUB, D., HOFFSOMMER, R. D., SLATES, H. L. AND WENDLER, N. L.: 16β-Methyl cortical steroids. J. Amer. chem. Soc. **80:** 4435, 1958.

729. TAUB, D., HOFFSOMMER, R. D. AND WENDLER, N. L.: Cortical steroids substituted at C_{12}. J. Amer. chem. Soc. **78:** 2912–2913, 1956.

729a. TAMM, J. AND VOIGT, K. D.: Experimentelle und klinische Untersuchungen mit synthetischen wasserlöslichen Steroidkonjugaten beim Menschen. Acta endocr., Copenhagen **54:** suppl., 1–80, 1960.

730. TAUBENHAUS, M. AND MORTON, J. V.: Influence of steroids upon osmotic pressure changes in isolated frog skin pouches. Proc. Soc. exp. Biol., N. Y. **98:** 162–164, 1958.

730a. TEMPLE, S., HOLLANDER, V. P., HOLLANDER, N. AND STEPHENS, M. L.: Estradiol activation of uterine reduced diphosphopyridine nucleotide oxidase. J. biol. Chem. **235:** 1504–1509, 1960.

730b. THOMAS, L.: Cortisone and infection. Ann. N. Y. Acad. Sci. **56:** 799–814, 1953.

731. THOMAS, P. Z., FORCHIELLI, E. AND DORFMAN, R. I.: The reduction *in vitro* of 17α-hydroxypregnenolone (3β,17α-dihydroxy-Δ^5-pregnen-20-one) by rabbit skeletal muscle. J. biol. Chem. **235:** 2797–2800, 1960.

732. THOMPSON, J. F. AND MIKUTA, E. T.: The effect of cortisone and hydrocortisone on the tryptophan peroxidase-oxidase activity of rat liver. Endocrinology **55:** 232–233, 1954.

733. THORN, G. W., ENGEL, L. L. AND LEWIS, R. A.: The effect of 17-hydroxycorticosterone and related adrenal cortical steroids on sodium and chloride excretion. Science **94:** 348–349, 1941.

734. THORN, G. W., FORSHAM, P. H., FRAWLEY, T. F., HILL, S. R., ROCHE, M., STAEHLIN, D. AND WILSON, D. L.: The clinical usefulness of ACTH and cortisone. New Engl. J. Med. **242:** 783–793, 824–834, 865–872, 1950.

735. THORN, G. W., RENOLD, A. E. AND WINEGRAD, A. I.: Some effects of adrenal cortical steroids on intermediary metabolism. Brit. med. J. **2:** 1009–1017, 1957.

736. THORN, G. W., SHEPPARD, R. H., MORSE, W. I., REDDY, W. J., BEIGELMAN, P. M. AND RENOLD, A. E.: Comparative action of aldosterone and 9α-fluorohydrocortisone in man. Ann. N. Y. Acad. Sci. **61:** 609–619, 1953.

737. TOLKSDORF, S.: Laboratory evaluation of anti-inflammatory steroids. Ann. N. Y. Acad. Sci. **82:** 829–835, 1959.

738. TOLKSDORF, S., BATTIN, M. L., CASSIDY, J. W., MACLEOD, R. M., WARREN, F. H. AND PERLMAN, P. L.: Adrenocortical properties of $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,11,20-trione (Meticorten) and $\Delta^{1,4}$-pregnadiene-11β,17α,21-triol-3,20-dione (Meticortelone). Proc. Soc. exp. Biol., N. Y. **92:** 207–214, 1956.

739. TOMICH, E. G., WOOLLETT, E. A. AND JENKINS, L. (Glaxo Laboratories, Ltd., Middlesex, England). Personal communication, 1960.

740. TOMIZAWA, H. H., NARAHARA, H. T., GIBBONS, C. A. AND WILLIAMS, R. H.: A metabolic study of hydrocortisone in rats. Proc. Soc. exp. Biol., N. Y. **85:** 51–53, 1954.

741. TOMKINS, G. M.: A mammalian 3α-hydroxysteroid dehydrogenase. J. biol. Chem. **218:** 437–447, 1956.

742. TOMKINS, G. M.: Enzymatic mechanisms of hormone metabolism. I. Oxidation-reduction of the steroid nucleus. Recent Progr. Hormone Res. **12:** 125–133, 1956.

743. TOMKINS, G. M.: The enzymatic reduction of Δ^4-3-ketosteroids. J. biol. Chem. **225:** 13–24, 1957.

744. TOMKINS, G. M.: Enzymatic metabolism of corticosteroids. Ann. N. Y. Acad. Sci. **82:** 836–845, 1959.

745. TRAVIS, R. H. AND SAYERS, G.: Metabolism of cortisol-4-C^{14} in the dog heart-lung preparation. Endocrinology **62:** 816–821, 1958.

