SOME EFFECTS OF HORMONES ON CELLS AND CELL CONSTITUENTS[1]

JAY TEPPERMAN AND HELEN M. TEPPERMAN

Department of Pharmacology, State University of New York, Upstate Medical Center, Syracuse, New York

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ACTH	Adrenocorticotrophic hormone
AIB	α-Aminoisobutyric acid
3,5-AMP	Adenosine-3',5'-monophosphate
ATP	Adenosine triphosphate

[2] Boldface numbers refer to those shown in Figure 1, which is described in the Introduction. Interrogation marks indicate highly speculative classification of material.

CoA	Coenzyme A
DNA	Deoxyribonucleic acid
DPN	Oxidized diphosphopyridine nucleotide
DPNH	Reduced diphosphopyridine nucleotide
EDTA	Ethylenediamine tetraacetic acid
FAD	Oxidized flavine adenine dinucleotide
FADH	Reduced flavine adenine dinucleotide
LH	Luteinizing hormone
RNA	Ribonucleic acid
TPN	Oxidized triphosphopyridine nucleotide
TPNH	Reduced triphosphopyridine nucleotide
TSH	Thyroid stimulating hormone
UDPG	Uridine diphosphoglucose
UTP	Uridine triphosphate

"Whatever his philosophical or theological views, it is sensible and expedient for a physiologist, using that term in the wider sense, when investigating an organ, a structure, a response or an adaptation, to ask what its functional significance is, its relation to other parts of the machinery, its purpose in connection with behavior, survival or inheritance. If his conscience, or his politics, forbids that much teleology, he had better take up something else; for in biology he will miss most of what is interesting."—A. V. Hill (113)

INTRODUCTION

If one defines pharmacology as the study of the effects of chemical agents on living systems, pharmacology includes endocrinology, for no clear line can be drawn between chemicals of endogenous origin and those of exogenous origin. Such distinctions are particularly meaningless at the cellular level, for, in its response to chemical substances, the cell does not differentiate between chemicals that are produced in a remote tissue and similar ones which are ingested or injected. In the present stage of the development of their art the cellular pharmacologist and the cellular endocrinologist have the same aims, use the same methods which they borrow shamelessly from anyone who has the ingenuity to develop them, erect similar constructs, and maintain a lively interest in the newest discoveries of the biochemist in the hope that he will lead them to a better understanding of the cellular mechanisms of action of drugs and hormones. Both often share the uneasy suspicion that the biochemical machinery upon which a substance may act has not yet been described. It is approximately as frustrating to attempt to describe the effects of anesthetics in biochemical terms at this time as it is to give a plausible biochemical description of the anti-inflammatory action of hydrocortisone. Theories concerning the cellular effects of hormones, then, must be of interest to those who are interested primarily in the effects of non-hormonal agents. Conversely, it is entirely possible that a better understanding of the intimate effects of cardiac glycosides on heart muscle cells will lead to clearer insights into the mechanism of action of steroid hormones.

There are many ways in which the subject under review can be treated. All of them are arbitrary and each has evident drawbacks. It is more or less customary to make a catalogue of the generally accepted hormones and give an
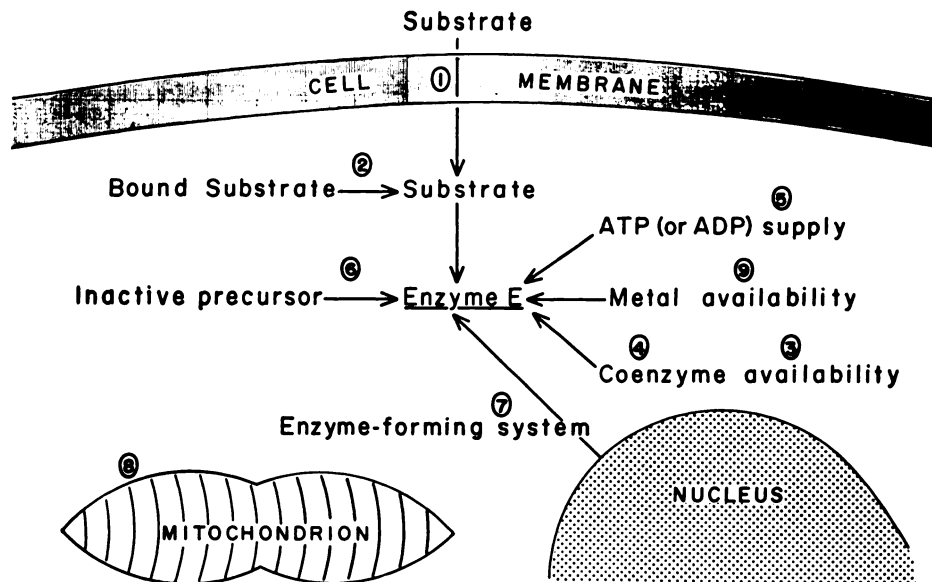
Fig. 1. A map of the terrain under review. (See table of contents and introduction for explanation.)

account of recent research on each in the hope that juxtaposition of apparently unrelated data will suggest a larger design to the reader than it often does to the reviewers. Or, one could divide the hormones into proteins, peptides, amino acids, and steroids on the theory that structurally similar substances might be expected to act similarly. The recent developments in this field have suggested an alternative approach: *a grouping of reports based on theory of cellular mechanism of action rather than on hormone identity or classification.* This stratagem has the obvious difficulty of fragmenting the discussion of individual hormones (sometimes into several widely scattered fragments), but it will have the considerable advantage of focusing attention on the heuristic value (or limitations) of certain mechanistic hypotheses which may be useful not only in analyzing the effect of hormones on cells but in reflecting upon the effects of chemical agents generally on cells.

Inspection of Figure 1 by the reader before he attempts to read the individual sections of this essay may be of value. This is, in effect, a diagrammatic outline of the paper, showing its scope as well as its limitations. The three cell constituents indicated by capital letters represent anatomical structures. The lower case is used to designate biochemical locus or mechanism of action without a specified anatomical or architectural position in the cell. The essential assumption states that Enzyme E represents one or more enzymes which catalyze one or more rate-limiting reactions in a hormone-sensitive cell, and that the general level of cellular activity rises or falls depending on the velocity of the reaction mediated at this step. The numbers from 1 to 9 indicate some of the mechanisms which

may be involved in controlling the velocity of the reaction mediated by Enzyme E. Only two of these (6,7) involve an actual change in the amount of apoenzyme. All of the others can alter the reaction velocity *without* a concomitant change in the amount of Enzyme E. They are, therefore, of particular interest in the consideration of hormonal effects which are seen soon after the confrontation of the cell by the hormone. The table of contents of the review is arranged according to the key numbers of Figure 1. The numbers in parentheses mean merely that the mechanism is a complex one and can best be described in terms of combinations of those shown in the diagram.

As this scheme evolved during the time the reviewers were monitoring current literature it became more and more difficult to fit the parathyroid hormone into it, because studies of this agent have not yet progressed to the point at which they can be analyzed in these terms. However, they appear to be approaching this stage very rapidly, and we have therefore included a brief discussion of the parathyroid hormone mainly, in all candor, because of our personal interest in it.

The object of the game, as everyone knows, is to be able to give a precisely defined mechanism of action which clearly describes the interaction of the hormone with some cellular component at the molecular level—in a word, the *primary* mechanism of action to which all else is secondary. If we were restricted to a discussion of primary mechanisms within the limitations of this definition, there would be no review to write, for the primary mechanism is unknown for any hormone. This review, then, deals with secondary mechanisms, some of them exceedingly close to what must be primary mechanisms, and some of them many reactions removed from the crucial, initial transaction between the hormone and its sensitive cell.

The material selected for discussion in this review obviously reflects the interests and prejudices of the reviewers, as well as their inability to assimilate all potentially relevant material, and, in that sense, the selection has been arbitrary. We hope that it will not be regarded as capricious.

1. Membrane permeability hypotheses

a. *Effect of insulin on muscle*. Insulin has been discussed, studied and reviewed (33, 34, 58, 121, 139, 155, 157, 198, 224, 246, 247, 248, 249, 291) practically continuously since its discovery. A summary of theories of the action of insulin reveals some of the cyclically changing attitudes of students of the general problem of the cellular action of hormones. Transitory enthusiasms for specific mechanisms have tended to follow emergent patterns of thought about cellular biology.

At first, before very much was known about the enzymatic machinery of cells, and before insulin was discovered, Höber postulated a glucose permeability theory of the mechanism of action of insulin which he referred to as "the membrane theory of diabetes." This hypothesis was recently rediscovered in retrospect after some of the developments to be described below (see Levine, 157, for references).

During the exciting period when the intermediary metabolism of carbohydrate

began to be described in detail and when many of the enzymes of the glycolytic cycle were isolated and purified, many investigators inevitably began to search for effects of insulin and other hormones on individual enzymes and enzyme complexes, frequently in re-constituted systems. Green (92) made the formal suggestion that hormones may work by influencing specific rate-limiting enzymes, although this assumption was implicit in much of the work that was in process at the time. This point of view reached its zenith with the report from Cori's laboratory that insulin exerts its effect by overcoming pituitary or adrenal inhibition of the enzyme hexokinase (42, 207, 208).

Very soon disenchantment with the hexokinase hypothesis became widespread. The theory offered no plausible explanation of the well-known hypersensitivity of hypophysectomized animals to insulin. Furthermore, careful attempts to reproduce some of the experiments on which the theory was based were unsuccessful (250, 251). It was during this period that the validity of approaching the problem of hormone effects at the cellular level by way of study of broken cell preparations came into serious question, and it was postulated that meaningful observations of hormone effects can be made only on an architecturally organized cell component (157). This view developed concurrently with the dawning realization among biochemists, who spent a large amount of their time and energy taking cells apart, that the living cell doubtless has a meaningful and distinctive architecture, and that the spatial relationships of enzymes, co-enzymes, substrates and ions within the organelles and subdivisions of the cell play an important role in the cell's function. The collective consciousness was ready to entertain the idea that cells can be stimulated from a low level of acivity to a high one not by way of an influence on the enzymatic machinery in the cell but by providing more substrate for the existing machinery to work on.

Many hormones accelerate or excite or stimulate certain chemical processes in cells, a fact which is implicit in the etymologic derivation of the word "hormone." However, most enzymes in cells are capable of dealing with larger substrate concentrations than are likely to be found in the cell at any given instant. This is particularly true of enzymes which function in reaction sequences in which the reaction products are "siphoned off" by an adjacent enzyme system. Under these circumstances the rate at which substrate is presented to the interior of the cell largely determines the rate of its use (see Krebs, 141).

Thus, the widely known experiments of Levine and his colleagues (87, 157, 158, 159) could not have been done at a more opportune time. These workers infused certain hexoses and pentoses into eviscerated-nephrectomized dogs and showed that they were confined very largely to the extracellular fluid space. Following the administration of insulin, however, the sugars rapidly entered intracellular water. These effects were readily dissociable from the process of primary phosphorylation since some of the sugars were phosphorylated to a negligible degree. Thus, the explicit suggestion was made that insulin may work in muscle by facilitating the transfer of glucose from the extracellular fluid into the cell.

A very large amount of subsequent work has tended to validate this extraordinarily prescient suggestion. Wick and others (292, 293) confirmed and extended the observations of the Chicago group. The observations of Haft et al. (96) on the uptake of sugars by rat diaphragm in vitro were in general agreement with the in vivo findings of the Levine and Wick groups, although there were some differences of opinion on certain points, particularly on the behavior of fructose in the system. Generally confirmatory observations were made by Ross (223), who studied the effect of insulin on the transfer of sugars into the aqueous humor of the eye. The permeability hypothesis received substantial support from the work of Park and his colleagues (195, 196, 197) who found that the entry of glucose into muscle cells was accelerated under conditions of glucose concentration or temperature that would have precluded any effect on the hexokinase system.

Kipnis and his colleagues (127) and Norman et al. (194) have made a significant contribution to the problem of control of penetration of glucose into muscle cells by pointing out that study of the cut-end diaphragm preparation, which has been a favorite object of investigation for many years, may yield misleading information. These workers compared the responsiveness to various hormones of the usual cut-fiber hemi-diaphragms with diaphragm preparations in which all of the insertions of the muscle were intact. Under these conditions they found that the effect of insulin on increasing the rate of penetration of pentose into muscle was greater in the "intact" diaphragm than it was in the conventional hemi-diaphragm system. The "intact" preparation appears to offer an opportunity to isolate the transport step more completely from intracellular events which might be influenced through the cut ends of fibers. Of course, studies on the beating heart, such as those of Fisher and his colleagues (74), of the Park group (198), have all of the advantages of the intact diaphragm, but it may not be valid to extrapolate results on the heart to skeletal muscle, as the reactivity of the two types of muscle fibers to hormones may be very different.

Thus, there is a large body of experimental evidence to support Levine's permeability or transport hypothesis of the mechanism of action of insulin. In muscle, at least, the transport step and the hexokinase step appear to have been separated clearly (126), and many of the experiments cited above point to an effect of insulin on the former. If the cell wall is indeed the anatomical locus of action of insulin, all secondary effects, such as those on protein synthesis, on lipogenesis, cholesterogenesis and so on ought to be explicable on the basis of an increased rate of glucose entry into insulin-sensitive cells. Unfortunately, isolated observations have been made from time to time which do not fit comfortably into a unitarian hypothesis of insulin action on glucose transport, or on amino acid transport. In general, these observations fall into two categories: first, descriptions of undeniable effects of insulin in certain broken cell preparations which simply did not contain a permeability barrier to glucose; and second, studies of insulin effects on the incorporation of amino acids into proteins under conditions which would preclude the possibility that the observed effects are secondary to an enhancement of either glucose or amino acid transport.

For some reason, insulin effects on broken cell preparations can be seen best in pigeon breast muscle minces. To the older accounts of Krebs and Eggleston (142) and Shorr and Barker (238), who found that insulin sustains the rate of oxygen consumption of minced muscle in the presence of substrates like citrate and pyruvate, we can now add the interesting observations of Bessman and Fitzgerald (20), that insulin significantly retarded glycogenolysis in pigeon breast muscle homogenates incubated with glucose. In some of their experiments there was a net synthesis of glycogen in the system. Many attempts to make similar observations have been made with muscle of other species, but the authors are unaware of reports of success. These insistent data simply cannot be ignored as indicating merely idiosyncratic behavior of pigeons, and they must be taken into consideration in the erection of a unitarian hypothesis of insulin action.

Many investigators have reported insulin effects in muscle preparations under circumstances in which an antecedent or concurrent effect on glucose transport would have been unlikely. For example, insulin stimulates the incorporation of labelled precursors into proteins and peptides by diaphragms of fed normal rats when no glucose is added to the medium and when the preparation has been soaked in glucose-free buffer before exposure to insulin (136, 240, 299). More recently, Wool and Krahl (298) performed an ingenious experiment in which the protein anabolic effect of insulin was observed after the labelled amino acids had gained entry into the cell, and in the absence of glucose in the medium. They injected C^{14} amino acids intraperitoneally before removing the diaphragm for incubation; then, the diaphragms were removed and hemisected, and one half was incubated in insulin-free medium while the other half was incubated with insulin. There was a significantly greater incorporation of the labelled amino acid into the protein of the insulin-treated tissue. The authors contend that contamination of the extracellular fluid with the labelled amino acid could not have contributed to the result.

In another experiment, Manchester and Krahl (174) demonstrated the *in vitro* stimulation by insulin of the incorporation of C^{14} from citrate, α-ketoglutarate, succinate, isobutyrate, propionate, pyruvate, acetate, formate, and CO_2 into protein of rat diaphragm. Any labelled amino acids which found their way into protein in these experiments must have been formed intracellularly, and, again, the anabolic effect must have been achieved by a mechanism other than one involving stimulation of transport of glucose or amino acids. Similarly, Krahl (140) has described accelerated incorporation of amino acids into protein of adipose tissue under the influence of insulin in glucose-free medium. This observation is of interest in the light of Barrnett's finding that insulin stimulation of pinocytosis and endoplasmic reticulum proliferation in adipose tissue *in vitro* occurs in the absence of added glucose, and is thus unlikely to be secondary to an effect of insulin on glucose transport into that tissue (11).

Other kinds of experiments have been cited as evidence against the transport hypothesis. For instance it has been pointed out by Shaw and Stadie (233, 234) and by Beloff-Chain (17) that insulin treatment often appears to stimulate

selectively the process of glycogen deposition, while the rate of substrate traffic over other potential routes of glucose-6-PO$_4$ disposal may not be markedly affected by the hormone. The argument appears to be that once glucose gains admission to the cell interior there ought to be a quick and impartial increase in the rate of use of the material for glycogen deposition, pyruvate formation, CO$_2$ formation and so on. The fact that preferential routes of glucose disposal may be seen in some experimental circumstances does not seem to the authors to be inconsistent with the transport hypothesis. Little is known about the traffic control system which determines the fate of metabolites which may be disposed of by alternative routes. The "set" of a control system may be such as to favor glucose disappearance by way of one route over another when the intracellular glucose concentration increases sharply. In fact, changing conditions of substrate supply may feed back on some part of the control system in such a way as to modify the controlling influence in a new direction (see section on lipogenesis below). In the case of adipose tissue glucose-1-carbon to CO$_2$ was stimulated preferentially by *either* insulin or high concentrations of glucose (123).

However, even without the "alternative route" objections we have seen that there are nagging little fragments of information that make it difficult to accept the hypothesis that all observed effects of insulin are secondary to a primary effect on increasing the transport of glucose across the cell membrane. Krahl (139), who has done as much constructive thinking about this problem as anyone, refuses to ignore data which cannot be explained by this hypothesis. Acknowledging that insulin appears to have important effects at the cell surface, he recognizes that there are deep reverberations of the effect of insulin within the cell. He suggests that the attachment of insulin to elements of the cell surface may trigger a "propagated disturbance" which spreads throughout the cell and acts in such a manner as to make existing enzymes, substrates and ATP more readily available to one another than they were before the encounter with insulin. This suggestion becomes more attractive if one considers the cell surface to include the lining of all of the canaliculae in the endoplasmic reticular system, a sort of cytologic Norwegian coastline. Actions of hormones at such an extensive cell "surface" would place the hormone in rather close spatial relationship to the protein synthetic machinery of the cell, and, indeed, in actual physical contact with mitochondria and other organelles. This growing awareness of the possible significance of the reticular canal system as an extension of the cell surface is discussed by Siekevitz (239). Also, Peters (203) has suggested the possibility that hormones may work by achieving a "re-orientation" of the "cytoskeleton" of cells which would permit simultaneous acceleration of a number of enzymatically controlled reactions.

Recently an important new study has enabled us to speculate about the nature of the non-transport-linked effects of insulin in what eventually may prove to be a constructive way. It should be emphasized here that all of the work on the effect of insulin on glucose transport has emphasized the fact that the entrance of glucose into the cell is a process which can be dissociated from its primary phosphorylation. There has been little emphasis on the biological nature of the

transport machinery, or on the mechanism by which insulin might influence its function. The experiments of Randle and Smith (215, 216) suggest something about the possible nature of the transport apparatus that may be influenced by insulin. These authors found that anaerobiosis markedly stimulates glucose uptake by diaphragm in bicarbonate medium. (This finding is of interest in view of the well-known fact that working muscle can use large amounts of glucose in the absence of insulin (44, 88, 120, 121).) Furthermore, under aerobic conditions glucose uptake was increased by a variety of inhibitors, including 2,4-dinitro-phenol, arsenite, cyanide, salicylate—all of which interfere with oxidative phosphorylation. There was an additional increase when insulin was added to the inhibitors, but the total effect was less than that of insulin alone. Cyanide, salicylate and the other inhibitors also promote the intracellular accumulation of free xylose. The authors make the arresting suggestion that the entry of glucose into the cell is actively restrained under resting conditions, and that this restraint is dependent on a continuing supply of phosphate bond energy. In other words, the basic design of the sugar transport equipment in the cell's surface is such that the movement of free glucose into or out of the cell is slowed or inhibited by a mechanism which functions only during the application of ATP energy to it. While there may be rare experimental circumstances when this mechanism appears to have the effect of keeping a sugar in muscle cells (296) most of the time the extracellular glucose concentration is much higher than the intracellular, and the mechanism functions as a "keeper-out-ase." Insulin may do its work by combining with some element of the "keeper-out-ase" machinery at the cell surface and thus prevent the application of energy to it. Of course, it is entirely possible that insulin, anaerobiosis and the inhibitors may affect the transport of glucose into the cell by entirely unrelated mechanisms. But this possibility need not inhibit us from using the hypothesis of Randle and Smith as the basis of a unitarian hypothesis of insulin action that embraces observed glucose transport effects and the non-transport-linked effects described above as well.

Most of the observations of insulin effects in the absence of obvious glucose transport changes have been made in intact cell preparations and are concerned chiefly with the protein anabolic effect of insulin. This may be studied either by measuring the rate of incorporation of labelled precursors into protein (as in the experiments of Krahl, Wool, Manchester and their colleagues, cited above) or by observing the synthesis of new cell structures with the electron microscope, as Barrnett has done in adipose tissue cells. One can begin to visualize a common theme in these data if one makes two assumptions: 1) that the amino acid activating step is the rate-limiting step in protein and peptide synthesis and 2) that the availability of ATP may be rate-limiting at the rate-limiting step. Now, if ATP energy is being continuously applied to the "keeper-out-ase" apparatus and insulin treatment has the effect of deflecting ATP away from a component of the transport system, the result will be an increase in the effective ATP concentration within the cell. Even if only very minute amounts of ATP are involved in these events, the anatomical sites of two processes involved (glucose transport and protein synthesis, for example) may be contiguous, so that a very

small net increase in ATP availability for the whole cell interior might constitute a very substantial increase at some local, strategic site. It is important to note that, within the frame of this theory, insulin effects a dissociation of ATP from "keeper-out-ase" even in the absence of glucose, and, thus, previously seques- tered ATP would be available for accelerated amino acid activation with re- sulting increased incorporation into protein. By bringing the energetics of the transport system into the discussion, Randle and Smith have made it possible for us to imagine how insulin may influence the permeability of the cell surface for glucose, and, at the same time, how the hormone might influence many metabolic activities deep within the cell. Stadie (247, 248) and Weil-Malherbe (291) have reviewed the general subject of the effect of insulin on phosphorylating mechanisms in cells. The hypothesis presented here offers an opportunity to relate many of the older observations to the transport idea.

These speculations can be extended to include data obtained in cell-free systems. Perhaps bits and pieces of functioning "keeper-out-ase" machinery persist in some homogenates, especially if they are prepared from pigeon breast muscle. Possibly they continue to abstract ATP from the total system, both that which may have been present at the beginning of the incubation period and that which may be generated during the experiment. Added insulin may break the connections between the persistently functioning fragments of transport ma- chinery and ATP, and thus increase the effective concentration of this critical material. It would be less difficult to devise experimental tests of this hypothesis in homogenates than it would in intact cell preparations.

We can confidently predict that we will soon see not only a detailed description of the nature of the glucose transport machinery of the muscle cell surface but also an elucidation of the mechanism of interaction of the insulin molecule with its surface receptor.

b. Effect of insulin on liver. The recent discovery of the oral insulin substitutes has stimulated renewed interest in many aspects of carbohydrate metabolism, but most particularly in the bewildering field of insulin effects on the liver. It is difficult to do more than document the confusion that exists in this area at pres- ent, but no attempt to arrive at a comprehensive theory of the effect of insulin can be made without examination of the disputed role of insulin in the metabo- lism of the liver.

It is an arresting fact that, although the central role of the liver in carbohy- drate metabolism is one of the most ancient facts of metabolic physiology (it reaches back over more than a century to Claude Bernard), we can find a whole spectrum of beliefs ranging from the idea that insulin probably does not have a direct effect on the liver at all (Levine and Fritz, 156) to the suspicion that the liver may be one of the most important target organs for insulin on the grounds that this organ abstracts a very considerable amount of insulin from the portal blood, some of which comes directly from the pancreas (76, 171, 188). Readers who are particularly interested in this field are referred to the review by Levine and Fritz cited above, and to one by Cahill *et al.* (31) in which the collective point of view of the Hastings school is summarized. The subject of insulin and the liver

is a favorite topic for discussion at symposia on diabetes and carbohydrate metabolism (3, 75).

Before considering the nature of the effect of insulin on the liver it is necessary to deal with the evidence for a direct effect of insulin on that organ. It seems to the reviewers that such an effect has been shown unequivocally in a large enough number of experimental circumstances to make untenable the extreme view that insulin works on the liver only by way of some antecedent effect on peripheral tissue metabolism. The addition of insulin to rat liver slices *in vitro* has been shown by a number of investigators (23, 26, 178, 184) to accelerate lipogenesis from a variety of non-fat precursors. Berthet *et al.* (19) and Cahill *et al.* (30) both report an enhanced incorporation of glucose into glycogen in rabbit liver slices on the addition of insulin *in vitro*. Most of the successful attempts to demonstrate direct effects of insulin on liver slices have been carried out on fed liver. Liver slices obtained from fasted or diabetic rats have not shown consistent effects of *in vitro* insulin addition when lipogenesis was used as the indicator of an insulin effect (23, 27, 184). However, when the incorporation of glycine-1-C^{14} into glutathione was studied in liver slices of diabetic rats, Krahl (136) found that glucose addition partially repaired the diabetic defect but that the combination of glucose and insulin fully restored the capacity of the slices to the normal level. Another possibility of partial repair of a diabetic defect by insulin addition *in vitro* is found in the report of Scaife and Migicovsky (228), who described an increased fatty acid synthesis and diminished cholesterol synthesis upon the addition of insulin to homogenates prepared from either normal or diabetic rats.

It is possible, of course, to cite many experiments in which no effect of insulin was observed on liver slices or on perfused livers when a variety of indices of insulin effect was under study (see Krahl, 138 for review), but Haft and Miller (95) did describe direct effects on insulin in perfused livers obtained from diabetic rats as long as the livers were not unduly traumatized when they were removed, and as long as the animal donors were not in ketosis. When this evidence is added to that already cited, it is difficult to see how one can invoke any number of negative experiments in support of the proposition that insulin acts on the liver only by way of a primary effect on extrahepatic tissues. Acceptance of the view that insulin has a direct effect on the liver does not necessarily mean that correction of the extrahepatic effects in the diabetic is not enormously important in the regression of certain adaptive changes which occur in the diabetic liver under insulin treatment. One can think of two such peripheral defects, namely, impaired protein synthesis in muscle and increased rate of release of free fatty acids from adipose tissue, which doubtless contribute to the diabetic disturbance in liver *in vivo* by flooding that tissue with amino acids and fatty acids. The damming of this flood by insulin treatment in itself might permit a certain amount of recompensation to occur in the diabetically decompensated liver cell. It is possible, in fact, that some of the adaptive rearrangements of the biochemical machinery in liver cells may be induced by the overwhelming quantity of amino acids and fatty acids that are presented to the diabetic liver for disposal. The rather long time it takes for insulin to effect certain changes in the livers of diabetic rats (102, 220)

may well involve extrahepatic as well as hepatic effects of the hormone. The reality of the direct hepatic effects, however, does not appear to be in question.

If the reader is willing to concede that the liver is a target organ for insulin it is necessary to ask what function insulin performs in this tissue. From the *in vitro* experiments cited above it is permissible to conclude that insulin has certain effects on the incorporation of acetate into fatty acids and on the incorporation of amino acids into peptides and proteins. These can be regarded as secondary effects of the hormone, and the former will be discussed in some detail below. The nature of the primary hepatic effect of insulin is one of the most significant questions in metabolic physiology at the present time.

There are two reasons for postulating that the hormone's effect in liver may be quite different from its effect in two other important target tissues: namely, muscle and adipose tissue. First, the time scale of the repair of the diabetic defect extends over a period of hours in liver, but only over a period of minutes in muscle (135) or in adipose tissue (294). This might mean that the adaptive rearrangement of enzymatic machinery that occurs in the liver of the diabetic animal (for example, the fall in glucose phosphorylation (219), and the rise in glucose-6-phosphatase (4)) may mask any immediate insulin effect, and may require a finite time for repair so that an insulin effect can become manifest. This does not necessarily mean that insulin does not have a fast effect, and that the hormone may not, in fact, be primarily responsible for initiating the chain of events which eventually results in a restoration of the enzymic pattern of the tissue to "normal."

A second difficulty is harder to reconcile with a comprehensive theory of the mechanism of action of insulin that would account for the events observed in muscle, adipose tissue and liver. While the muscle data fit the hypothesis that insulin may work by facilitating the transport of glucose into the cell, Cahill and his colleagues (30) contend that a permeability barrier to glucose, such as exists in muscle, does not exist in the liver cell. The liver cell is described as being freely permeable to glucose, and insulin apparently has no effect on the distribution of the sugar across the cell membrane. Other compounds, such as fructose, mannose, galactose, sorbitol and mannitol entered the cell, while maltose, sucrose and raffinose were barred from the intracellular compartment. These observations, and others referred to in Cahill's report, make permeability or transport hypotheses of insulin action in the liver cell difficult to support. Partisans of a unitarian hypothesis can only fall back to a previously prepared, but precarious, position: namely, the canaliculae of the endoplasmic reticulum (239). Or, to put the possibility in more general terms, insulin may make glucose available to glucokinase and ATP by a mechanism similar to that seen in muscle. Analyses of whole liver tissue, such as those employed by the Boston group and by Gey (84), may not indicate how much of the intracellular glucose pool is readily available to the glucokinase system for phosphorylation.

Cellular mechanisms of action of hormones can be discussed most profitably only insofar as they can be reconciled with observed physiologic effects. At the present time there is no great unanimity of opinion about the effect of insulin on

the liver in intact animals. A number of investigators, using different experimental techniques and different species, have described what they interpret to be a diminution in hepatic glucose output following the administration of insulin. Bondy et al. (24) and Bearn et al. (14) used the hepatic venous catheterization technique in humans and described a decreased glucose output from the splanchnic bed following insulin administration, even to diabetics. Madison and Unger (172) compared the effect of insulin given intraportally and by way of a peripheral vein, in the anesthetized dog and, on the basis of changing glucose gradients across a limb, concluded that there is a decreased hepatic output of glucose following the administration of the hormone. For every positive experimental result of this sort it is possible to find a negative one; for example, the failure to find a diminished hepatic glucose output following insulin treatment in the dog (237).

The technique first suggested by Searle and Chaikoff (229) was applied to the insulin-liver problem by Dunn et al. (59). In this method, a single injection of C^{14} glucose is made and the specific activity of serial blood glucose samples is estimated. It is assumed that the linear decline in blood glucose specific activity is due to the contribution of unlabelled glucose by the liver. Thus, the plateauing of the decline in specific activity that is regularly seen following insulin administration in the dog is interpreted as an inhibition of the rate of glucose delivery to the circulation by the liver. DeBodo and his colleagues (45) used the same basic concept but modified the experimental design by injecting a priming trace dose of C^{14} glucose and then maintained the established radioactivity for several hours with a constant intravenous infusion of a mixture of C^{14}-glucose and C^{12}-glucose. By studying the course of the plasma glucose radioactivity it was possible to estimate the size of the body glucose pool, the glucose contribution by the liver to the plasma and the peripheral uptake of glucose by the tissues. These workers concluded on the basis of their experiments that diminution in delivery rate of glucose to the plasma by the liver after insulin contributed only very slightly to the observed hypoglycemia. This is certainly a reasonable conclusion from their data, but they do demonstrate one of the most striking effects of insulin on the liver that the present reviewers have seen: namely, a very sudden and very substantial increase in hepatic glucose outflow coincident with the termination of insulin infusion. Certainly, this suggests that some kind of a brake was being applied to hepatic glucose release during the insulin infusion, and it may well be that the DeBodo group have observed an effect of insulin on the liver that is similar to that seen by other workers, but one which expresses itself differently owing to differences in experimental conditions and design.

Shoemaker, Mahler and Ashmore (237) have studied this problem in the unanesthetized dog under conditions that permitted not only estimates of glucose gradients across the liver and splanchnic bed separately, but also measurements of flow rates. They do not feel that diminished hepatic glucose output follows insulin administration in their preparation, and they are inclined to the view that some of the dilution curves which have been used as the basis for suggesting primary hepatic effects of insulin may have been due to back diffusion of C^{14}-glucose from intestinal mucosal cells. The interested reader is referred to this

report for detailed criticism of most of the reports cited above which purport to show direct hepatic effects of insulin. Some of their objections seem to have been answered by the experiments of Madison *et al.* (171). In spite of the experiments of Shoemaker *et al.* (237) the reviewers hold the tentative opinion that insulin does indeed have a direct effect on the liver, but that this effect is difficult to demonstrate, particularly in experiments on intact animals.

If, after reviewing the assorted conflicting opinions presented here, one's conscience permits the concession that insulin has a direct effect on the liver cell, and that this effect expresses itself in a retention of glucose under some circumstances, how can one formulate a unitary hypothesis of the mechanism of action of insulin which would comprehend both muscle and liver effects? It is difficult to imagine that the hormone acts at entirely different biochemical sites in the two tissues. It is much more comforting to imagine that it acts on precisely the same molecular machinery in the two cell types but that this machinery is integrated into the vital economy of the cell in one way in muscle and in another way in the liver cell. From Randle's studies it is permissible to imagine that some energy-yielding mechanism is coupled to a device in the cell surface that functions to keep glucose either in or out of the cell when the energy is applied. Insulin permits the ingress or egress of glucose, depending on the experimental circumstances, by interfering with the application of energy to the device. Since this is a flight of fancy, perhaps the reader will permit us to suggest that the glucose "keeper-out-ase" in muscle is powered by ATP energy, and that insulin works by prohibiting the delivery of ATP energy to some component of the transport apparatus.

The situation in liver does not lend itself readily to the construction of even fanciful hypotheses. Most of the observed facts are consistent with the theory that, under ordinary circumstances, there is no readily demonstrable permeability barrier to glucose in the liver cell. There is suggestive evidence that insulin inhibits the release of glucose from the liver. The apparently diminished glucokinase activity in the insulin-deprived liver cell suggests that, in the absence of insulin, glucose, ATP, and glucokinase simply do not perform optimally as a functioning unit although the cell is obviously producing glucose from non-carbohydrate precursors at a rapid rate. This in itself suggests some sort of compartmentalization of crucial reactions in the cell. If insulin were to make glucose (and, possibly, ATP as well) available to the primary phosphorylating enzyme as it appears to do in muscle, some of the glucose would be salvaged for use within the liver cell itself. This glucose would enter the Embden-Meyerhof and Dickens-Lipman pathways, glycogen would be deposited, reduced pyridine nucleotide coenzyme production would be stimulated, fatty acid synthesis would be promoted and ketosis would be suppressed (see section on lipogenesis below). At the same time, the release of fatty acids by adipose tissue would be inhibited by the action of insulin, and, therefore, the delivery rate of fatty acids to the liver for metabolism would be sharply curtailed. Thus, the antiketogenic effect of insulin can be attributed to the combined effect of insulin on adipose tissue and liver cells. The extreme difficulty of demonstrating a direct effect of insulin on the liver of the diabetic animal may be related to the possibility that the action of insulin

is masked by an overactive glucose-6-phosphatase activity, and diminished glucose phosphorylation. In order for a convincing demonstration of an insulin effect in this tissue to be made there must be a prior regression of glucose-6-phosphatase activity, and possibly, some recovery of glucokinase activity, processes which, apparently, are time-consuming ones. It is also possible that these rearrangements are intimately related to fluctuations in gluconeogenesis rate which are known to occur in the insulin-deficient liver.

These speculations are to be regarded as no more than the expression of a wistful hope for a theory of action of insulin at the molecular level which will account for all of the observed effects of the hormone. Some encouragement is afforded by the recent elucidation of the structure of the insulin molecule (226).

c. Effect of various hormones on amino acid transport. Concurrently with the events described above, analogous hypotheses have developed concerning the role of hormones in amino acid transport and protein metabolism. Much of the information in this field has been summarized by Christensen and his colleagues (38, 193). The similarities in the thought processes of students of glucose transport and amino acid transport are striking: for example, the "non-utilizable" sugars of the former have their precise counterpart in a "non-utilizable" amino acid, α-aminoisobutyric acid ("AIB") favored by the latter (39). Furthermore, the inference is very strong in the writings of the two groups that once the appropriate substrate has been accumulated in the cell under the influence of a particular hormonal agent, all subsequent metabolic effects are the inevitable consequences of providing the interior of the cell with an increased amount of glucose in the one case or amino acids in the other. In both cases the hormones are presumed to exert their effects at or near the surface of the cell.

Christensen and his colleagues studied the concentrative transfer of C^{14}-labelled α-aminoisobutyric acid in most of their studies. The "non-utilizability" of this substance was inferred from the lack of appearance of $C^{14}O_2$ following its administration *in vivo* and by the fact that it appeared in the urine in apparently unmodified form as judged by its mobility on paper. Most observations were made from 20 to 39 hours after the administration of the amino acid "clinker," so that the effects observed may have been secondary to any number of modifications of the cell that might have occurred more acutely.

It was found that, with increasing age, the α-aminoisobutyric acid distribution space became smaller, and it could be shown that this was associated with a decreased tendency to concentrate the amino acid in the tissues of older rats. It appeared that the tissues of the younger, more rapidly growing animals showed more "amino acid hunger" than did those of elderly animals, a fact which suggests that the ability of tissues to concentrate amino acids may be related, in an informational sense, to the going rate of protein synthetic activity of the cell (193).

A rather bewildering variety of hormones have been found to increase the intracellular accumulation of α-aminoisobutyric acid (193). For example, epinephrine produced an increased concentration in heart, liver, muscle, lung, duodenum, ovary, brain and kidney within two hours of injection, the amino acid having been given 30 hours previously. Similarly, pituitary growth hormone

(134, 193) administration resulted in increased tissue concentrations of α-amino-isobutyric acid in most tissues. Even cortisone, which is not generally regarded as anabolic in muscle, caused an increased accumulation of the compound in that tissue, as well as in liver. Estradiol caused a marked enhancement of intracellular accumulation of the test acid in the uterus 20 hours after injection, but not in the liver. Kipnis and Noall (129) describe enhancement of transport of AIB into muscle by insulin added *in vitro*.