746. TRETHEWIE, E. R. AND TRAUTNER, E. M.: Effect of succinate on the number of circulating eosinophils in man and in the cat. Aust. J. exp. Biol. med. Sci. **34:** 109–120, 1956.

747. TROOP, R. C.: Influence of gonadal hormones on metabolism of cortisone. Endocrinology **64:** 671–675, 1959.

747a. TROWELL, O. A.: The action of cortisone on lymphocytes *in vitro*. J. Physiol. **119:** 274–285, 1953.

748. TRUELOVE, S. C. AND WITTS, L. J.: Cortisone and corticotrophin in ulcerative colitis. Brit. med. J. **1:** 387–394, 1959.

749. TULLNER, W. W.: Mediation of progestational action of amphenone by the rabbit adrenal. Proc. Soc. exp. Biol., N. Y. **98:** 157–160, 1958.

750. TULLNER, W. W. AND HERTZ, R.: High progestational activity of 19-norprogesterone. J. clin. Endocrin. **12:** 916–917, 1952.

750a. ULICK, S. AND LIEBERMAN, S.: Evidence for the occurrence of a metabolite of aldosterone in urine. J. Amer. chem. Soc. **79:** 6567–6568, 1959.

751. ULICK, S. AND SOLOMON, S.: The synthesis of aldosterone from progesterone by the amphibian adrenal. J. Amer. chem. Soc. **82:** 249, 1960.

752. ULRICH, F. AND LONG, C. N. H.: Effects of stress on serum C^{14} levels in rats following administration of hydrocortisone-4-C^{14} and corticosterone-4-C^{14}. Endocrinology **59:** 170–180, 1956.

753. UMBREIT, W. W.: Influence of adrenalectomy and cortisone treatment on enzymatic reactions in rat tissues. Ann. N. Y. Acad. Sci. **54:** 569–574, 1951.

754. UMBREIT, W. W. AND TONHAZY, N. E.: The metabolic effects of cortisone. II. The adenylate system. J. biol. Chem. **191:** 257–261, 1951.

755. UMBREIT, W. W. AND TONHAZY, N. E.: The metabolic effects of cortisone. I. The oxidation of proline. J. biol. Chem. **191:** 249–256, 1951.

756. Ussing, H. H.: Ion transport across biological membranes. In: Ion Transport across Membranes, ed. by H. T. Clarke. Academic Press, N. Y., 1954.

757. Valette, G. and Huidobro, H.: Histaminase and the adrenal cortex. C. R. Soc. Biol., Paris **151**: 1305–1306, 1957.

758. Vander, A. J., Malvin, R. L., Wilde, W. S., Lapides, J., Sullivan, L. P. and McMurray, V. M.: Effects of adrenalectomy and aldosterone on proximal and distal tubular sodium reabsorption. Proc. Soc. exp. Biol., N. Y. **99**: 323–325, 1958.

759. Velarde, E., Iriarte, J., Ringold, H. J. and Djerassi, C.: Steroids. CXIII. 6-Methyl oestrogens. J. org. Chem. **24**: 311–313, 1959.

760. Velardo, J. T.: Influence of purified adrenal hormones and adrenocorticotrophin on progesterone in decidual tissue formation. Amer. J. Physiol. **190**: 408–412, 1957.

761. Velluz, L., Muller, G., Jequier, R. and Plotka, C.: Ethynylated derivatives related to adrenosterone and a steroidal effect on experimental hyperlipaemia. J. Amer. chem. Soc. **80**: 2026, 1958.

762. Vermeulen, A.: Adrenal hypofunction after corticoid withdrawal. Acta endocr., Copenhagen **28**: 321, 1958.

763. Villee, C. A.: Oestrogens and uterine enzymes. Ann. N. Y. Acad. Sci. **75**: 524–534, 1959.

764. Villee, C. A., White, V. K. and Hastings, A. B.: Metabolism of C^{14}-labelled glucose and pyruvate by rat diaphragm muscle *in vitro*. J. biol. Chem. **195**: 287–297, 1952.

764a. Villee, C. A., Hagermann, D. D. and Joel, P. B.: An enzymatic basis for the physiologic functions of estrogens. Recent Progr. Hormone Res. **16**: 49–69, 1960.

765. Villotti, R., Djerassi, C. and Ringold, H. J.: Steroids. CXXIII. 19-Nor-6-methylandrostane derivatives. J. Amer. chem. Soc. **81**: 4566–4570, 1959.