These are extremely interesting observations and this field certainly deserves further exploration. However, the connection between these experiments and the physiologic effects of the hormones under study remains to be established. On the face of it, it would appear unlikely that all of these hormones, dissimilar as they are structurally and in their overall effects, work in exactly the same way. Moreover, there is no certainty that the criteria of "non-utilizability" of α-amino-isobutyric acid used by these authors necessarily rule out the possibility that the substance may function as a "biochemical monkey wrench" in the cell's machinery, or that its behavior under the circumstances outlined is representative of that of the biologically important amino acids. Manchester and Young (175), in fact, have questioned the validity of the assumption that AIB behaves like a naturally occurring amino acid.

Finally, while analogies between amino acid and glucose metabolism may be helpful, they may also be misleading. Therefore, it is pertinent here to stress certain differences between the glucose transport facts and those of amino acid transport. It has already been pointed out (Randle) that anoxia causes an influx of glucose into muscle cells, a fact which is consistent with the hypothesis that energy is expended to keep glucose out of these cells. But Noall and Kipnis (192) found that, during anaerobiosis, the rate of AIB entry into muscle cells was markedly diminished, indicating that the muscle cell membrane is relatively impermeable to the free diffusion of amino acids. This finding suggests that energy may be expended in the process of concentratively transferring amino acids into the cell. Another important difference is one already referred to: namely, the fact that glucose transport effects can be seen extremely rapidly after insulin administration, whereas effects on α-aminoisobutyric acid accumulation have been described from 2 to 20 hours after the administration of various hormones. This leaves a very substantial amount of time unaccounted for in the latter case and indicates the need for caution in assigning great physiologic significance to the very provocative studies of Christensen and his colleagues.

d. An insulin-like effect of ACTH on the adrenal cell. An interesting example of the borrowing of a mechanism of action of one hormone for the construction of a working hypothesis for that of another is seen in the report of Eichhorn *et al.* (60). These workers postulated that ACTH may influence the permeability of adrenal cells in much the way that insulin affects that of muscle cells. They found that hypophysectomy markedly diminished the entry of D-xylose into the intracellular water of the adrenal, and that ACTH administration markedly increased xylose entry to levels approaching those found in stressed animals with intact pituitaries. (Neither hypophysectomy nor ACTH affected xylose distribution in

tissues other than the adrenal.) This response was seen to occur within 20 to 30 minutes after ACTH administration when there was no influence on inulin space.

A more detailed description of recent studies on the effect of ACTH on the metabolism of adrenal cells will be given below (see section 3b). The demonstration of an enhanced permeability of the adrenal cell to sugar is an extraordinarily interesting one, since it offers a better explanation of the *sustained* effect of ACTH on adrenal cells than does the phosphorylase activation hypothesis to be described. However, it is necessary to inquire whether the insulin-like effects of ACTH described by the Worcester group represent the primary effects of ACTH on the adrenal cell or whether they represent an effect secondary to profound intracellular rearrangements and adjustments which revolve around phosphorylase activation.

e. An apparent transport effect secondary to modification of the cell's inner structure. Recently, Hechter and his colleagues (109, 110) have alerted us to the possibility that apparent differences in permeability of cell membranes, particularly with respect to cations, may not be due so much to a selective transport by the membranes as to differences in the internal architecture of the cell. They have suggested that hormone effects which appear to result in alterations of the permeability of cells to sodium and potassium ions, for example, may be exerted throughout the cell, rather than specifically at the cell surface. They point to the fact that certain minerals selectively concentrate potassium (and reject sodium) because potassium fits better into particular sites on the crystal lattice. The extrapolation from the behavior of incubated feldspar minerals to the interior of living cells may be a larger one than many observers are willing to make at this time, but Hechter's suggestion seems to the reviewers to be heuristically stimulating and useful. It is fanciful to suggest that hormones may influence apparent permeability of membranes not by influencing the selective transport of cations at the cell surface but by modifying the character of some intracellular lipoprotein lattice so that a greater or smaller number of cations of a certain sort could be bound by it. It is within neither the scope of this review nor the competence of the reviewers to enter a discussion of the degree of freedom or bondage of intracellular ions. However, this hypothesis, which is reminiscent in some ways of the suggestion of Peters (203) about the possibility of hormone effects by way of alterations in the "cytoskeleton," and of Krahl's "propagated disturbance" referred to above (139), has the virtue of freshness and originality, and it is the kind of fancy that may one day appear to be prescient in retrospect.

f. Pinocytosis: a specific transport mechanism? Following the suggestion of Christensen and his colleagues (193) that certain hormones may act by accelerating the transport of amino acids across the cell membrane Leake and Pomerat (150) advanced the interesting speculation that such transport might have been influenced by a stimulation of the process of pinocytosis, or "cell drinking," which was first described by Lewis (161). Christensen and his colleagues (40) cited the work of Heinz (111) in support of the view that pinocytosis could not possibly account primarily for the accumulation of glycine in ascites tumor cells, since half of this amino acid in such cells was exchanged in about 2 minutes. For this

to occur by pinocytosis, the cell would have had to incorporate several times its own volume of suspending fluid. Although the objections of the Michigan group to the idea of a large participation of pinocytosis in amino acid transport seem convincing, this brief comment on the subject is included in this essay because it illustrates once again the recurrence of ideas in this field.

Studies on the mechanism of action of insulin on adipose tissue have included interesting observations on pinocytosis in cells of the epididymal fat pad of the rat. Ball and his colleagues (9) have described a relationship between the evolution of CO_2 from fat pads incubated *in vitro* and the glucose and insulin concentration of the medium. Their system proved to be responsive to extremely low concentrations of insulin; *i.e.*, those of the order of 10^{-5} to 10^{-3} units per ml. Barrnett and Ball (12), using this technique of monitoring the insulin effect, studied morphologic changes produced in adipose tissue cells on *in vitro* addition of the hormone. Within 20 minutes after the addition of 10^{-3} units per ml of insulin a definite metabolic change had occurred as revealed by CO_2 evolution and electron microscopy of the tissue showed characteristic morphologic changes. While the control tissue incubated without insulin showed a dense granular cytoplasm with a small population of organelles, the cytoplasm of tissue incubated with insulin showed a loss of granularity and the development of a system of membrane-bound vesicles which appeared as pinocytotic invaginations at the plasma membrane. A system of canalicular channels and vesicles had developed deeper in the cytoplasm. The authors suggest that insulin stimulates pinocytosis, and imply that this may be the mechanism by which additional amounts of glucose are presented to the cell interior. However, it is impossible to state at the present time which is cause and which effect. It is conceivable that the morphologic changes seen by the authors are secondary to either a transport effect of insulin at the cell membrane or to a metabolic effect somewhere in the cell. Whatever the ultimate explanation turns out to be this is an extraordinarily provocative and interesting report, and the concurrent observation of biochemical and morphologic events in cells so unique that this study may well serve as a model for many future attempts to analyze effects of hormones on cells.

2. The release of bound substrate intracellularly

a. *ACTH and steroid hormone precursor.* In their studies on the mode of action of ACTH Koritz and Péron (131) found that freezing quartered rat adrenals for 5 minutes and subsequently incubating them prevented the stimulatory action of ACTH on corticoid hormone output by the gland segments. However, incubating pre-frozen glands with TPN and glucose-6-phosphate resulted in the production of far more hormone than did ACTH stimulation of unfrozen glands. Addition of ACTH to TPN and glucose-6-phosphate produced no further increment in corticoid output. On the basis of these observations the authors conclude that TPNH availability (see section 3b below) may not be the only determinant of the rate of hormone synthesis in the adrenals. They suggest that ACTH may do two things: 1) make more TPNH available for crucial hydroxylations involved in hormone biosynthesis, and 2) release hormone precursor material intracellularly from some previously unavailable form. They visualize transitory freezing as simulat-

ing the latter effect of ACTH. A number of speculative possibilities on how this might be accomplished are offered by the authors. Although the authors expressed some doubt about this interpretation in a later paper (132) this is an intriguing suggestion and it offers a mechanism which would function in much the same way as a sudden permeability increase; that is, pre-existing enzymes, working considerably below their substrate-handling capacity, would be stimulated by being offered an increased amount of substrate for disposal.

Some years ago the reviewers encountered a vaguely similar situation in a study of the effects of gonadotrophins on the testes of immature rats (270, 271). In that study it was found that animals treated with purified pituitary LH showed a very marked increase in the esterified cholesterol content of the testes. Histochemical examination revealed that this increase was limited to the androgen-producing cells of the interstitial islands. Many investigators believe that esterified cholesterol is the hormone precursor in the adrenal cortices (227) and it is possible that this is so in the steroid hormone producing cells of the testis. The mechanism of the increase in cholesterol ester under the influence of LH was never established (i.e., whether increased rate of abstraction of pre-esterified cholesterol from the blood, increased synthesis or esterification intracellularly) but the observation remains as a possible example of a circumstance in which the rate of hormone production in cells of the male gonad may be stimulated by a mechanism that involves a primary increase in availability of hormone precursor intracellularly.

3. Hormonal effects via increased coenzyme availability

a. Insulin and lipogenesis. When acetyl CoA is formed in cells there are many alternative routes for its disposal. It may: a) be oxidized by way of the Krebs cycle, b) participate in acetylation reactions, c) serve as a precursor for cholesterol or other steroids, d) undergo reductive synthesis to long-chain fatty acids, and e) condense to form the coenzyme A precursors of ketone bodies. It is obvious that one or another of these routes is favored in different physiologic circumstances, and that an understanding of the intracellular control mechanisms which determine the disposal route of choice at a given time would be of potential value in the analysis of similar "decisions" that may be made in the metabolism of other substrates.

A role of insulin in the formation of fat from glucose was suggested by the work of Drury (57). Later workers (253, 269) confirmed Drury's observations and pointed to a failure of lipogenesis in the diabetic animal and the correction of this defect by insulin treatment. Other studies suggested a very close relationship between lipogenesis and the availability of carbohydrate to the animal. For example, high carbohydrate diets were found to stimulate fatty acid synthetic activity in liver and adipose tissue (177, 272). When glucose was provided in excess even in diets only moderately high in carbohydrate, as in the overeating of experimental hypothalamic (gold thioglucose) obesity, the lipogenic activity of liver slices was greatly enhanced (179, 272). Conversely, starvation caused a "defect" in lipogenesis (105, 118, 177, 272).

When Lynen (170) described the reaction sequence for fatty acid oxidation it

was widely believed that fatty acid synthesis proceeds by the reverse of the oxidative pathway, thus: 2 acetyl CoA $\rightleftharpoons$ acetoacetyl CoA $\rightleftharpoons$ β-hydroxybutyryl CoA $\rightleftharpoons$ crotonyl CoA $\rightleftharpoons$ butyryl CoA. It had been established that flavine adenine dinucleotide (FAD) functioned as a coenzyme in the reaction butyryl CoA $\rightleftharpoons$ crotonyl CoA, and the reaction was written as a reversible one, accompanied by the equilibrium FAD $\rightleftharpoons$ FADH$_2$. The participation of DPNH at the β-ketoreductase step of the synthesis (β-hydroxyacyl-CoA-dehydrogenase) was clearly established, and the potential significance of the availability of reduced coenzyme as a control mechanism for the synthesis of fatty acids was explicitly recognized. In fact, Helmreich et al. (112) had found that the concentration of reduced diphosphopyridine nucleotide was depressed in diabetic liver, and suggested that this might be the mechanism for the reduction in lipogenesis rate seen in this tissue. Although the interpretation of the experimental findings was questioned by the authors recently (116) this was an important suggestion because it helped to alert the collective intelligence to the possible meaning of subsequent developments.

In the same year, two important complementary observations were made by workers in different laboratories. Shaw, Dituri and Gurin (232) suggested that the reaction crotonyl CoA $\rightarrow$ butyryl CoA might be the locus of an important block in fatty acid synthesis in cell-free systems prepared from diabetic liver. At about the same time, Langdon (144) unequivocally established the fact that this reaction specifically requires TPNH, and not FADH. Inevitably, TPNH was quickly added to DPNH in the erection of hypotheses that are basically similar to the Lynen-Helmreich speculations described above.

Meanwhile, the direct oxidative pathway of glucose catabolism first suggested by Lipmann (164) and Dickens (47) had been described in great detail (for review see 176). Since TPNH is generated by the first two steps of the direct oxidative pathway ("hexose-monophosphate shunt," "phosphogluconate oxidative pathway," Dickens-Lipmann pathway) attempts were made to explain the obligatory coupling of glucose oxidation and fatty acid synthesis in terms of variations in the continuing supply of hexosemonophosphate-shunt-produced TPNH. There was a nice division of labor in this enterprise, since some of the workers were primarily interested in the defect in lipogenesis that occurs in the tissues of diabetic and starved animals (186, 235, 241, 242, 243, 294) while others were especially concerned with certain physiologic situations in which the tissues form new fat from glucose or acetate at extremely rapid rates (182, 183, 268, 272).

Milstein (186), using the differentially-labelled glucose technique of estimating the extent of direct glucose oxidation over the HMP shunt, demonstrated a diminished utilization of glucose by way of the shunt in adipose tissue of the alloxan diabetic rat. He suggested that the lipogenic defect in diabetes may be due to a failure of the TPNH supply. Milstein's observations were confirmed by Winegrad and Renold (294) who extended them to include estimations of the incorporation of the first and sixth carbons of glucose into fatty acids. They concluded that insulin stimulated the oxidation of glucose by both the HMP shunt and the Embden-Meyerhof pathway in about the same proportion and again emphasized

the key role of TPNH in the reductive synthesis of fatty acids. In a more recent paper (295) bovine growth hormone was added to rat epididymal fat pads *in vitro* and was found to increase glucose utilization without stimulating lipogenesis. Carbon dioxide formation from C-6 was particularly stimulated in the growth hormone-treated tissues, (certainly, a non-HMP shunt pathway!) a fact which re-emphasizes the importance of the route of glucose utilization in the stimulation of lipogenesis.

Another piece of experimental evidence which supports the TPNH availability hypothesis of lipogenesis control was offered by Siperstein (241, 242, 243) who studied lipogenesis and cholesterol synthesis in suitably fortified homogenates in which glucose utilization was "steered" over either the Embden-Meyerhof pathway by DPN addition or the hexosemonophosphate shunt by TPN addition. He found that DPN addition caused only a small increase in lipogenesis rate, while the addition of TPN caused a very striking enhancement of both fatty acid synthesis and cholesterol formation from acetate-1-C^{14}.

From a study of "supernormal" lipogenesis rate in liver slices of aurothioglucose obese mice, and of rats trained to eat their entire day's ration in one hour (272) Tepperman and Tepperman were led to a re-investigation of the "re-feeding phenomenon." Many investigators (for example see 231) had observed that the rate of formation of fatty acids in both liver and adipose tissue is enormously accelerated in animals which have been fasted for 48 to 72 hours and then re-fed a high carbohydrate, low-fat diet. Liver slices of such rats form fat from acetate at rates 500 to 1,000 % of control rates. It seemed likely that the apparent "training" of the fat-forming apparatus that occurs in chronic overnutrition, periodic overfeeding and re-feeding might be related to the production of TPNH at extremely rapid rates. The re-feeding situation afforded an excellent opportunity to investigate this point, since liver slices of rats at the end of a 48-hour fast form no detectable fat from added glucose or acetate, while slices obtained from 24- or 48-hour re-fed rats show strikingly "supernormal" rates of fat formation. A simultaneous study of lipogenesis rate, re-glycogenation of the liver and the TPN reducing ability of dialyzed supernates of liver homogenates in the presence of glucose-6-phosphate ("shunt dehydrogenase activity") showed that enhanced lipogenesis was associated with markedly increased shunt dehydrogenase activity (268). Further studies (273) with glucose-1-C^{14} and glucose-6-C^{14} showed a progressively increasing proportion of glucose metabolism by way of the shunt with increasing lipogenic activity during 48 hours of re-feeding. These observations were considered to be circumstantial evidence in favor of the view that very rapid rates of lipogenesis may be set by the ready availability of lavish amounts of TPNH.

McLean, in her elegant studies of the pathways of glucose catabolism in rat mammary gland, has carried the analysis one step further. In addition to her time plot of increasing hexosemonophosphate shunt dehydrogenase activity and lipogenic activity of developing lactating mammary gland, she has analyzed the glands for oxidized and reduced DPN and TPN and has demonstrated an extremely high content of TPNH per total gland at the height of lactation (183).

There is, concurrently, a comparable increase in DPN. It is suggested that very active lipogenesis is among the consequences of the increased amount of TPNH and the more rapid hydrogen turnover of this coenzyme is implicit in the finding of increased shunt enzyme activity.

Early in the development of the TPNH availability hypothesis of lipogenesis control there was no obvious reason for doubting that fatty acids are synthesized by way of a metabolic route which is the opposite of the degradation pathway. The rate-limiting step in synthesis was believed to be the reaction crotonyl CoA → butyryl CoA, but TPNH was also believed to be essential for all subsequent 2-carbon additions up to the final long-chain fatty acid product (230). Recently, however, Brady (25) and Wakil and his colleagues (288, 290) have explored another pathway of fatty acid biosynthesis whose distinctive features include a biotin-dependent CO_2-fixation by acetyl CoA with the formation of malonyl CoA as a short-lived intermediate, aldol condensation of malonyl CoA and acetaldehyde with a loss of the CO_2 that had been fixed in the first reaction, and the transformation of the resulting β-hydroxybutyryl CoA to crotonyl CoA. The Brady-Wakil synthetic route makes the TPNH-availability control hypothesis even more attractive than it had been, for the reverse-of-oxidation pathway requires TPNH only at the ethylene reduction steps, whereas the CO_2 fixation pathway requires an additional mole of TPNH at the acetyl CoA → acetaldehyde step (275, 288).

The comparative physiologic importance of the two pathways is not yet understood. Both Brady and Wakil worked with pigeon liver systems and a quantitative estimate of the activity of the two routes in mammalian liver and adipose tissue remains to be made. It is of some interest that Popják and Tietz (205) found that malonate stimulated fatty acid synthesis in the mammary gland. Siperstein (241) has expressed the view that the reverse-of-oxidation pathway occurs in the mitochondria, whereas the Brady-Wakil synthesis occurs in extra-mitochondrial locations in the cell. Wakil has suggested that the extra-mitochondrial system may be most important for fatty acid synthesis from small molecules, whereas the mitochondrial synthetic apparatus may be used predominantly for chain-lengthening operations of longer chain fatty acids (289).

The original problem posed for the purpose of this essay was: how does the lack of insulin impair fatty acid synthesis and how does the replacement of insulin to deprived tissues restore the rate of synthesis to normal, and even supernormal levels? Clearly, a tentative answer may be given to this question, and an important effect of a hormone on cells can be described, in the absence of a clear understanding of the primary biochemical locus of action of insulin. For the rate regulatory machinery for lipogenesis is built into the very life-fabric of the cell. Synthesis fails when carbohydrate utilization becomes minimal, and is restored when glucose oxidative mechanisms come into play. When the biochemists worked out many of the details of the reaction sequences and their co-factor requirements, attention was quickly focused on the hexosemonophosphate shunt as a crucial route of glucose oxidation because TPNH is generated by the operation of this pathway. At the present time it would be a dangerous oversimplification to assert

that the signal for active lipogenesis is a ready supply of TPNH, while the cell responds to TPNH deficiency by shutting down production of fatty acids. The true situation may be unimaginably more complex than this (302-307) but the general outline of this hypothesis is being discussed extensively. Certainly, it has the appeal of the teleologically attractive, because it states, in effect, that the larger the glucose disposal problem a cell has, the more TPNH it forms and the better it becomes at disposing of glucose by the fat synthesis route. It is an admirable trick, a kind of biochemical pulling of self up by the bootstraps.

Over and above the application of these ideas to the immediate problems described above, and to the closely related problems of ketosis and clinical diabetes, there remains the possibility that the control of critical reaction velocities in cells by coenzyme balance may be one of the most fundamental methods by which an orderly, and, at the same time, elastic pattern of chemical reactions is maintained within the cell. A beautiful discussion of this idea is given by H. A. Krebs in a recent essay (141).

b. ACTH and steroidogenesis. Recent studies on the mechanism of action of adrenocorticotrophic hormone (ACTH) on adrenal cortical cells have represented a confluence of a number of lines of investigation. A theory of the biochemical mechanism of action of ACTH has been constructed by juxtaposing information about the biosynthesis of adrenal steroids (55, 225), the coenzyme availability hypothesis of metabolic control, and certain analogies with the mechanism of action of glucagon and epinephrine on the liver.

Haynes and Berthet (107) incubated steer adrenal cortical slices in the presence of low concentrations (0.1 unit/ml) of commercial ACTH. At the end of the incubation period adrenal hormones were extracted and estimated colorimetrically. Such slices incubated with ACTH, then chilled, homogenized, and assayed for phosphorylase activity showed increases of from 30% to several hundred-fold over those of similar preparations that had been incubated without ACTH. No compound tested other than ACTH (including epinephrine, glucagon, insulin, bovine albumin, globulin, sodium salicylate) had this effect, and ACTH did not have a glucagon-like effect on liver cell preparations. Studies with adrenal homogenates suggested to the authors that steroidogenesis was stimulated by TPNH generating systems. Haynes and Berthet advanced the intriguing suggestion that ACTH activated adrenal phosphorylase, and that the glucose-6-phosphate resulting from this activation was metabolized by way of the hexosemonophosphate shunt which is extremely active in the adrenal (85). The TPNH thus generated increased the rate of steroidogenesis by providing essential coenzyme for a number of TPNH-specific hydroxylation reactions that occur in the intermediate steps between cholesterol and finished hormone (55, 225). Thus, the control of the rate of steroidogenesis was visualized as similar to the control of lipogenesis rate described above, the reaction velocities of a number of critically placed steps in the synthesis being determined by the availability of TPNH.

Koritz and Péron demonstrated that corticosteroid production by rat adrenal segments could be markedly stimulated under circumstances in which there was

no change in the rate of incorporation of glycine-1-C^{14} into adrenal gland protein (133). In a later paper (131) these authors explored some of the implications of the Haynes-Berthet hypothesis in the rat adrenal-segment system. They appeared to be impressed by the possibility that ACTH might work by regulating the available supply of TPNH, but they felt that all of the observed effects of ACTH could not be accounted for by this mechanism. Adrenal tissue maximally stimulated with ACTH responded to TPN and glucose-6-PO_4 addition, which suggests that TPNH availability was limiting in this circumstance. However, tissues maximally stimulated with TPN and glucose-6-PO_4 responded to ACTH with an increased output of steroid hormones. This suggests that a factor other than TPNH generation—possibly hormone precursor availability—was limiting. The authors suggest that TPNH production is not the only factor under ACTH control, but that ACTH might act also by making additional corticoid precursor available (see section 2a above).

Stimulated by the remarkable studies of Sutherland and his group on glucagon and epinephrine, Haynes (106) found an accumulation of adenosine-3',5'-monophosphate (3,5-AMP) in beef adrenal slices on the addition of ACTH. When this compound, which is the same one Sutherland et al. found in liver under the influence of glucagon or epinephrine, was added to adrenal slices phosphorylase activity was markedly increased. He suggested that the phosphorylation activation that occurs in the adrenal under the influence of ACTH is mediated by 3,5-AMP. However, there is a striking tissue specificity in the function of phosphorylase activation, for ACTH does not activate liver phosphorylase and glucagon or epinephrine does not activate the adrenal enzyme.

This series of experiments was brought to a beautiful stage of resolution when Haynes, Koritz and Péron (108) joined forces to demonstrate that rat adrenal sections which respond to ACTH with an increase in cortical hormone output respond similarly to the addition of 3,5-AMP! Undoubtedly there is much to be learned about the precise mechanism of action of ACTH, but one cannot help but feel that the studies described here will figure centrally in any future understanding. If one ignores, for the moment, the possibility that ACTH may work, in part, by liberating bound hormone precursor intracellularly, and thus make it available for steroidogenesis, the concept suggested by these studies is that of an effect by way of coenzyme availability. Therefore, ACTH functions first to release a substance which, in turn, participates in the activation of an enzyme, phosphorylase. This results in an increased intracellular concentration of glucose-6-phosphate. Again, as in the case of lipogenesis control, the things that happen at this point have a certain inevitability about them that is built into the adrenal cell. For it happens that the adrenal cell has an exceedingly high hexosemonophosphate dehydrogenase activity, which means that a substantial part of any glucose-6-phosphate pool in the cell is likely to be metabolized by way of the direct oxidative pathway. The TPNH generated by this event is made available to the hydroxylating reactions which specifically require it, and steroidogenesis is accelerated. It was difficult to relate this hypothesis to suggestions that ACTH stimulation occurs very early in the transformation of cholesterol to steroid

hormone (55). Now Halkerston *et al.* (100) have demonstrated a TPNH require-
ment for the conversion of cholesterol to progesterone, as well as for hydroxyla-
tion reactions beyond.

How can this be reconciled with the failure of Koritz and Péron to demonstrate
an immediately enhanced glycine-C^{14} incorporation into adrenal protein (131)
when the fact that the adrenals hypertrophy under the influence of ACTH is
well known? In fact, Logan *et al.* (165) have demonstrated clearly that the
incorporation of P^{32} into the RNA of rat adrenals is markedly depressed by
hypophysectomy and restored to normal four hours after one injection of ACTH.
One can visualize the eventual hypertrophy of the gland as secondary to the
primarily induced metabolic hyperactivity described here. Possibly a sudden
increase in hormone production produces a situation in which a large proportion
of the enzymes of the cell are saturated with substrate molecules at any instant.
This information may be conveyed to the enzyme-forming machinery which
responds by producing new enzymes, ultimately resulting in hypertrophy of the
cells. It is possible that pituitary factors in addition to ACTH may play a role
in this process.

It is interesting to find that similar ideas are being expressed about the mech-
anism of action of TSH on the thyroid. Field *et al.* (71) have given evidence for
the existence of the hexosemonophosphate shunt pathway in the thyroid, and
for its stimulation by TSH on *in vitro* addition. They suggest that many of the
observed effects of TSH on thyroid cells (*i.e.*, stimulation of iodine trapping,
stimulation of the incorporation of iodine into the organic fraction, thyroid
hormone release, and enhanced phospholipid synthesis) may be secondary to a
primary stimulation of the hexosemonophosphate shunt with an increased rate
of generation of TPNH.

4. Hormone as coenzyme

a. Estrogen effect on transhydrogenase. During the past few years an exciting
series of experiments has led to the suggestion of a possible biochemical locus of
action of estrogens. The digging in this field has been energetic and the finds
rich, but many fragments of essential information are missing and it is still
difficult to reconcile the recent discoveries with our knowledge of the physiologic
and biochemical effects of estrogens. It has been suggested that estrogens func-
tion as coenzymes in a specific reaction, and that they exert their physiologic
effects by modifying the intracellular balance and availability of coenzymes
essential for many vital reactions.

Following the lead of earlier workers who had described increased oxygen
consumption of estrogen-treated tissues, Villee and Hagerman (284) demon-
strated that estradiol increases the rate of conversion of both pyruvate-2-C^{14}
and acetate-1-C^{14} to $C^{14}O_2$ in slices of human placenta. On the basis of this find-
ing they guessed that estrogen stimulates some reaction in the tricarboxylic
acid cycle. Accordingly, they added citric acid to whole homogenates of placenta
and demonstrated a marked increase in citrate oxidation on the addition of small
amounts of estrogen in this system. Further studies (280, 283) showed that the
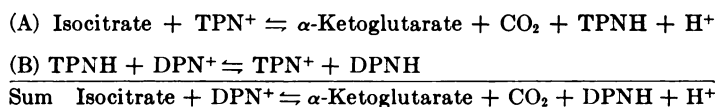
estrogen-sensitive system was in the non-particulate part of the cell, and it was tentatively localized at oxidation of isocitric acid to α-ketoglutarate *via* oxalo-succinic acid. A DPN requirement was established and the estrogen-stimulated reaction was believed to be the following:

$$\text{Isocitrate} + \text{DPN} \to \alpha\text{-Ketoglutarate} + CO_2 + \text{DPNH} + H^+$$
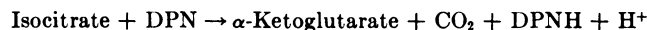
The extent of stimulation by the estrogens could be estimated by following this reaction spectrophotometrically at 340 mμ (DPN reduction), by measuring citrate disappearance, or α-ketoglutarate accumulation. The most arresting property of the system was that it could be stimulated by the merest suspicion of estrogen, *i.e.*, 4 $\times$ 10^{-9} M! The response of the system to increasing concentrations of estrogen is typically sigmoid, and Villee and his colleagues have used it as the basis for an assay of estrogen in urine and tissues (91, 99, 167).

Attempts at purification of the estrogen-sensitive enzyme revealed the necessity of an additional co-factor, either UTP or ATP, and it was shown that ATP served as a phosphate donor in the system (285). Talalay and Williams-Ashman (262) showed that the requirement for an additional co-factor in Villee's system could be met by TPN. Furthermore, if one used *substrate* amounts of DPN, *catalytic* amounts of TPN, and a TPNH-generating substrate (*either* isocitrate or glucose-6-phosphate), extremely small concentrations of estrogen had a stimulating effect on the reaction. It appeared likely that the stimulating effect of ATP or UTP in Villee's earlier experiments had been due to the formation of minute amounts of TPN from DPN as a result of phosphate donation by the ATP or UTP.

Talalay and Williams-Ashman suggested that estrogen functions as a coenzyme in the transfer of hydrogen from one pyridine nucleotide coenzyme to the other. They reformulated the isocitrate reaction as follows:

(A) Isocitrate + TPN$^+$ $\rightleftharpoons$ α-Ketoglutarate + CO$_2$ + TPNH + H$^+$

(B) TPNH + DPN$^+$ $\rightleftharpoons$ TPN$^+$ + DPNH
_____
Sum   Isocitrate + DPN$^+$ $\rightleftharpoons$ α-Ketoglutarate + CO$_2$ + DPNH + H$^+$

They remarked that any substrate-enzyme combination that is capable of generating TPNH can function in this system as well as isocitric acid–isocitric dehydrogenase, particularly glucose-6-phosphate and its dehydrogenase. They visualized the estrogen effect as being related entirely to step (B), the transhydrogenation reaction, and they proposed the scheme for the participation of estrogen in this reaction shown in Figure 2.

This reaction is written as a reversible one, but in the earliest formal publication from the Chicago laboratory there is a strong hint that the authors were intrigued by the possibility that the estrogens might exert their dramatic anabolic effects in target organs by shifting the balance of pyridine nucleotide coenzymes in the direction of TPNH, thus providing a specific coenzyme which is known to be required for many reductive syntheses. However, Villee attempted to demonstrate such reversibility without success. In his system (282) a DPN-linked

I.   ESTRADIOL + DPN⁺ ⇌ DPNH + H⁺ + ESTRONE

2.   ESTRONE + TPNH + H⁺ ⇌ TPN⁺ + ESTRADIOL
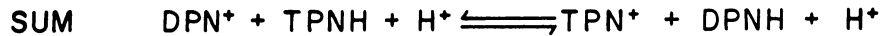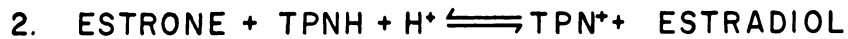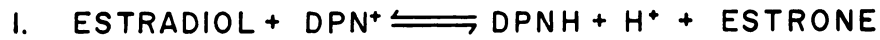
SUM   DPN⁺ + TPNH + H⁺ ⇌ TPN⁺ + DPNH + H⁺

FIG. 2. The Talalay-Williams-Ashman hypothesis of the mechanism of estrogen's participation in a transhydrogenation reaction.

enzyme, lactic dehydrogenase, was added to substrate amounts of TPN, catalytic amounts of DPN and the transhydrogenase from placenta, but no stimulation was seen on estrogen addition. From the Boston vantage point the equilibrium of the estrogen-sensitive transhydrogenase appeared to favor the direction TPNH + DPN⁺ → DPNH + TPN⁺. Villee suggests (281) that, if the TPN⁺ supply is rate-limiting because most of the cell's supply of TPN is maintained in the reduced state (86), the conversion to TPN⁺ by a transhydrogenase would permit the oxidation of larger amounts of such substrates as glucose-6-phosphate and isocitrate. At the same time more DPNH would be made available to the electron transport machinery of the cell, which evidently (124) can produce ATP energy more effectively from DPNH than it can from TPNH. This would have the effect of providing a sudden surge of energy which might be used for water imbibition, protein synthesis, or other known energy-requiring effects of estrogens. This is an attractive hypothesis, but it must be an oversimplification of the facts, for, during the first six hours of treatment, when striking metabolic effects of estrogen can be shown in the uterus, Roberts and Szego (222) found no increase in Q_{O_2}. This suggests that many processes, such as protein synthesis, amino acid activation and fatty acid synthesis can be stimulated without a concomitant increase in oxidative metabolism. Such an increase would be required by the Villee hypothesis if one assumes that any considerable amount of extra DPNH suddenly became available to the oxidative phosphorylation machinery of the mitochondria as a result of accelerated transhydrogenation.

In this connection, the reviewers (274) following the lead of Glock and Mc-Lean's (85) demonstration that the livers of adult female rats contain more glucose-6-phosphate dehydrogenase activity than do those of either males or immature females, have observed a diminution in glucose-6-phosphate dehydrogenase activity in livers of oophorectomized, adult female rats. There was a

partial restoration of activity in the castrate females on estrogen administration. McKerns (181) has shown increased substrate traffic over the hexosemonophosphate shunt in the livers of estrogen-treated rats. These observations suggest that in the liver, at least, a substantial shift of hydrogen atoms from DPNH to TPN is not likely to occur under the influence of estrogen in physiologic doses, for, if this has happened, one would expect an inhibition of metabolism by way of the HMP shunt, rather than a stimulation.

Much investigative energy has gone into attempts to answer the question: is the estrogen-sensitive transhydrogenase system described by Talalay and Williams-Ashman identical with the human placental estradiol-17-β-dehydrogenase system described by Langer and Engel (145, 146)? Talalay and his colleagues (117, 261, 262) and Hollander et al. (114, 115) reported that the evidence favored the view that the two systems—estrogen-mediated transhydrogenation and the Langer-Engel enzyme—are identical. Hagerman and Villee (97, 98, 286), however, claim to have achieved a separation of the two activities by starch block and continuous flow curtain electrophoresis. This controversy emphasizes current uncertainty about the physiologic significance of the estrogen-coupled transhydrogenase reaction; i.e., whether the reaction represents a mechanism for modifying estrogen structure or the primary biochemical locus of action of an important steroid hormone. The concentrations of estrogen which are effective in these reconstructed systems are so small (4 × 10^{-10} M; see 115a) that they suggest biocatalysis rather than a route of hormone disposal, but the amounts of hormone that must be metabolized by the cells of the body are very small indeed. It remains to be seen whether the participation of estrogen in the transhydrogenase system represents a fleeting instant in the fate of the estrogen molecule or a kind of biochemical switch turned on by the hormone in order to stimulate the metabolic activity of the estrogen-sensitive cell.

Considerable doubt was thrown on the estrogen-as-transhydrogenase-coenzyme hypothesis by Stein and Kaplan (252). These workers compared the activities of the TPNH-DPN transhydrogenase in the mitochondrial and soluble fractions of rat liver, and found that the level of activity in the latter fractions (i.e., those that have been shown to be estrogen-sensitive) was less than 1% of that observed in the mitochondrial fraction. The abstract of their paper, a laconic gem in an age of verbal surfeit, is here quoted in toto: "It appears that the α-hydroxy steroid dehydrogenases are not significant entities in promoting transhydrogenase reactions in animal tissues." This decision may be somewhat harsh because it is based on studies of a tissue which is not generally regarded to be estrogen-sensitive. Moreover, it is hasty to conclude that what appears to be insignificant in a cell-free system is necessarily insignificant in its proper cytoarchitectural context.

5. Hormone effect via ATP availability

a. Protein anabolic effect of insulin. A model of an ATP availability hypothesis was presented in section 1 in connection with certain effects of insulin (on the incorporation of labelled amino acids into protein) which could not be explained

on the basis of enhanced glucose or amino acid transport. It should be emphasized that this speculation was based upon the assumption that the supply of ATP may be rate-limiting at strategic sites in the cell in the activation of amino acids preparatory to their synthesis into protein.

b. *Effect of estrogens on amino acid activating enzymes.* For many years students of carbohydrate metabolism have considered the glucokinase reaction as a possible locus of action of insulin, growth hormone, and adrenal cortical hormones. A lively interest in this possibility is apparent in the papers of Kipnis and Cori (126, 128). Recently, an analogous idea in the field of protein metabolism has been advanced by McCorquodale and Mueller (180), who have demonstrated increased amino acid-activating enzyme activity in the 105,000 × g supernatant fractions prepared from whole homogenates of rat uteri in animals treated with estrogen. This effect was observed as early as 3 hours after treatment and increased progressively over a 24-hour period. Seven separate amino-acid activating enzymes have been studied and the activities of all have been found to increase rapidly over the 24-hour period following treatment with a single dose of estrogen. The authors consider three possible mechanisms for the phenomenon: a) stimulation of *de novo* synthesis of enzymes by estrogen, b) activation of pre-existing enzyme, or c) the releasing of a bound, possibly particulate, enzyme into the operationally defined non-particulate compartment of the cell. This very interesting study raises a number of questions, the first of which involves the fact that multiple enzymes appear to be affected by the treatment. It would be easier to imagine that estrogen exerts its effect on the activating enzymes by some common mechanism rather than by stimulating each one individually. Possibly all of these activating enzymes share a co-factor the availability of which might constitute part of a signal to the enzyme forming machinery to make more enzyme. Conceivably, the activating enzymes could be responding secondarily to a primary effect on amino acid transport like the one suggested by Christensen *et al.* Alternatively, the amino acid transport effects could well be secondary to the amino acid activation enzyme increases described by the Madison workers. Possibly a sweeping of all available intracellular amino acids into synthetic pathways under the influence of the activating enzymes and ATP results in a secondary influx of larger amounts of α-aminoisobutyric acid into cells. This suggestion is consistent with the finding of the Ann Arbor group that extensive α-aminoisobutyric acid accumulations occur in rapidly growing tissues—tissues in which amino acid activation and protein synthesis are proceeding briskly. Possibly this permits the ingress and accumulation of an amino acid which cannot participate in the activation reaction.