766. Vischer, E., Meystre, C. and Wettstein, A.: Mikrobiologische Herstellung von 1-Dehydro-Steroiden. Helv. chim. acta **38**: 835–840, 1955.

766a. Vischer, E., Schmidlin, J. and Wettstein, A.: Microbiologische Spaltung razemischer Steroide. Synthese von *d*-Aldosteron. Experientia **12**: 50–52, 1956.

767. Vogt, M.: Hexoestrol and 'Amphenone B' on morphology and function of rat adrenal cortex. Yale J. Biol. Med. **29**: 469–479, 1957.

768. Voigt, K. D. and Schroeder, W.: Nitrogen-containing steroids in aqueous adrenal cortical extracts. Nature, Lond. **176**: 599–600, 1955.

769. Wade, A. P., Slater, J. D. H., Kellie, A. E. and Holliday, M. E.: Urinary excretion of 17-KS following rectal infusion of cortisol. J. clin. Endocrin. **19**: 444–453, 1959.

770. Wallace, E. Z., Silverberg, H. I. and Carter, A. C.: Effect of ethinyl oestradiol on plasma 17-hydroxycorticosteroids, adrenocorticotrophin responsiveness, and hydrocortisone clearance in man. Proc. Soc. exp. Biol., N. Y. **95**: 805–808, 1957.

771. Wallach, S., Englert, E., Jr. and Brown, H.: Adrenocortical function in diabetic acidosis. Metabolism **6**: 107–115, 1957.

772. Ward, L. E., Polley, H. F., Slocumb, C. H. and Hench, P. S.: Comparative effects of hydrocortisone, hydrocortisone acetate and cortisone acetate in rheumatoid arthritis. J. Lab. clin. Med. **40**: 959, 1952.

773. Warner, R. C., Webber, J., de Bodo, R. C. and Kurtz, M.: Hypophysectomy and subsequent administration of growth hormone and ACTH on serum proteins of dogs. Amer. J. Physiol. **190**: 121–128, 1957.

774. Weaver, J. A.: Changes induced in the thymus and lymph nodes of the rat by the administration of cortisone and sex hormones and by other procedures. J. Path. Bact. **69**: 133–139, 1955.

775. Weber, G., Allard, C., de Lamirande, G. and Cantarow, A.: Increased liver glucose-6-phosphatase activity after cortisone administration. Biochim. biophys. Acta **16**: 618–619, 1955.

776. Wegelius, O. and Teir, H.: Effect of cortisone, adrenalectomy and hypophysectomy on tissue eosinophil count in glandular stomach of rat. Acta endocr., Copenhagen **28**: 197–204, 1958.

777. Weinges, K. and Maske, H.: Pancreatectomy and action of cortisone in guinea-pigs. Arch. exp. Path. Pharmak. **231**: 440–454, 1957.

778. Weisenborn, F. L.: Quoted in: Steroids, by L. F. Fieser and M. Fieser, p. 599. Reinhold, New York, 1959.

779. Welt, I. D., Stetten, D., Jr., Ingle, D. J. and Morley, E. H.: Effect of cortisone upon rates of glucose production and oxidation in the rat. J. biol. Chem. **197**: 57–66, 1952.

779a. West, C. D., Tyler, F. H., Brown, H. and Samuels, L. T.: The role of the liver and the kidneys in the metabolism of intravenous testosterone by human subjects. J. clin. Endocrin. **11**: 897–912, 1951.

780. West, H. F. and Newns, G. R.: Cortisone acetate v. cortisol in the treatment of rheumatoid disease. Lancet (July 24), 168–169, 1954.

780a. Westerhof, P. and Rerink, E. H.: Sterols XVI. The synthesis and properties of 9β,10α-androstanes. Rec. Trav. chim. Pays Bas **79**: 794–806, 1960.

781. Westin, B. and Odeblad, E.: Effect of some steroid hormones on incorporation of ^{35}S in mouse uterus following administration of radio-sulphate; autoradiographic study. Acta path. microbiol. scand. **45**: 129–132, 1959.

782. Wettstein, A.: Homologe der Keimdrüsenhormone. IV. Höhere Homologe des Pregnenolons und Progesterons. Helv. chim. acta **23**: 1371–1379, 1940.

783. Wettstein, A.: Über Steroide. 24. Über $\Delta^{4,6}$-3-Ketone der Androstan- und Pregnan-Reihe. Helv. chim. acta **23**: 388–399, 1940.