Another possible mechanism for the observed effects on amino acid activation is suggested by the construction of a bridge between the transhydrogenase function of estrogen (see discussion above) and its stimulating effect on amino acid activation. If transhydrogenase activity were stimulated in the direction of suddenly increasing the supply of DPNH, a quick charge of ATP resulting from the oxidation of the DPNH by the electron transport system could result in a rapid increase in amino acid activation if ATP availability were limiting this

process. This hypothesis has the additional advantage of offering an explanation for the other early metabolic effects of estrogens described by Mueller (189) and others which are difficult to relate to amino acid activation as the prime mover.

6. Hormonal activation of an inactive enzyme precursor

a. *Effects of glucagon and epinephrine on phosphorylase.* Recent studies on the effects of glucagon and epinephrine on cells and broken cell preparations emphasize the point that the student of cytologic endocrinology may not wait for pertinent basic biochemical mechanisms to be described, but may be motivated by his interest in hormonal effects to discover such mechanisms for himself. This story has been reviewed by Berthet (18), but this essay would be incomplete without a brief account of a series of investigations that has yielded much useful information and has given great pleasure to many observers.

Sutherland and Cori (254, 255) established the fact that phosphorylase catalyzes the rate-limiting step in the glycogenolytic process in liver slices, and that the glycogen breakdown stimulated by glucagon and epinephrine is accompanied by an activation of hepatic phosphorylase. This was true whether the hormones were given to intact animals or added to slices *in vitro*. Subsequent studies by Sutherland and his colleagues (213, 257, 300) characterized liver phosphorylase as a phosphoprotein which can be dephosphorylated to an inactive form by a phosphatase. The inactive form can be reactivated by a specific kinase in conjunction with ATP. Ordinarily, most of the protein is present in the biocatalytically inert form. Glucagon and epinephrine have the effect of shifting the equilibrium sharply in the direction of the active enzyme.

A series of ingenious experiments (212, 256) involving recombinations of particulate and non-particulate fractions of homogenates treated with the hormones revealed that some particulate fraction is stimulated to produce a nucleotide, which has been identified as adenosine-3',5'-monophosphate or cyclic adenylic acid (3,5-AMP) from ATP. It is this nucleotide which is specifically responsible for the activation of liver phosphorylase. There is little doubt that a similar sequence of events occurs in heart and skeletal muscle (15, 16, 211) and in adipose tissue (278) in response to appropriate hormones. Although the precise mechanism by which glucagon and epinephrine increase the availability of 3,5-AMP to the cell is unknown, there is no doubt that the efforts of workers on this problem have raised the level of sophistication of the questions that can now be asked in this field by several orders of magnitude.

The historical development of these ideas began with the physiologic observation of glycogenolysis, and it is, therefore, not surprising that there is some hope of reconciling the biochemical facts with physiology, at least in the liver. At first, it was difficult to understand how an activation of phosphorylase would result in the breakdown of liver glycogen since the reaction catalyzed by this enzyme has been regarded as reversible since its discovery by the Cori's. Berthet (18) states that tissue analyses have shown that the concentrations of glucose-1-phosphate and phosphate are such that glycogen breakdown would always be favored by phosphorylase activation. Furthermore, it has been ob-

served that glycogen synthesis occurs in muscle or liver only when most of the phosphorylase is in inactive form. These reassuring thoughts have not prevented proponents of the phosphorylase activation school of glucagon mechanism from hopefully looking at the possibility that the uridine diphosphoglucose (UDPG) pathway (153, 279) may prove to be the quantitatively important route of glycogen synthesis while the phosphorylase catalyzed reaction may be the main stream of glycogenolysis. There is no doubt that this arrangement would be a very beautiful one from the point of view of susceptibility to hormonal control. For example, the possibility that insulin might have a selective effect on the UDPG pathway of glycogen synthesis has already been considered (221). At the time of writing these must be considered merely as interesting speculations.

Perske and his colleagues (202) have questioned the role of phosphorylase activation in epinephrine-induced hyperglycemia. They found no significant difference in the phosphorylase activity of livers of hyperglycemic rats injected with epinephrine and that of livers of rats in which the hyperglycemia was inhibited by dihydroergotamine. Again, they stress the reversibility of the phosphorylase reaction, and remark that under equilibrium conditions that favor glycogen deposition, phosphorylase activation should increase the glycogen deposition. That this does not occur was shown by Teng et al. (267) who found that liver slices under the conditions favorable for glycogen deposition produce little glycogen on addition of epinephrine to the buffer. Furthermore, doubt is cast on the rate-limiting role of phosphorylase in the glycogen-to-glucose pathway. "It seems apparent that other, as yet obscure, events must occur upon epinephrine administration which change the equilibrium conditions to those strongly favoring glucose formation or which activate a second alternative pathway for glycogenolysis" (202). They come very close to invoking the Leloir-Cardini UDPG pathway (153) as a possible alternate route of glycogenolysis, as well as glycogenesis. Obviously, the physiologic significance of this pathway is destined to be explored extensively in the next few years.

A synthesis of well-established physiologic observations on skeletal, cardiac, and smooth muscle and the activation of phosphorylase into a single, inclusive hypothesis remains a difficult one. Epinephrine has a potent positive inotropic effect on cardiac muscle, and so, it now appears, does glucagon (69). Similarly the well-known relaxing effect of epinephrine on intestinal smooth muscle has been duplicated with glucagon (69). Epinephrine and isoproterenol increase the force of contraction of the potassium-depressed rat diaphragm (65). Haugaard and his colleagues (103) have found a rough parallelism between the ability of sympathomimetic amines to stimulate the heart and to increase active phosphorylase activity. Ellis (64) in a thoughtful review of this physiology-biochemistry interphase, concludes that the activation of glycogenolysis may be important for the muscular effects of epinephrine, but that such effects probably are not due to a stimulation of energy production through the glycolytic pathway. He cites a number of experiments which suggest some sort of relationship between potentiated contraction and the presence of hexosephosphates in cells, and suggests the possibility that contractile performance in muscle may be

improved by the presence of hexosephosphates which accumulate in epinephrine-treated muscle. The present reviewers are also interested in the possibility that the changes in intracellular potassium which are associated with glycogenolysis may play a role in the contractility changes seen in muscle under the influence of epinephrine. A clear exposition of the relationship, if any, between positive inotropic effect or inhibition of smooth muscle and phosphorylase activation remains to be made.

Even without an obvious association between epinephrine- or glucagon-mediated phosphorylase activation and the physiologic events in tissues in which this occurs, the description of phosphorylase activation by the hormones given by Sutherland and his school remains an exciting achievement. As a model system in which an enzyme can be reversibly activated and inactivated depending on the availability of a coenzyme-like substance whose very presence is elicited by hormones, it invites speculation about the possibility of the existence of similar control systems in other biochemical situations. The extension of this concept to the adrenal cortical cell (see section 3b above) may be but the first application of many to come.

7. Hormonal effects on enzymes via enzyme-forming systems

a. *Effects of growth hormone, estrogens, androgens, and pituitary trophic hormones on RNA, cell protein, enzymes, and mitotic apparatus.* Certainly, the velocity of the reaction mediated by Enzyme E in Figure 1 can be varied by varying the amount of the apoenzyme itself. During the past twenty years a very large amount of literature has accumulated on the general subject of the modification of the enzymic patterns of cells under the influence of hormones. Fortunately, it is unnecessary to review this literature in detail, since Knox, Auerbach and Lin have recently presented an exhaustive survey of this subject (130). For the present, it is only necessary to attempt to bring within the perspective of this essay the fact that multiple and individual changes in the activity of enzymes may occur in hormone-deprived and hormone-treated tissues.

If one makes the assumption that a change in an enzyme concentration in a cell represents some sort of modification of the feedback relationship that exists between every formed enzyme and the machinery which forms it (in the manner colorfully described by Potter, 206) it is exceedingly difficult to visualize how a hormone can exert its *primary* effect by changing the total concentration of apoenzymes. The fact that many enzyme changes are frequently found in tissues of hormone-treated animals (130), whether they are treated with thyroxine, growth hormone or with a tissue specific hormone like estrogen or androgen, suggests that the enzyme changes seen are likely to be many reactions removed from the primary biochemical locus of action of the hormone, and that the enzyme-forming machinery itself may be involved in the sequence of events that leads to an alteration of the enzyme profile of the cell. Similarly, the many striking effects of hormones on cell division and proliferation, some of which have been reviewed by Swann (258), can reasonably be regarded as secondary effects of hormones. The fact that these effects are secondary does not mean

that they are of secondary interest.. Moreover, while an intimate description of hormonal effects on cell division and on the enzyme formation in cells may have to await a clearer understanding of the fundamental biology of these processes, it is just as likely that students of hormonal effects in this area will make substantial contributions to our understanding ot the processes themselves. A biochemically dormant cell which is awakened by the administration of a hormone seems to us to be an ideal system in which to study many problems related to protein synthesis and the participation of RNA in the formation of proteins.

For example, Di Stefano et al. (49), using microspectrophotometric techniques of analysis in rat liver, demonstrated that hypophysectomy results in a fall in nuclear and cytoplasmic volumes, nuclear and cytoplasmic RNA and nuclear and cytoplasmic protein. Treatment with a purified bovine growth hormone preparation restored all values to normal levels or slightly higher. In a subsequent study (52) the same group followed the time course of recovery of the same parameters at two-day intervals over a period of six days of treatment. At two days nuclear RNA and protein had returned to control levels, anticipating changes in the cytoplasm by two days. By the fourth day, in fact, nuclear RNA values were significantly supernormal. It is of some interest that nuclear and cytoplasmic effects of growth hormone were separable in time and that the former were seen before the latter. This finding has been questioned by Greenbaum et al. (93) who reported a 30% increase in RNA turnover in growth-hormone treated rats, but doubted that the events in the nucleus preceded those in the cytoplasm. However, this statement appears to be founded on specific activity data stated by the authors to be without statistical significance.

In the light of these effects on nucleic acid metabolism many of the enzymatic defects found in the tissues of hypophysectomized animals and their correction on administration of a variety of types of replacement therapy (see 130 for a review of some of these) can be attributed to secondary effects of the hormones on the protein-synthesizing, or enzyme-forming machinery of the cells. That this is indeed a secondary or derivative effect is indicated by the fact that with growth hormone, at least, a number of readily measurable metabolic changes occur very quickly on administration of the hormone; for example, depression of the α-amino acid concentration of the blood (163, 185), fat mobilizing activity (154), contra-insulin effects (137) and many others. No measurable effect of growth hormone on nucleic acid was seen before 12 to 24 hours of growth hormone treatment. Since the primary biochemical locus of action of growth hormone is quite unknown, it would be futile to speculate on the connection between the primary and secondary effects of the hormone. It is similarly difficult to explain certain effects of growth hormone on ploidy distribution pattern and mitotic activity of hepatic cells, a subject which has been studied by Di Stefano and Diermeier (50, 51), by Bass et al. (13), and by Cater et al. (32). From these studies it is certain that growth hormone has profound effects not only on the protein-synthesizing component of cells but also on the reproductive equipment itself.

Balis et al. (7) have extended the study of growth hormone effects on the liver

to a study of the incorporation of labelled amino acids into microsomal ribonucleoprotein particles and into the intracellular soluble proteins and serum proteins. In their experiments the nucleic acid purines reached maximal activity much later than the protein of the particles. Growth hormone administration to rats before preparation of the fractions increased the labelling of proteins but not in parallel with changes in activity of the purines of the particles. They suggest that the synthesis of total protein and the nucleic acid of the RNA protein particle do not necessarily proceed simultaneously.

Effects of hormones on nucleic acids, protein synthesis, cell division and growth of tissue are not limited to those of growth hormone. Estrogens, androgens, and pituitary trophic hormones are all anabolic hormones for certain sensitive tissues. Conversely, adrenal cortical hormones, which have profound catabolic or anti-anabolic effects on cells have been shown to have readily demonstrable effects on nucleic acid metabolism and cell division in certain cells.

As an example of a study of the effect of estrogens on protein synthesis and nucleic acid metabolism one can cite the report of Mueller (189), who demonstrated an increase in the rate of incorporation of glycine-2-C^{14} and other labelled amino acids into protein of surviving uterine segments as a result of estradiol pretreatment *in vivo*. He also showed striking enhancement of the rate of incorporation of formate-C^{14}, glycine-2-C^{14}, and serine-3-C^{14} into nucleic acid purines in the same kind of system. Similarly, stimulatory effects on the incorporation of $C^{14}O_2$ into nucleic acid pyrimidines were shown. It should be pointed out that none of these effects was demonstrable before the fifth hour of estradiol pretreatment, whereas significant effects on amino acid activating enzyme activity referred to above (180) were found 3 hours after injection. The histamine-like effects of estrogen can be seen much earlier (236, 245, 260). The coenzyme function of estrogens can be demonstrated on *in vitro* addition. The time sequence of development of these various effects of estrogen should be helpful in establishing which of these is closest to the primary effect of the hormone. But even if the primary molecular locus of action of the hormone were precisely known we are left with the complex problem of elucidating the chain of events which link it with some of the striking secondary effects we have described. Other interesting studies on the effects of estrogen on nucleic acid metabolism of the rat uterus are those of Telfer (266) who suggests that estrogen may increase mitochondrial mass.

Although the authors have not found reports of studies on androgen-sensitive tissues similar to Mueller's on the uterus, there is a report on a striking effect of androgen treatment on DNA and RNA content and concentration in the seminal vesicles of castrate rats (Rabinovitch *et al*. 209). The time scale of these changes again suggests that they are secondary to effects which must have occurred much earlier, but, again, the nature of the primary effect is completely unknown.

Thyroid stimulating hormone is a growth hormone for the thyroid gland, and Alfert *et al*. (1) have done karyometric and microspectrophotometric studies of rat thyroid cell nuclei in glands obtained from animals in different functional states of thyroid activity. The most striking effect seen in this tissue is an increase

in nuclear size which is attributable to an increase in non-histone protein content. That TSH functions as a growth hormone is obvious from inspection of the thyroid gland in a thiouracil-treated animal. Fiala *et al.* (70) describe changes in cytoplasmic RNA in the thyroid after TSH treatment. Again, the relationship between the growth effect of this trophic hormone and its primary effect is quite unknown. The relationship between the metabolic effects of ACTH on the adrenal cortical cell and its anabolic effect on adrenal tissue was discussed above. That this relationship involves changes in nucleic acid metabolism is suggested by the work of Symington and Davidson (259).

Studies on the effects of cortisone in the rat by Einhorn *et al.* (61) and by Lowe and Williams (169) showed markedly inhibited restoration of nucleic acids in regenerating liver with the former group, and a disappearance of parenchymal basophilia and a disappearance of RNA from the microsomes and mitochondria of the liver with the latter. These effects, too, may be many steps removed from the primary biochemical locus of action of cortisone.

At present it is possible to speculate on these effects of hormones on cells only in a very tentative and general way. Effects on the machinery of cell division and on the rate of protein synthesis could follow modifications of the permeability of cells to critical substrates, changes in availability of phosphate bond energy, alterations in the balance of any number of essential coenzymes or shifts in the concentration of ions or trace metals. A generalized acceleration of the metabolic activity of the cell—as, for example, by simply speeding up a single rate-limiting step—could easily activate the protein synthetic apparatus for reasons which were previously suggested in the section on ACTH. Work hypertrophy, whether of muscle or secretory cell, may prove to be interpretable according to general laws when more is known about the feedback control of enzyme-forming systems.

8. Hormonal effects on mitochondrial structure and function

a. Thyroxine and phosphorylation-uncoupling. In considering the effects of hormones or other chemical agents on the mitochondria it is useful to imagine that the metabolic activity of these organelles may be influenced in ways that are very similar to those we have discussed in connection with the comparative macrocosm of the cell itself. Availability of substrates, coenzymes and metals to the mitochondrial membrane or to its working interior may be influenced by hormones. Modification of the intramitochondrial environment by changes in the permeability of its membrane to water and soluble substances may alter profoundly its biochemical performance. Availability of ATP appears to play an important role in the functional integrity of the mitochondrion, but, in this case, it seems fair to assume that the crucial ATP is locally produced within the organelle by oxidative phosphorylation, and that disordered mitochondrial function can result from interference with this process. Finally, the mitochondrion-duplicating apparatus of the cell plays a role in hormonal effects, since it has been clearly established in the case of the thyroid hormone, at least, that the feeding of desiccated thyroid to rats results in an increased number of mitochondria per unit of tissue (Lardy and Maley, 148). Some idea of the scope of

the new science of mitochondriology can be obtained by reading the fascinating essay of Ernster and Lindberg (68), who deal not only with the effect of chemical agents on mitochondria, but also with their static and dynamic morphology, their relation to other parts of the cell, their chemical composition and their metabolic activity. The mitochondrion, in fact, is being studied now much as the cell has been studied—*i.e.*, as an organized unit with a distinctive and meaningful architecture, the function of which is integrated into the general activity of its environment with extraordinary precision and subtlety.

An account of the development of the phosphorylation-uncoupling hypothesis of the mechanism of action of thyroid hormones has been given by Lardy and Maley (148). According to these authors, the biochemical basis of the physiologic activity of thyroid hormone(s) may be related to their ability to "uncouple" oxidative phosphorylation, *i.e.*, to diminish the yield of trapped phosphate-bond energy per unit of oxygen consumed by mitochondria. Differences in efficiency of phosphorylation are readily demonstrable when mitochondria of normal and hyperthyroid rats are compared. Similar effects can be seen on *in vitro* addition of thyroid hormones, but the concentrations used are of the order of 10^{-5} M, which are rather large in comparison with the tissue concentrations one would expect from the administration of therapeutically effective doses of these hormones.

The uncoupling hypothesis of the mechanism of action of thyroid hormones has been greeted with reserve by physiologists and clinicians, and some of the flavor of their skepticism can be sampled in the discussion of Lardy's Laurentian Hormone Conference paper cited above. An important negative observation has been the failure to see differences in P/O ratios on comparison of mitochondrial suspensions prepared from the tissues of hypothyroid and normal animals. Yet, the well-known effects of thyroid hormone deprivation are extremely striking and it is difficult to conceive of them as the result of possibly supernormal P/O ratios in the tissues of hypothyroid animals. Moreover, uncoupling agents, such as dinitrophenol, do not repair the defect of thyroid hormone lack although they raise oxygen consumption. This in itself would suggest that, if the uncoupling phenomenon has physiologic meaning in thyroid hormone-treated animals, there are at least two, and possibly many more, forms of uncoupling.

Two broadly distinguishable groups of phosphorylation-uncoupling substances have been described. The first, of which thyroxine is a prototype, causes mitochondrial swelling which can be reversed on the addition of ATP *in vitro* (152). Another characteristic of compounds of this group is their failure to uncouple in a submitochondrial system prepared by the disruption of mitochondria in digitonin solutions. Compounds of the second group, typified by dinitrophenol, dicumarol, gramicidin, and pentachlorphenol, do not cause swelling of mitochondria and do effectively uncouple in submitochondrial particles in which the function of oxidative phosphorylation is preserved (43). Recently, Park *et al.* (199) and Bronk (29) described mitochondrial sonicates which were sensitive to the action of thyroxine and tri-iodothyronine. This apparent disagreement may indicate more about the possible differences between sonicates and digitonin

preparations than it does about the mechanism of action of thyroxine. Possibly the examination of various kinds of particles with the electron microscope in the manner described by Ziegler *et al.* (301) will reveal reasons for their variability of response to thyroxine.

We have already seen a great preoccupation with the idea that hormones may influence cellular metabolism by altering the balance of coenzymes in the cell. It is interesting to see that this idea, or something like it, has been extended to the mitochondrion. Ball and Cooper (8) studied a particulate preparation of heart muscle which contained an electron transport system and transhydrogenase but little or no TPNH-specific cytochrome reductase. This system did not react with oxygen in the presence of TPNH (or a TPNH generating system) unless trace amounts of DPN were added. Thyroxine, however, inhibited the DPN fortified system. They suggest that this inhibition might have the effect of detouring TPNH over pathways which are much less efficiently coupled than the one studied here (124). In fact, Phillips and Langdon have reported that the activity of TPNH-specific cytochrome C reductase is doubled in hyperthyroid liver (204).

Another interesting possible competitive relationship between thyroxine and DPN was presented by Wolff and Ball (296), who described evidence for the inactivation of malic dehydrogenase, and, consequently, a failure of oxalacetate formation in fresh heart homogenates. The inactivation of the enzyme is visualized as a competition between thyroxine and DPN for the enzyme, possibly *via* the zinc component of the dehydrogenase, although Wolff and Wolff (297) were unable to prove that the hormone interacted with zinc specifically. In any case, the "stimulatory" effect of thyroxine on succinate oxidation in heart and kidney mitochondria *in vitro* can be regarded as the result of the following sequence: inhibition of malic dehydrogenase → diminished formation of oxalacetate → lowered concentration of oxalacetate, a succinic dehydrogenase inhibitor, → release of inhibition of succinate oxidation. This extraordinarily ingenious scheme may or may not have a connection with the physiologic effect of thyroxine, but it is certainly possible that it can stand as a caveat (if one were needed) for investigators who add hormones to reconstructed systems and observe striking effects, for some of these may be due simply to chemical interactions between the hormone and some crucial component of the system, and may be quite irrelevant to the problem of the locus of action of the hormone *in vivo*. This is not to be construed as a criticism of the work of the authors cited, who are as aware as anyone of the difficulties of interpretation in their experiments.

Emmelot and Bos (66) have stated that thyroxine "labilizes the functional integrity of the mitochondrion" rather than acting specifically on phosphorylation uncoupling. They appear to regard the release of DPN from enzymatically active sites as a symptom of the functional disorganization of the mitochondrion, but it is equally possible that inhibition of crucial DPN-dependent dehydrogenases may contribute to the abnormality of the mitochondrion. A disordered production of DPNH, diminished production of ATP and other concomitant changes could lead to profound alterations in the characteristics of the mito-

chondrial envelope and modifications of its permeability. The reversal of swelling by ATP addition referred to above suggests that the proper functioning of the membrane may be dependent on an expenditure of energy just as some functions of membranes of cells may be metabolically driven.

All of these studies on the inhibitory effects of thyroxine on DPN-linked dehydrogenases were done in reconstructed systems. Their pertinence to physiology would be emphasized by a demonstration of changes of the sort described above in preparations from animals injected with the hormone.

Another attempt to relate thyroid function to the coenzyme balance of the cell has been made by Lee et al. (151), who found that liver mitochondria of thyroid-fed rats oxidize α-glycerophosphate five times as rapidly as do mitochondria of euthyroid animals. The activity of soluble α-glycerophosphate dehydrogenase is not changed. The authors make the interesting suggestion that the α-glycerophosphate-dihydroxyacetone-phosphate system may function as an electron shuttle between the extramitochondrial portion of the cell and the inside of the mitochondrion. In their view the product of α-glycerophosphate oxidation in the mitochondrion, dihydroxyacetone phosphate, returns to the cytoplasm to oxidize more DPNH, and an altered relationship is set up that favors increased availability of extramitochondrial pyridine nucleotide to the electron transport machinery of the mitochondrion.

So far, none of the suggested biochemical mechanisms of action for thyroxine has had an irresistible appeal for physiologists. Clearly, the brain is very centrally involved in the syndromes of both thyroid hormone lack and thyroid hormone excess, and Timiras and Woodbury (276) have shown that modifications of thyroid function in either direction produce striking differences in the excitability of that organ. Yet there are no reports of biochemical alterations in brain produced by hypothyroidism or thyroid hormone administration. The most striking features of the hyperthyroid state, in fact, are those that suggest the extreme sensitivity of the tissues of the hyperthyroid animal or man to the autonomic mediators, adrenergic as well as cholinergic—irritability, tremors, sweating, tachycardia, diarrhea, and so on. It is difficult to see how any suggested biochemical mechanism of action can be invoked to explain these phenomena. Nor is it easy to make the mental connection between phosphorylation uncoupling hypotheses and such a phenomenon as the initiation of metamorphosis in a tadpole.

It is just possible that new insight into the mechanism of action of thyroid hormone at the biochemical level could be gained by making the assumption that the final expression of the effect of thyroxine really represents a collaborative enterprise which involves either epinephrine or norepinephrine and thyroxine. Brewster et al. (28) reported that surgical sympathetic blockade obliterated the increase in heart rate, and certain other circulatory indicators of hyperthyroidism in experimentally hyperthyroid animals, but that the differences between euthyroid and hyperthyroid animals became clearly apparent when either epinephrine or norepinephrine was infused intravenously. Ramey et al. (214) found that the administration of sympatholytic blocking agents prevented the usual rise in

oxygen consumption following treatment with thyroxine. On the other hand, Goodman and Knobil (90) found that they could not induce a rise in plasma nonesterified fatty acids with epinephrine in the hypophysectomized monkey unless they had previously treated the animal with TSH or tri-iodothyronine. Again, the effect was obtainable only when the tissue was under the influence of both hormones. It would be of some interest to use these data in the design of experiments on the mechanism of action of thyroxine at the cellular level.

9. Hormonal effects on ions as activators or as essential structural components

a. Thyroid and magnesium, and assorted related subjects. The subtitle of this section is a sort of verbal Rorschach Test ink blot, and could elicit a very wide variety of responses from people who have a direct or tangential interest in this subject. It is far beyond the scope of this essay to consider the profound effects of adrenal cortical hormones on sodium and potassium movements in the body. A few of the influences of parathyroid hormone on calcium and phosphorous metabolism which could be considered in this section will be discussed briefly below.

Our present concern is with metallic components of enzyme systems in which the metal presumably plays some activating role. It is conceivable that hormones could influence reaction velocities by sequestering a metallic activator out of a reaction or by combining with some cellular component in such a way as to make available a larger amount of the metallic ion. Chenoweth, in a recent review in this journal (37), has described some of the profound effects on metabolism that are observed when chelating agents exert their effects on cells.

A relationship between magnesium and thyroid function was suggested by the report of Soffer *et al.* (244) and that of Vitale *et al.* (287). The latter group, having found an increased magnesium requirement in animals maintained in a cold environment, studied the effect of supplementing magnesium intake on the growth inhibition in young rats produced by the addition of thyroxine to the diet. They found that thyroxine-induced suppression of growth was partially overcome by the magnesium supplement, and that the amount of magnesium required for reversal was related to the amount of thyroxine added. Furthermore, oxidative phosphorylation was impaired in mitochondria prepared from the hearts of the thyroxine-treated rats, but it was normal in similar preparations obtained from animals on thyroxine plus high magnesium diet.

In a subsequent study an obscure triangular relationship was demonstrated among three substances: thyroxine, magnesium, and vitamin B_{12}. Gershoff *et al.* (83) found that thyroxine lowered the concentration of vitamin B_{12} in tissues, and that this effect was reversed by increasing the magnesium of the diet. Also, B_{12}, like magnesium, partially reversed the effect of thyroxine administration on oxidative phosphorylation in heart mitochondria. The mechanisms involved in these relationships are unknown, but, with older information, they point to some functional connection between thyroxine and magnesium.

There have been a number of reports that attribute a metal-binding role to thyroxine. For example, Gemmill (80) found that small amounts of thyroxine

stimulated the rate of oxidation of ascorbic acid by squash ascorbic acid oxidase. He found also (82) that the inhibition of cupric-ion catalyzed oxidation of ascorbic acid is overcome by thyroxine, and that the inhibition resulted from the formation of a thyroxine-metal complex. Interestingly, 3,5,3'-tri-iodothyronine also complexed with metal, but 3,5-di-iodothyronine, thyronine, and 3,5-di-iodotyrosine did not (81, 82). Additional studies on thyroxine as a chelating reagent were done by Frieden and Naile (79), who explain Gemmill's original ascorbic oxidase "stimulation" experiments on the basis of the fact that the system as it is usually prepared is functioning at lower-than-maximum levels due to the presence of inhibiting heavy metal impurities which are chelated out not only by thyroxine, but also by other metal binding reagents, such as CN^-, EDTA, cysteine, and proteins.

Another interesting instance of a chelating action of thyroxine was postulated on the basis of reports by Askonas (5) and Kuby et al. (143). Lardy (147) suggests that thyroxine inhibits ATP-creatine phosphorylase by removing soluble Mg^{++} from the system.

The temptation is strong to suggest, on the basis of the data examined above, that the effect of thyroxine on oxidative phosphorylation may somehow involve chelation of Mg^{++} by the hormone. However, Maley and Lardy (173), using concentrations of thyroactive substances in the range of 10^{-5} M, found depression of P/O ratios with both thyroxine and tri-iodothyronine in rat kidney mitochondria with glutamate as a substrate. Lardy (147) reports that tri-iodothyronine does not form an insoluble chelate with Mg^{++} and that further additions of Mg^{++} do not overcome the uncoupling effect of either hormone, and he states quite explicitly his belief that the effects of thyroxine and of tri-iodothyronine on oxidative phosphorylation are *not* the result of removing Mg^{++} from the system.

The question of the possible involvement of Mg^{++} in the action of the thyroid hormone at the cellular level must remain an open one. This section is included in the discussion merely for the purpose of pointing to the possibility that critical reaction velocities may be influenced not only by forces which play on the apoenzyme, on its substrate load, on coenzyme or ATP availability and so on, but also by alterations in the ionic environment in which the reactions proceed.

Other interesting and thought-provoking relationships between hormones and ions have been described. For example, Péron and Koritz (201) have demonstrated a calcium requirement for the action of ACTH on the adrenals. Similarly, Lopez et al. (166) have shown that the lipolytic effect of ACTH (but not that of epinephrine) is abolished when adipose tissue is depleted of calcium. The effect of the hormone is restored on the addition of calcium, but not magnesium.

There is a suggestion that the effect of insulin on isolated rat diaphragm can be modified by alterations in the ionic environment of the tissue. Bhattacharya (21) reported that insulin increases the uptake of glucose by isolated rat diaphragm only in the presence of certain metallic ions such as sodium, potassium, rubidium, caesium, and magnesium. In a later report (22) the same author demonstrated that preincubation of diaphragms at 38°C in sucrose-bicarbonate

buffer resulted in a loss of insulin sensitivity and contraction of the muscle. The addition of low concentrations of magnesium prevented both of these effects, and calcium counteracted the effect of magnesium.

One special case of a possible ion effect may be mentioned in a speculative spirit. In addition to their importance in the maintenance of the integrity of many intracellular reactions, certain ions may participate very centrally in the maintenance of the functional integrity of cell surfaces and membranes. By virtue of their potential effect on the ionic constituents of cell membranes it is entirely possible that certain hormones may have a profound effect on the permeability characteristics of the membranes, and, therefore, on many processes that take place deep within the cell. The reviewers are unaware of any postulated hormonal action of this specific type, but recently they were privileged to see a magnificent time-lapse motion picture of fibroblasts in tissue culture prepared by Dornfeld (56). This investigator discovered that he could observe and photograph dramatic changes in shape and surface activity (*i.e.*, a rounding of the cell and a "bubbling" of the cell surface similar to that seen in some hot springs) of fibroblasts cultivated *in vitro* simply by adding EDTA to the nutrient medium or by changing the bathing fluid to one low in calcium concentration. The latter effect showed a longer latent period than the former, suggesting a slower withdrawal of calcium from the cell. According to Dornfeld's hypothesis, calcium is a structural component of the lipoprotein complex of the cell wall, and the cell's ability to maintain its polygonal shape and quiescent attitude are related to the relatively slow permeability of its surface to water and, presumably, solutes as well. When the calcium is chelated out of the membrane, or leached out, as in the case of low calcium media, the membrane becomes much more freely permeable, the cell imbibes water and swells, and the cell surface exhibits an astonishing amount of intricate "bubbling" activity. It is easy to imagine that the ionic composition and the physiologic behavior of cell membranes might be altered by hormones either from the outside, or, secondarily, by virtue of metabolic changes induced in the cell by some other primary effect of the agent. One can only hope that Dornfeld and his colleagues will find ways of cultivating cells of target tissues and of examining them on the addition of their respective hormones *in vitro*.

10. Parathyroid hormone

For many years efforts of physiologists interested in studying the actions of the parathyroid glands were concentrated on establishing the tissue site of the primary effects of the hormone. Now direct effects of parathyroid hormone on bone metabolism have been demonstrated unequivocally by two types of experiment. First, Barnicot (10) and Chang (35), using rats and mice, produced local decalcification of bone by implanting bits of parathyroid tissue next to it. Second, it has been established in a long series of experiments that parathyroid hormone-induced changes in serum calcium levels are independent of either a) serum phosphate alterations resulting from phosphaturia induced by hormone administration (122), or b) the very presence of the kidney in the animal at the time of

administration of parathyroid extract or removal of the glands (94, 187, 265). Typical histological changes in bone tissue in response to parathyroid extract administration have also been described in nephrectomized rats (119). Control of phosphate excretion by action of parathyroid hormone directly on the kidney has been shown by Levinsky and Davidson (160), who took advantage of the existence of a renal portal circulation in the chicken to demonstrate an increased excretion of phosphate by the kidney tubule perfused with blood coming from the leg into which parathormone had been injected, compared to phosphate excretion by the other kidney. Recent experiments have also indicated that this hormone directly affects absorption of calcium from the intestine (217, 264). Settlement of the kidney vs. bone argument, together with recent successes in the preparation of highly purified parathyroid hormone (6, 218), have made it likely that rapid advances in our understanding of the cellular mechanisms by which parathyroid hormone exerts its effects on bone, kidney and intestine can now be expected.

Lotspeich (168) has summarized the evidence for the direct action of parathyroid on phosphate excretion by the kidney independent of any effect on glomerular filtration rate and renal blood flow. Experiments of Nicholson (191) on dogs with chemically produced nephrosis in various portions of the tubule appear to localize the action of parathormone to the distal tubule. The hormonal effects are accompanied by an increased turnover of various phosphate fractions of the kidney, changes in the uridine diphosphate being most striking (46). Certain changes in the kidney enzymes of animals treated with parathyroid extract have also been found, including a decrease in succinic dehydrogenase activity (67), also reported in bone tissue of rats treated with parathyroid extract (149). The relationship of these findings to alterations in phosphate excretion produced by the hormone is certainly not apparent now and may become clear only when the mechanism of phosphate excretion is better understood (see Lotspeich, 168, for a statement of the present knowledge in this area).

Neuman et al. (73, 190) have recently proposed a theoretical explanation for the action of parathyroid hormone on bone cells. They were impressed, as many others have been, by the relationship between calcium and citrate in bone and other tissues. The high citrate content of bone first described by Dickens (48) and the observation by many investigators mentioned by Dickens, as well as by more recent studies (54), that under certain circumstances the levels of calcium and citrate in blood plasma fluctuate together stimulated Neuman and his collaborators to investigate further the possible role of citrate metabolism in bone physiology. It had been shown that administration of parathyroid extract to animals produces an increase in blood citrate as well as calcium (2, 48, 62, 162), and that raising the level of citrate in the blood by citrate injection (36, 89) or by treatment with fluoroacetate (78) produces decalcification of bone and changes in blood and urine calcium similar to those observed following treatment with parathyroid extract. Elliott and Talmage (63, 263), in studies on the composition of the peritoneal fluid of nephrectomized rats, also found evidence for a relationship between calcium and citrate concentrations. Neuman

and his collaborators (73) extended these observations by showing *in vitro* that the presence of citrate in the medium increased the solubility of bone mineral, and that the increase in blood calcium of dogs given parathyroid extract was preceded by a rise in citrate concentration. By sampling the blood entering and leaving bone, Firschein *et al.* (72) showed that bone added citrate to blood and that this contribution increased markedly in dogs following the injection of parathyroid extract. Using this evidence they postulate that the parathyroid hormone stimulates bone cells to produce citrate which increases the solubility of bone salt, and that is the basis of the effect of the hormone on bone metabolism. Neuman presents this idea as a working hypothesis potentially helpful in designing future experiments. Some details of the original idea have already been shown to be incorrect. The production of citrate by bone was believed by Dixon and Perkins (53) and by Neuman (190) to be due to a high concentration of bone citrogenase together with a relatively low isocitric dehydrogenase activity. However Perkins and Dixon (200) themselves showed that alterations in blood calcium of parathyroidectomized rats occurred long before enzymatic changes predicted by this theory could be detected, and more recently the application of newer methods has shown that the isocitric dehydrogenase of bone is much higher than originally believed (277). Various reports of the relationship between calcium and citrate in the blood are also difficult to explain by the Neuman hypothesis. Elliott and Freeman (62), for example, found that comparable hypercalcuria produced by vitamin D and by parathyroid extract was accomplished by a much smaller citrate rise in the latter case. Harrison and Harrison (101) reported a fall in serum calcium following the administration of citrate to rachitic infants with no change in serum citrate. Freeman and Chang (77) found that nephrectomy produced the same increase in citric acid whether or not the parathyroid glands were present, although the rise in calcium was not seen in the absence of these glands. The relationship of these ions in the blood may not, however, reflect accurately the concentrations locally in bone. Kenny *et al.*, who studied bone resorption in the simpler environment of tissue culture (125), reported large increases in the release of citrate into the medium with relatively minor changes in calcium concentration. This observation established the capacity of bone cells to produce citrate but its relationship to bone resorption is not clear. The problem of the mechanism of action of the parathyroid hormone has been immensely complicated by the fact that most of the observations have been made under conditions which made the interpretation of changes in concentration of critical metabolites very difficult indeed. Citrate, for example, may very well play a central role in calcium mobilization, but it is such an ubiquitous metabolite that a rise or fall in its blood concentration may or may not be related to concurrent changes in the state of bone. Recently, Raisz, Au and J. Tepperman (210) have demonstrated that very thin strips of calvarium obtained from normal, hypoparathyroid and hyperparathyroid weanling rats neatly reflect the parathyroid status of the donor animals when such strips are incubated in Krebs-Henseleit bicarbonate buffers over a period of 8 hours. Analysis of the bath fluid at the end of the incubation period shows high levels of calcium in

beakers containing skull fragments of hyperparathyroid rats, lower levels in association with hypoparathyroidism and intermediate values for the controls. These differences, which are highly significant statistically, persist even on incubation under 95 % nitrogen and 5 % CO_2, but the buffer calcium is markedly diminished and most of the intergroup differences disappear when the tissue is preheated to 80°C for 10 minutes. Analysis of the bath fluid for citrate reveals no correlation between the amount of citrate present in the vessel and the extent of calcium mobilized from the surviving bone fragments. *In vitro* addition of fluoroacetate produced striking increases in bath fluid citrate concentration, but depressed calcium mobilization. The authors hope that the study of bone fragments *in vitro* will yield information which will be useful in evaluating many of the intact animal experiments described above, and that this technique will serve as a vehicle for the posing of the problem of the mechanism of action of parathyroid hormone in cellular terms.