784. Wettstein, A.: Höhere Homologe des Progesterons und Desoxycorticosteron-Acetats. Helv. chim. acta **24**: 311–317, 1941.

785. Wettstein, A.: Conversion of steroids by micro-organisms. Experientia **11**: 465–479, 1955.

786. Wettstein, A.: Chemie und Biologie neuer Corticoide. Verh. dtsch. Ges. inn. Med. **62**: 214–227, 1956.

787. Wettstein, A. and Anner, G.: Advances in the field of adrenal cortical hormones. Experientia **10:** 397–416, 1954.
788. White, H. L. and Rolf, D.: Whole tissue electrolyte analyses in normal and adrenalectomized rats. Amer. J. Physiol. **180:** 287–295, 1955.
789. Whitehouse, M. W. and Lash, J. W.: Effect of cortisone and related compounds on the biogenesis of cartilage. Nature, Lond. **189:** 37–39, 1961.
789a. Wiest, W. G., Fujimoto, G. I. and Sandberg, A. A.: Metabolism of progesterone-4-C^{14} in a postmenopausal woman with a biliary fistula. J. clin. Endocrin. **18:** 972–980, 1958.
790. Wiggins, R. A., Hutchin, M. E., Carbone, J. V. and Doolan, P. D.: Effect of spirolactone SC 8109 on renal function in normal human subjects. Proc. Soc. exp. Biol., N. Y. **100:** 625–627, 1959.
791. Wilbrandt, W.: Zur Frage der Beziehung zwischen Herzglykosid- und Corticosteroidwirkungen. Pharm. Acta Helvet. **33:** 485–500, 1958.
792. Wilbrandt, W.: Digitalis und Ionentransporte. Bad Oeznhausener Gespräche **3:** 94–107, 1959.
793. Wilbrandt, W.: Permeability and transport systems in living cells. J. Pharm., Lond. **11:** 65–79, 1959.
794. Wilbrandt, W.: Die Bedeutung der Corticosteroide für Ionentransporte. Schweiz. med. Wschr. **89:** 363–367, 1959.
795. Wilbrandt, W.: Permeabilität und Stofftransporte. Fortschr. Zool. **12:** 28–127, 1960.
796. Wilbrandt, W. and Weiss, E. M.: Antagonismus zwischen Herzglykosid und Corticosteroiden am Froschhautpotential. Arzneim.-Forsch. **10:** 409–412, 1960.
797. Wilds, A. L., Hoffman, C. H. and Pearson, T. H.: Steroid analogs lacking ring C. V. Some analogs of testosterone and androstenedione. J. Amer. chem. Soc. **77:** 647–651, 1955.
798. Wilds, A. L. and Shunk, C. H.: Steroid analogs lacking ring C. II. Some analogs of progesterone and desoxycorticosterone. J. Amer. chem. Soc. **72:** 2388–2395, 1950.
799. Wilds, A. L., Shunk, C. H. and Hoffman, C. H.: Synthesis of physiologically active analogs of testosterone. J. Amer. chem. Soc. **71:** 3266–3267, 1949.
800. Williams, A. W.: Influence of cortisone on the healing of gastric ulcers. J. Path. Bact. **67:** 259–261, 1954.
801. Williams, M. F.: The vascular architecture of the rat uterus as influenced by estrogen and progesterone. Amer. J. Anat. **83:** 247–307, 1948.
802. Williams-Ashman, H. G., Cassman, M. and Klavins, M.: Two enzymic mechanisms for hydrogen transport by phenolic oestrogens. Nature, Lond. **184:** 427–429, 1959.
802a. Willmer, E. N.: Steroids and cell surfaces. Biol. Rev. **36:** 368–398, 1961.
803. Wilson, D. L.: Direct effects of adrenal cortical steroids on electrolyte content of rabbit leucocytes. Amer. J. Physiol. **190:** 104–108, 1957.
804. Wilson, D. L., Frawley, T. F., Forsham, P. H. and Thorn, G. W.: The functional relationship between the pancreatic islets and the adrenal cortex in man. Proc. Amer. Diabetes Ass. **10:** 25–34, 1950.
805. Wilson, E. and Tishler, M.: Synthesis of Δ^1-allopregnene-17α,21-diol-3,11,20-trione-21-acetate. J. Amer. chem. Soc. **74:** 1609–1610, 1952.
806. Wilson, H., Fairbanks, R., Scialabba, D., McEwen, C. and Ziff, M.: Metabolites of hydrocortisone and cortisone in synovial fluid in rheumatoid arthritis. J. clin. Endocrin. **16:** 86–113, 1956.