CONCLUSION

In this review we have tried to capture some of the flavor of current thought about the problem of the effects of hormones on cells. We hope, too, that we have conveyed the idea of the operation of a collective consciousness upon which the unfolding historical record impinges. One can find evidences in the fragments of this essay of such a thing as a refractory period of the collective consciousness to certain ideas, and of its sensitization to others. The indivisibility of biologic knowledge is the one insistent theme that recurs in the searches we have described, and there is a bidirectional flux of ideas into and out of the small corner of biology we have discussed. Transhydrogenase had to be discovered before an effect of estrogen could be demonstrated on it, but the search for a mechanism of action of glucagon led to a better understanding of phosphorylase activation.

Another recurrent theme is related to the "built-in" responsiveness of cells stimulated by hormones. Again and again we have seen instances of cells responding to hormonal stimulation with an elaborate sequence of interconnected biochemical events, some of them many reactions removed from the primary interaction of the hormone with its receptor at the molecular level. These intricate responses are based upon the genetically determined, autonomous, and elastic system of biochemical controls which permit the cell to function in an orderly way. In cybernetic terms they must involve an unbelievably large number of "bits" of information, of yes-no decisions. It is upon these "built-in" intracellular controls that the neural and hormonal adjustments are superimposed, and it is, therefore, rather surprising to note that the local regulation of cell metabolism has only recently come into its own as a field of intensive research (41). There is just as much rampant yearning for understanding of the primary mechanisms of action of hormones, as ever there was, but there is a growing awareness of the fact that some of the secondary effects of hormones that are mediated by way of the cell's own control mechanisms have a considerable intrinsic beauty of their own. The treasures inside the box, in fact, are no less interesting than the interaction between the hormonal key and its lock.

REFERENCES

1. Alfert, M., Bern, H. A. and Kahn, R. H.: Hormonal influence on nuclear synthesis. Acta anat. 23: 185-205, 1955.
2. Alwall, N.: Studien über die Regulation vom Citronensäuregehalt des Blutserums IV. Über die Einwirkung von Parathormon auf den Citronensäuregehalt des Blutserums, ein Beitrag zur Frage des Zusammenhangs zwischen Calcium und Citronensäure. Acta med. scand. 116: 337-339, 1944.
3. Ashmore, J. (Ed.): Insulin and the oral hypoglycemic agents. Metabolism 8: 469-685, 1959.
4. Ashmore, J., Hastings, A. B., Nesbett, 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.
5. Askonas, B. A.: Effect of thyroxine on creatine phosphokinase activity. Nature, Lond. 167: 933-934, 1951.
6. Aurbach, G. D.: Isolation of parathyroid hormone after extraction with phenol. J. biol. Chem. 234: 3179-3181, 1959.
7. Balis, M. E., Samarth, K. D., Hamilton, M. G. and Petermann, M. L.: Role of ribonucleoprotein particles in protein synthesis and the effects of growth hormone. J. biol. Chem. 233: 1152-1155, 1958.
8. Ball, E. G. and Cooper, O.: The oxidation of reduced triphosphopyridine nucleotides as mediated by the transhydrogenase reaction and its inhibition by thyroxine. Proc. nat. Acad. Sci., Wash. 43: 357-364, 1957.
9. Ball, E. G., Martin, D. B. and Cooper, O.: The effect of insulin on glucose utilization as measured by the manometric determination of carbon dioxide output. J. biol. Chem. 234: 774-780, 1959.
10. Barnicot, N. A.: The local action of the parathyroid and other tissues on bone in intracerebral grafts. J. Anat., Lond. 82: 233-248, 1948.
11. Barrnett, R. J.: Personal communication.
12. Barrnett, R. J. and Ball, E. G.: Morphologic and metabolic changes produced in rat adipose tissue in vitro by insulin. Science 129: 1282, 1959.
13. Bass, A. D., McArdle, A. H. and Grisham, J.: Effect of hypophysectomy on liver nucleoproteins. Proc. Soc. exp. Biol., N. Y. 89: 7-9, 1955.
14. Bearn, A. G., Billing, B. H. and Sherlock, S.: Hepatic glucose output and hepatic insulin sensitivity in diabetes mellitus. Lancet 2: 698-701, 1951.
15. Belford, J. and Feinleib, M. R.: Phosphorylase activity of heart muscle under various conditions affecting force of contraction. J. Pharmacol. 127: 257-264, 1959.
16. Belford, J. and Feinleib, M. R.: Phosphorylase activity in heart muscle. The Pharmacologist 1: No. 2, p. 62, 1959.
17. Beloff-Chain, A., Catanzaro, R., Chain, E. B., Masi, I., Pocchiari, F. and Rossi, C.: The influence of insulin on carbohydrate metabolism in the isolated diaphragm muscle of normal and alloxan diabetic rats. Proc. roy. Soc. B 143: 481-503, 1955.
18. Berthet, J.: Some aspects of the glucagon problem. Amer. J. Med. 26: 703-714, 1959.
19. Berthet, J., Jaques, P., Hers, H. G. and de Duve, C.: Influence de l'insuline et du glucagon sur la synthèse du glycogène hépatique. Biochim. biophys. Acta 20: 190-200, 1956.
20. Bessman, S. P. and Fitzgerald, J.: An effect of insulin on glycogen metabolism in cell free systems. Fed. Proc. 18: 192, 1959.
21. Bhattacharya, G.: Influence of ions on the uptake of glucose and on the effect of insulin on it by rat diaphragm. Nature, Lond. 183: 324-325, 1959.
22. Bhattacharya, G.: Role of magnesium in the insulin-sensitivity of the rat diaphragm in vitro. Nature, Lond. 184: 1401-1402, 1959.
23. Bloch, K. and Kramer, W.: The effect of pyruvate and insulin on fatty acid synthesis in vitro. J. biol. Chem. 173: 811-812, 1948.
24. Bondy, P. K., Bloom, W. L., Whitner, V. S. and Farrar, B. W.: Studies of the role of the liver in human carbohydrate metabolism by venous catheter technic II. Patients with diabetic ketosis, before and after the administration of insulin. J. clin. Invest. 28: 1126-1133, 1949.
25. Brady, R. O.: The enzymatic synthesis of fatty acids by aldol condensation. Proc. nat. Acad. Sci., Wash. 44: 993-998, 1958.
26. Brady, R. O. and Gurin, S.: The biosynthesis of radioactive fatty acids and cholesterol. J. biol. Chem. 186: 461-469, 1950.
27. Brady, R. O., Lukens, F. O. W. and Gurin, S.: Hormonal influence upon the in vitro synthesis of radioactive fatty acids. Science 113: 413-415, 1951.
28. Brewster, W. R., Isaacs, J. P., Osgood, P. F. and King, T. L.: The hemodynamic and metabolic interrelationships in the activity of epinephrine, nor-epinephrine and the thyroid hormones. Circulation 13: 1-20, 1956.
29. Bronk, J. R.: Some effects of thyroxine on oxidative phosphorylation in submitochondrial particles and intact mitochondria. Biochim. biophys. Acta 27: 667-668, 1958.

30. CAHILL, G. F., JR., ASHMORE, J., EARLE, A. S. AND ZOTTU, S.: Glucose penetration into liver. Amer. J. Physiol. 192: 491-496, 1958.
31. CAHILL, G. F., ASHMORE, J., RENOLD, A. E., AND HASTINGS, A. B.: Blood glucose and the liver. Amer. J. Med. 26: 264-282, 1959.
32. CATER, D. B., HOLMES, B. E. AND MEE, L. K.: The effect of growth hormone upon cell division and nucleic acid synthesis in the regenerating liver of the rat. Biochem. J. 66: 482-486, 1957.
33. CHAIKOFF, I. L.: Metabolic blocks in carbohydrate metabolism in diabetes. Harvey Lect. 47: 99-125, 1952.
34. CHAIN, E. B., BELOFF-CHAIN, A. AND POCCHIARI, F.: Present knowledge of the mechanism of the mode of action of insulin. Selected scientific papers from the Istituto Superiore di Sanita 1: 389-535, 1956.
35. CHANG, H. Y.: Grafts of parathyroid and other tissues to bone. Anat. Rec. 111: 23-47, 1951.
36. CHANG, T. S. AND FREEMAN, S.: Citric acid and its relation to serum and urinary calcium. Amer. J. Physiol. 160: 330-334, 1950.
37. CHENOWETH, M. B.: Chelation as a mechanism of pharmacological action. Pharmacol. Rev. 8: 57-87, 1956.
38. CHRISTENSEN, H. N.: Active transport, with special reference to the amino acids. Perspectives in Biol. & Med. 2: 228-242, 1959.
39. CHRISTENSEN, H. N., ASPEN, A. J. AND RICE, E. G.: Metabolism in the rat of three amino acids lacking α-hydrogen. J. biol. Chem. 220: 287-294, 1956.
40. CHRISTENSEN, H. N., NOALL, M. N., RIGGS, T. R. AND WALKER, L. M.: Letter in reply to Leake and Pomerat (150). Science 127: 163, 1958.
41. CIBA FOUNDATION: Symposium on the Regulation of Cell Metabolism, ed. by G. E. W. Wolstenholme and C. M. O'Connor. Little, Brown and Co., Boston, 1959.
42. COLOWICK, S. P., CORI, G. T. AND SLEIN, M. W.: The effect of adrenal cortex and anterior pituitary extracts and insulin on the hexokinase reaction. J. biol Chem. 168: 583-596, 1947.
43. COOPER, C. AND LEHNINGER, A. L.: Oxidative phosphorylation by an enzyme complex from extracts of mitochondria V. The adenosine triphosphate-phosphate exchange reaction. J. biol. Chem. 224: 561-578, 1957.
44. CORI, G. T., CLOSS, J. O. AND CORI, C. F.: Fermentable sugar in heart and skeletal muscle. J. biol. Chem. 103: 13-24, 1933.
45. DEBODO, R. C., STEELE, R., ALTSZULER, N., DUNN, A., ARMSTRONG, D. T. AND BISHOP, J. S.: Further studies on the mechanism of action of insulin. Metabolism 8: 520-530, 1959.
46. DEVERDIER, C. H.: The incorporation of labelled phosphate into different compounds in the rat kidney. The effect of parathyroid extract on an in vivo system. Acta physiol. scand. 39: 1-11, 1957.
47. DICKENS, F.: Mechanisms of carbohydrate oxidation. Nature, Lond. 138: 1057, 1936.
48. DICKENS, F.: The citric acid content of animal tissues, with reference to its occurrence in bone and tumor. Biochem. J. 35: 1011-1023, 1941.
49. DI STEFANO, H. S., BASS, A. D., DIERMEIER, H. F. AND TEPPERMAN, J.: Nucleic acid patterns in rat liver following hypophysectomy and growth hormone administration. Endocrinology 51: 386-393, 1952.
50. DI STEFANO, H. S. AND DIERMEIER, H. F.: Effects of restricted food intake and growth hormone on rat liver proteins and nucleic acids. Endocrinology 64: 448-454, 1959.
51. DI STEFANO, H. S. AND DIERMEIER, H. F.: Effect of hypophysectomy and growth hormone activity on ploidy distribution and mitotic activity of rat liver. Proc. Soc. exp. Biol., N. Y. 92: 590-594, 1956.
52. DI STEFANO, H. S., DIERMEIER, H. F. AND TEPPERMAN, J.: Effects of growth hormone on nuleic acid and protein content of rat liver cells. Endocrinology 57: 158-167, 1955.
53. DIXON, T. F. AND PERKINS, H. R.: Citric acid and bone metabolism. Biochem. J. 52: 260-265, 1952.
54. DIXON, T. F. AND PERKINS, H. R.: Citric acid and bone. In: The Biochemistry and Physiology of Bone, ed. by G. H. Bourne, pp. 309-323. Academic Press, New York, 1956.
55. DORFMAN, R. I.: Biosynthesis of adrenocortical steroids. Cancer, N. Y. 10: 741-745, 1957.
56. DORNFELD, E. J.: Surface responses in cultured fibroblasts elicited by EDTA. J. biophys. biochem. Cytol. 4: 243-250, 1958.
57. DRURY, D. R.: The role of insulin in carbohydrate metabolism. Amer. J. Physiol. 131: 536-543, 1940.
58. DRURY, D. R. AND WICK, A. N.: Mechanisms of insulin action. Diabetes 4: 203-206, 1955.
59. DUNN, D. F., FRIEDMAN, B., MAASS, A. R., REICHARD, G. A. AND WEINHOUSE, S.: Effects of insulin on blood glucose entry and removal rates in normal dogs. J. biol. Chem. 225: 225-237, 1957.
60. EICHHORN, J., HALKERSON, I. D. K., FEINSTEIN, M. AND HECHTER, O.: Increase in sugar permeability in the rat adrenal produced by ACTH. Abstracts Endocrine Society Meetings, June 1959, pp. 33-34.
61. EINHORN, S. L., HIRSCHBERG, E. AND GELLHORN, A.: Effects of cortisone on regenerating rat liver. J. gen. Physiol. 37: 559-574, 1954.
62. ELLIOTT, J. R. AND FREEMAN, S.: Relative effect of vitamin D and parathyroid extract on plasma calcium and citric acid of normal and thyroparathyroidectomized dogs. Endocrinology 59: 196-200, 1956.
63. ELLIOTT, J. R. AND TALMAGE, R. V.: Removal of Ca⁴⁰ and Ca⁴⁵ from bone by citrate as influenced by the parathyroids. Endocrinology 62: 709-716, 1958.
64. ELLIS, S.: Relation of biochemical effects of epinephrine to its muscular effects. Pharmacol. Rev. 11: 469-479, 1959.
65. ELLIS, S., DAVIS, A. H. AND ANDERSON, H. L., JR.: Effects of epinephrine and related amines on contraction and glycogenolysis of the rat's diaphragm. J. Pharmacol. 115: 120-125, 1955.
66. EMMELOT, P. AND BOS, C. J.: Diphosphopyridine nucleotide from mitochondrial dehydrogenases. Exp. Cell Res. 14: 132-141, 1958.
67. ENGFELDT, B., GARDELL, S., HELLSTRÖM, J., IVEMARK, B., RHODIN, J. AND STRANTH, J.: Effect of experimentally induced hyperparathyroidism on renal function and structure. Acta endocr. Copenhagen 29: 15-26, 1958.

68. ERNSTER, L. AND LINDBERG, O.: Animal mitochondria. Annu. Rev. Physiol. 20: 13–42, 1958.
69. FARAH, A. AND TUTTLE, R.: J. Pharmacol. 129: 49, 1960.
70. FIALA, S., SPROUL, E. E. AND FIALA, A. E.: Regulation by anterior pituitary hormones of nucleic acids in dependent endocrine glands. Proc. Soc. exp. Biol., N. Y. 94: 517–520, 1957.
71. FIELD, J. B., PASTAU, I., JOHNSON, P. AND HERRING, B.: Biochem. and Biophys. Res. Communications 1: 284–287, 1959.
72. FIRSCHEIN, H., MARTIN, G., MULRYAN, B. J., STRATES, B. AND NEUMAN, W. F.: Mechanism of action of parathyroid hormone I on ion gradients. J. Amer. chem. Soc. 80: 1619–1623, 1958.
73. FIRSCHEIN, H. E., NEUMAN, W. F., MARTIN, G. R. AND MULRYAN, B. J.: Studies on the mechanism of action of the parathyroid hormone. Recent Progr. Hormone Res. 15: 427–454, 1959.
74. FISHER, R. B. AND LINDSAY, D. B.: The action of insulin on the penetration of sugars into the perfused heart. J. Physiol. 131: 526–541, 1956.
75. FORSHAM, P. H. (Ed.): Current trends in research and clinical management of diabetes. Ann. N. Y. Acad. Sci. 82: 191-644, 1959.
76. FRAWLEY, T. F., SHELLEY, T. F., RUNYAN, J. W., JR., MARGULIES, E. J. AND CIUCOTTI, J. J.: Further studies on the significant role of the liver in sulfonyl urea hypoglycemia. Ann. N. Y. Acad. Sci. 82: 460–478, 1959.
77. FREEMAN, S. AND CHANG, T. S.: Effect of thyroparathyroidectomy and vitamin D upon serum calcium and citric acid of normal and nephrectomized dogs. Amer. J. Physiol. 160: 341–347, 1950.
78. FREEMAN, S. AND ELLIOTT, J. R.: The effect of fluoroacetate upon the plasma citrate response to parathyroidectomy and nephrectomy. Endocrinology 59: 190-195, 1957.
79. FRIEDEN, E. AND NAILE, B.: Interaction of phenylmercuric chloride with thyroxine and related compounds. Arch. Biochem. Biophys. 48: 448–457, 1954.
80. GEMMILL, C. L.: The effect of thyroxine on ascorbic acid oxidation. J. biol. Chem. 192: 749–754, 1951.
81. GEMMILL, C. L.: Comparison of activity of thyroxine and 3,5,3'-triiodo-thyronine. Amer. J. Physiol. 172: 286–290, 1953.
82. GEMMILL, C. L. AND PLUNKETT, R. L.: The kinetics of the inhibition by thyroxine of the cupric chloride catalyzed oxidation of ascorbic acid. Arch. Biochem. Biophys. 36: 434–441, 1952.
83. GERSHOFF, S. N., VITALE, J. J., ANTONOWICZ, I., NAKAMURA, M. AND HELLERSTEIN, E. E.: Studies of interrelationships of thyroxine, magnesium and vitamin B₁₂. J. biol. Chem. 231: 849–854, 1958.
84. GEY, K. F.: The concentration of glucose in rat tissues. Biochem. J. 64: 145–150, 1956.
85. GLOCK, G. E. AND MCLEAN, P.: Levels of enzymes of direct oxidative pathway of carbohydrate metabolism in mammalian tissues and tumors. Biochem. J. 56: 171-175, 1954.
86. GLOCK, G. E. AND MCLEAN, P.: Levels of oxidized and reduced diphosphopyridine nucleotide and triphosphopyridine nucleotide in animal tissues. Biochem. J. 61: 388-390, 1955.
87. GOLDSTEIN, M. S., HENRY, W. L., HUDDLESTUN, B. AND LEVINE, R.: Action of insulin on transfer of sugars across cell barriers: common chemical configuration of substances responsive to action of the hormone. Amer. J. Physiol. 173: 207-211, 1953.
88. GOLDSTEIN, M. S., MULLICK, V., HUDDLESTUN, B. AND LEVINE, R.: Action of muscular work on transfer of sugars across cell barriers: comparison with action of insulin. Amer. J. Physiol. 173: 212-216, 1953.
89. GOMORI, G. AND GULYAS, E.: Effect of parenterally administered citrate on the renal excretion of calcium. Proc. Soc. exp. Biol., N. Y. 56: 226-228, 1944.
90. GOODMAN, H. M. AND KNOBIL, E.: Mobilization of fatty acids by epinephrine in normal and hypophysectomized rhesus monkeys. Proc. Soc. exp. Biol., N. Y. 100: 195-197, 1959.
91. GORDON, E. E. AND VILLEE, C. A.: An in vitro assay for estradiol-17 and estrone. Endocrinology 58: 150-157, 1956.
92. GREEN, D. E.: Enzymes and trace substances. Advanc. Enzymol. 1: 177-198, 1941.
93. GREENBAUM, A. L., GRAYMORE, C. N. AND SLATER, I. F.: The effect of pituitary growth hormone on the level and turnover of nucleic acids and phospholipids of the rat's liver. J. Endocrin. 15: 235-242, 1957.
94. GROLLMAN, A.: The role of the kidney in the parathyroid control of the blood calcium as determined by studies on the nephrectomized dog. Endocrinology 55: 166-172, 1954.
95. HAFT, D. E. AND MILLER, L. L.: Alloxan diabetes and demonstrated direct action of insulin on metabolism of isolated perfused rat liver. Amer. J. Physiol. 192: 33-42, 1958.
96. HAFT, D., MIRSKY, I. A. AND PERISUTTI, G.: Influence of insulin on uptake of monosaccharides by the isolated rat diaphragm. Proc. Soc. exp. Biol., N. Y. 82: 60-62, 1953.
97. HAGERMAN, D. D. AND VILLEE, C. A.: Separation of human placental estrogen-sensitive transhydrogenase from estradiol-17-β-dehydrogenase. J. biol. Chem. 234: 2031-2036, 1959.
98. HAGERMAN, D. D., VILLEE, C. A. AND WELLINGTON, F. M.: Separation of human placental estrogen-sensitive transhydrogenase from estradiol-17-β-dehydrogenase. Fed. Proc. 18: 240, 1959.
99. HAGERMAN, D. D., WELLINGTON, F. M. AND VILLEE, C. A.: Estrogens in marine invertebrates. Biol. Bull., Woods Hole 112: 180-183, 1957.
100. HALKERSTON, I. D. K., EICHHORN, J. AND HECHTER. O.: TPNH requirement for cholesterol side chain cleavage in adrenal cortex. Arch. Biochem. Biophys. 85: 287-289, 1959.
101. HARRISON, H. E. AND HARRISON, H. C.: Further studies on the effects of citrate feeding on the calcium, phosphorus and citrate metabolism of rachitic infants. J. Pediat. 41: 756-765, 1952.
102. HASTINGS, A. B., RENOLD, A. E. AND TENG, C.: Effects of ions and hormones on carbohydrate metabolism. Recent Progr. Hormone Res. 11: 381-400, 1955.
103. HAUGAARD, N., KUKOVETZ, W. R. AND HESS, M. E.: The effect of sympathomimetic amines on phosphorylase activity of the isolated rat heart. Pharmacol. Rev. 11: 466-468, 1959.
104. HAUGAARD, N. AND MARSH, J. B.: Mechanism of insulin action. Charles C Thomas, Springfield, Ill., 1953.