807. Winter, C. A., Ingram, W. R. and Gross, E. G.: Effect of pitressin injections upon the serum electrolyte and water exchange of cats with diabetes insipidus and adrenal insufficiency. Amer. J. Physiol. **127:** 64–70, 1939.
808. Winternitz, W. W., Dintzis, R. and Long, C. N.: Further studies on the adrenal cortex and carbohydrate metabolism. Endocrinology **61:** 724–741, 1957.
809. Wolff, H. P., Koczorek, K. R. and Buchborn, E.: Hyperaldosteronism in heart disease. Lancet **2:** 63–66, 1957.
810. Woodbury, D. M.: Extrarenal effects of desoxycorticosterone, adrenocortical extract and adrenocorticotrophic hormone on plasma and tissue electrolytes in fed and fasted rats. Amer. J. Physiol. **174:** 1–19, 1953.
810a. Woodbury, D. M.: Relation between the adrenal cortex and the central nervous system. Pharmacol. Rev. **10:** 275–357, 1958.
811. Woodbury, D. M. and Koch, A.: Effects of aldosterone and deoxycorticosterone on tissue electrolytes. Proc. Soc. exp. Biol., N. Y. **94:** 720–723, 1957.
812. Wool, I. G. and Weinshelbaum, E. I.: Incorporation of C^{14}-amino acids into protein of isolated diaphragms: role of the adrenal steroids. Amer. J. Physiol. **197:** 1089–1092, 1959.
813. Yates, F. E.: Central venous congestion on sodium, potassium and water metabolism in rat; response to deoxycorticosterone. Amer. J. Physiol. **194:** 57–64, 1958.
814. Yates, F. E., Urquhart, J. and Herbst, A. L.: Impairment of enzymic inactivation of adrenal cortical hormones following passive venous congestion of the liver. Amer. J. Physiol. **194:** 65–71, 1958.
815. Zachariae, L. and Moltke, E.: Influence of hydrocortisone (Compound F) on mast cells of normal skin and healing wounds in the rabbit. Acta endocr., Copenhagen **16:** 300–304, 1954.
816. Zander, J., Forbes, T. R., Neher, R. and Desaulles, P.: Über biologisch aktive Progesteronmetaboliten im menschlichen Organismus. Klin. Wschr. **35:** 143, 1957.
816a. Zaffaroni, A.: The effect of alkyl- and electronegative-group substitution on steroidal hormone activity. Proc. Symp. 1st int. Congr. Endocrin., Advance Abstracts, pp. 139–145. Periodica, Copenhagen, 1960.
817. Zaffaroni, A., Ringold, H. J., Rosenkranz, G., Sondheimer, F., Thomas, G. H. and Djerassi, C.: Synthesis of 19-nor-delta^4-pregnene-11β,17α,21-triol-3,20-dione (19-nor-hydrocortisone) and related hormones. J. Amer. chem. Soc. **80:** 6110–6114, 1958.
818. Zarrow, M. X., Shoger, R. L. and Lazo-Wasem, E. A.: Rate of disappearance of exogenous progesterone from blood. J. clin. Endocrin. **14:** 645–652, 1954.
819. Zderic, J. A., Batres, E., Limon, D. C., Carpio, H., Lisci, J., Monroy, G., Necoecha, E. and Ringold, H. J.: Steroids. CXL. 11-Methyl steroids. J. Amer. chem. Soc. **82:** 3404–3409, 1960.

820. Zderic, J. A., Carpio, H. and Djerassi, C.: Steroids. CXIX. The preparation of some vicinal glycols in the cortical hormone series. J. org. Chem. **24**: 909–911, 1959.

821. Zderic, J. A., Carpio, H. and Djerassi, C.: Steroids. CXXX. Synthesis of 12β-hydroxy-prednisolone 11β,12β-acetonide 21-acetate. J. Amer. chem. Soc. **82**: 446–451, 1960.

822. Zderic, J. A. and Limon, D. C.: Steroids. CXXXI. A new series of 6-substituted progesterone analogs. J. Amer. chem. Soc. **82**: 2304–2308, 1960.

823. Ziff, M., Scull, E., Ford, D., McEwen, C. and Bunim, J. J.: Effects in rheumatoid arthritis of hydrocortisone and cortisone injected intra-articularly. Arch. intern. Med. **90**: 774–784, 1952.

824. Zizine, L.: Inhibition of compensatory adrenal hypertrophy by testosterone. C. R. Soc. Biol., Paris **150**: 1333–1334, 1956.

825. Zweifach, B. W. and Metz, D. B.: Validity and limitations of rat mesoappendix bioassay studies. Circulation Res. **3**: 121–132, 1955.