105. HAUSBERGER, F. X. AND MILSTEIN, S. W.: Dietary effects on lipogenesis in adipose tissue. J. biol. Chem. 214: 483–488, 1955.
106. HAYNES, R. C.: The activation of adrenal phosphorylase by the adrenocorticotrophic hormone. J. biol. Chem. 233: 1220–1222, 1958.
107. HAYNES, R. C., JR. AND BERTHET, L.: Studies on the mechanism of action of the adrenocorticotrophic hormone. J. biol. Chem. 225: 115–124, 1957.
108. HAYNES, R. C., JR., KORITZ, S. B. AND PÉRON, F. G.: Influence of adenosine 3',5'-monophosphate on corticoid production by rat adrenal glands. J. biol. Chem. 234: 1421–1423, 1959.
109. HECHTER, O. AND LESTER, G.: Cell permeability and hormone action. Recent Progr. Hormone Res. 16: to be published, 1960.
110. HECHTER, O., POLLERI, A., LESTER, G. AND GREGOR, H. P.: A model for cellular cation discrimination. J. Amer. chem. Soc. 81: 3798, 1959.
111. HEINZ, E.: Kinetic studies on the "influx" of glycine-1-C^{14} into the Ehrlich mouse ascites carcinoma cell. J. biol. Chem. 211: 781–790, 1954.
112. HELMREICH, E., HOLZER, H., LAMPRECHT, W. AND GOLDSCHMIDT, S.: Bestimmung stationärer Zwischenstoffkonzentrationen II. Die Entstehung der Ketokörper und ihre Beziehung zur Glykolyse. Hoppe-Seyl. Z. 297: 113–126, 1954.
113. HILL, A. V.: Lectures on the Scientific Basis of Medicine, 1954-55, vol. 4, pp. 1–17. The Athlone Press, London, 1956.
114. HOLLANDER, V. P., HOLLANDER, N. AND BROWN, J. D.: Inhibition of steroid mediated pyridine nucleotide transhydrogenase and 17-β-hydroxysteroid dehydrogenase by 2' adenylic acid. Proc. Soc. exp. Biol., N. Y. 101: 475–477, 1959.
115. HOLLANDER, V. P., HOLLANDER, N. AND BROWN, J. D.: Studies on the estrogen-sensitive enzyme system from placenta. J. Biol. Chem. 234: 1678–1684, 1959.
115a. HOLLANDER, V. P., NOLAN, H. AND HOLLANDER, N.: The structural specificity of the estrogen-sensitive enzyme system in placental homogenates. J. biol. Chem. 233: 580–582, 1958.
116. HOLZER, H.: Discussion of DICKENS, F., GLOCK, G. E. AND MCLEAN, P.: Some problems in the choice of oxidative pathways of carbohydrate metabolism. In: Ciba Foundation Symposium on the Regulation of Cell Metabolism, p. 185. Little, Brown and Co., Boston, 1959.
117. HURLOCK, B. AND TALALAY, P.: 3-α-hydroxysteroids as coenzymes of hydrogen transfer between di- and triphosphopyridine nucleotides. J. biol. Chem. 233: 886–893, 1958.
118. HUTCHENS, T. T., VAN BRUGGEN, J. T., COCKBURN, R. M. AND WEST, E. S.: The effect of fasting upon tissue lipogenesis in the intact rat. J. biol. Chem. 208: 115–122, 1954.
119. INGALLS, T. H., DONALDSON, G. AND ALBRIGHT, F.: The locus of action of the parathyroid hormone: experimental studies with parathyroid extract on normal and nephrectomized rats. J. clin. Invest. 22: 603–608, 1943.
120. INGLE, D. J., NEZAMIS, J. E. AND MORLEY, E. H.: Work output and blood glucose values in severely diabetic rats with and without insulin. Amer. J. Physiol. 165: 469–472, 1951.
121. INGLE, D. H., NEZAMIS, J. E. AND RICE, K. L.: Work output and blood glucose values in normal and in diabetic rats subjected to the stimulation of muscle. Endocrinology 46: 505–509, 1950.
122. JACOBS, E.: Sur le méchanisme d'action de la parathormone: effet du maintien d'un état d'hyperphosphorémie sur l'action hypercalcémiante de l'hormone. Arch. int. Pharmacodyn. 95: 225–233, 1953.
123. JEANRENAUD, B. AND RENOLD, A. E.: Studies on rat adipose tissue in vitro IV. Metabolic patterns produced in rat adipose tissue by varying insulin and glucose concentrations independently from each other. J. biol. Chem. 234: 3082–3087, 1959.
124. KAPLAN, N. O., SWARTZ, M. N., FRECH, M. E. AND CIOTTI, M. M.: Phosphorylative and nonphosphorylative pathways of electron transfer in rat liver mitochondria. Proc. nat. Acad. Sci., Wash. 42: 481–487, 1956.
125. KENNY, A. D., DRASKÓCZY, P. R. AND GOLDHABER, P.: Citric acid production by resorbing bone in tissue culture. Amer. J. Physiol. 197: 502–504, 1959.
126. KIPNIS, D. M.: Regulation of glucose uptake by muscle: functional significance of permeability and phosphorylating activity. Ann. N. Y. Acad. Sci. 82: 354–365, 1959.
127. KIPNIS, D. M. AND CORI, C. F.: Studies of tissue permeability III. The effect of insulin on pentose uptake by the diaphragm. J. biol. Chem. 224: 681–693, 1957.
128. KIPNIS, D. M., HELMREICH, E. AND CORI, C. F.: Studies of tissue permeability IV. The distribution of glucose between plasma and muscle. J. biol. Chem. 234: 165–170, 1959.
129. KIPNIS, D. M. AND NOALL, M. W.: Stimulation of amino acid transport by insulin in the isolated rat diaphragm. Biochim. biophys. Acta 28: 226–227, 1958.
130. KNOX, W. E., AUERBACH, V. H. AND LIN, E. C. C.: Enzymatic and metabolic adaptations in animals. Physiol. Rev. 36: 164–254, 1956.
131. KORITZ, S. B. AND PÉRON, F. G.: Studies on the mode of action of the adrenocorticotrophic hormone. J. biol. Chem. 230: 343–352, 1958.
132. KORITZ, S. B. AND PÉRON, F. G.: The stimulation in vitro by Ca^{++}, freezing and proteolysis of corticoid production by rat adrenal tissue. J. biol. Chem. 234: 3122–3128, 1959.
133. KORITZ, B., PÉRON, F. G. AND DORFMAN, R. I.: Influence of ACTH on cortical production and glycine-1-C^{14} incorporation into protein by rat adrenals. J. biol. Chem. 226: 643–650, 1957.
134. KOSTYO, J. L., HOTCHKISS, J. AND KNOBIL, E.: Stimulation of amino acid transport in isolated diaphragm by growth hormone added in vitro. Science 130: 1653–1654, 1959.
135. KRAHL, M. E.: The effect of insulin and pituitary hormones on glucose uptake in muscle. Ann. N. Y. Acad. Sci. 54: 649–670, 1951.

136. Krahl, M. E.: Incorporation of C^{14} amino acids into glutathione and protein fractions of normal and diabetic rat tissues. J. biol. Chem. 200: 99–109, 1953.

137. Krahl M. E.: Effect of pituitary hormones on metabolism of isolated tissues. In: Hypophyseal Growth Hormone, Nature and Actions, ed. by R. W. Smith, Jr., O. H. Gaebler, and C. N. H. Long, pp. 344–365. Blakiston, McGraw-Hill, New York, 1955.

138. Krahl, M. E.: Functions of insulin and other regulatory factors in peptide formation by animal cells. Recent Progr. Hormone Res. 12: 199–225, 1956.

139. Krahl, M. E.: Speculations on the action of insulin, with a note on other hypoglycemic agents. Perspectives in Biol. & Med. 1: 69–96, 1957.

140. Krahl, M. E.: Incorporation of C^{14} amino acid precursors into adipose tissue protein: An insulin stimulation not involving glucose or amino acid transport. Biochem. biophys. Acta 35: 556, 1959.

141. Krebs, H. A.: The steering of metabolic processes. Lectures on the Scientific Basis of Medicine 5: 126–142, 1955–56.

142. Krebs, H. A. and Eggleston, P.: The effect of insulin on oxidations in isolated muscle tissue. Biochem. J. 32: 913–925, 1938.

143. Kuby, S. A., Noda, L. H. and Lardy, H. A.: Adenosinetriphosphate-creatine transphorylase III. Kinetic studies. J. biol. Chem. 210: 65–82, 1954.

144. Langdon, R. G.: The biosynthesis of fatty acids in rat liver. J. biol. Chem. 226: 615–629, 1957.

145. Langer, L. J., Alexander, J. A. and Engel, L. L.: Human placental estradiol-17-β-dehydrogenase. J. biol. Chem. 234: 2609–2614, 1959.

146. Langer, L. J. and Engel, L. L.: Human placental estradiol-17-β-dehydrogenase. J. biol. Chem. 233: 583–588, 1958.

147. Lardy, H.: Effect of thyroid hormones on enzyme systems. The Thyroid. Brookhaven Symposia in Biology, No. 7, pp. 90–101, 1955.

148. Lardy, H. A. and Maley, G. F.: Metabolic effects of thyroid hormones in vitro. Recent Progr. Hormone Res. 10: 129–155, 1954.

149. Laskin, D. M. and Engel, M. B.: Bone metabolism and bone resorption after parathyroid extract. Arch. Path. (Lab. Med.) 62: 296–302, 1956.

150. Leake, C. D. and Pomerat, C. M.: Transport of molecules into cells against a concentration gradient. Science 127: 162–163, 1958.

151. Lee, Y. P., Takemori, A. E. and Lardy, H.: Enhanced oxidation of α-glycerophosphate by mitochondria of thyroid fed rats. J. biol. Chem. 234: 3051–3054. 1959.

152. Lehninger, A. L.: Reversal of various types of mitochondrial swelling by adenosine triphosphate. J. biol. chem. 234: 2465–2471, 1959.

153. Leloir, L. F. and Cardini, C. E.: Biosynthesis of glycogen from uridine diphosphate glucose. J. Amer. chem. Soc. 79: 6340–6341, 1957.

154. Levin, L. and Farber, R. K.: Hormonal factors which regulate the mobilization of depot fat to the liver. Recent Progr. Hormone Res. 7: 399–435, 1952.

155. Levine, R.: On the mechanisms of action of hormones on cells. Survey of Biological Progress 3: 185–211, 1957.

156. Levine, R. and Fritz, I. B.: The relation of insulin to liver metabolism. Diabetes 5: 209–222, 1956.

157. Levine, R. and Goldstein, M. S.: On the mechanism of action of insulin. Recent Progr. Hormone Res. 11: 343–380, 1955.

158. Levine, R., Goldstein, M. S., Huddlestun, B. and Klein, S. P.: Action of insulin on the permeability of cells to free hexoses, as studied by its effect on the distribution of galactose. Amer. J. Physiol. 163: 70–76, 1950.

159. Levine, R., Goldstein, M. S., Klein, S. and Huddlestun, B.: The action of insulin on the distribution of galactose in eviscerated nephrectomized dogs. J. biol. Chem. 179: 985–986, 1949.

160. Levinsky, N. G. and Davidson, D. D.: Renal action of parathyroid extract in the chick. Amer. J. Physiol. 191: 530–536, 1957.

161. Lewis, W.: Pinocytosis. Johns Hopk. Hosp. Bull. 49: 17–23, 1931.

162. L'Heureux, M. V. and Roth, G. J.: Influence of parathyroid extract on citric acid of serum. Proc. Soc. exp. Biol., N. Y. 84: 7–9, 1953.

163. Li, C. H., Geschwind, I. and Evans, H. M.: The effect of growth and adrenocorticotrophic hormones on the amino acid levels in the plasma. J. biol. Chem. 177: 91–95, 1949.

164. Lipmann, F.: Fermentation of phosphogluconic acid. Nature, Lond. 138: 588–589, 1936.

165. Logan, J. E., Heagy, F. C. and Rossiter, R. J.: Effect of hypophysectomy and administration of ACTH on the incorporation of radioactive phosphate into RNA nucleotides. Endocrinology 56: 455–460, 1955.

166. Lopez, E., White, J. E. and Engel, F. L.: Contrasting requirements for the lipolytic action of corticotropin and epinephrine on adipose tissue in vitro. J. biol. Chem. 234: 2254–2258, 1959.

167. Loring, J. M. and Villee, C. A.: The oestrogen content of human placenta. Acta endocrinol. 25: 371–376, 1957.

168. Lotspeich, W. D.: Metabolic Aspects of Renal Function, pp. 15–37. Charles C Thomas, Springfield, Ill., 1959.

169. 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.

170. Lynen, F.: Participation of coenzyme A in the oxidation of fat. Nature, Lond. 174: 962–965, 1954.

171. Madison, L. L., Combes, B., Strickland, W., Unger, R. and Adams, R.: Evidence for a direct effect of insulin on hepatic glucose output. Metabolism 8: 469–471, 1959.

172. Madison, L. L. and Unger, R. H.: The physiologic significance of the secretion of endogenous insulin into the portal circulation. I. Comparison of the effects of glucagon-free insulin administered via the portal vein and via a peripheral vein on the magnitude of hypoglycemia and peripheral glucose utilization. J. clin. Invest. 37: 631–639, 1958.

173. MALEY, G. F. AND LARDY, H. A.: Metabolic effects of thyroid hormones *in vitro* II. Influence of thyroxine and tri-iodothyronine on oxidative phosphorylation. J. biol. Chem. **204**: 435–444, 1953.

174. MANCHESTER, K. L. AND KRAHL, M. E.: Effect of insulin on the incorporation of C^{14} from C^{14}-labelled carboxylic acids and bicarbonate into protein of isolated rat diaphragm. J. biol. Chem. **234**: 2938–2942, 1959.

175. MANCHESTER, K. L. AND YOUNG, F. G.: Unpublished experiments cited in reference 174.

176. MARKS, P. A.: A newer pathway of carbohydrate metabolism; the pentose phosphate pathway. Diabetes **5**: 276–283, 1956.

177. MASORO, E. J., CHAIKOFF, I. L., CHERNICK, S. S. AND FELTS, J. M.: Previous nutritional state and glucose conversion to fatty acids in liver slices. J. biol. Chem. **185**: 845–856, 1950.

178. MASRI, M. S., LYON, L. AND CHAIKOFF, I. L.: Nature of the stimulating action of insulin on lipogenesis from acetate in fasted rat liver. J. biol. Chem. **197**: 621–624, 1952.

179. MAYER, J., HAGMAN, N. C., MARSHALL, N. B. AND STOOPS, A. J.: Fat metabolism in three forms of obesity. V. Hepatic lipogenesis *in vitro*. Amer. J. Physiol. **181**: 501–503, 1955.

180. McCORQUODALE, D. J. AND MUELLER, G. C.: Effect of estradiol on the level of amino acid activating enzymes in the rat uterus. J. biol. Chem. **232**: 31–42, 1958.

181. McKERNS, K. W. AND BELL, P. H.: The mechanism of action of estrogenic hormones on metabolism. Recent Progr. Hormone Res. **16**: to be published, 1960.

182. McLEAN, P.: Carbohydrate metabolism of mammary tissue. I. Pathways of glucose catabolism in the mammary gland. Biochim. biophys. Acta **30**: 303–315, 1958.

183. McLEAN, P.: Carbohydrate metabolism of mammary tissue. II. Levels of oxidized and reduced diphosphopyridine nucleotide and triphosphopyridine nucleotide in the rat mammary gland. Biochim. biophys. Acta **30**: 316–324, 1958.

184. MEDES, G., THOMAS, A. AND WEINHOUSE, S.: Nutritional factors in fatty acid synthesis by tissue slices *in vitro*. J. biol. Chem. **197**: 181–191, 1952.

185. MILMAN, A. AND RUSSELL, J.: Some effects of purified pituitary growth hormone on carbohydrate metabolism in the rat. Endocrinology **47**: 114–128, 1950.

186. MILSTEIN, S. W.: Oxidation of specifically labelled glucose by rat adipose tissue. Proc. Soc. exp. Biol., N. Y. **92**: 632–635, 1956.

187. MONAHAN, E. P. AND FREEMAN, S.: The maintenance of a normal serum calcium by the parathyroid gland in nephrectomized dogs. Amer. J. Physiol. **142**: 104–106, 1944.

188. MORTIMORE, G. E. AND TIETZE, F.: Studies on the mechanism of capture and degradation of insulin I^{131} by the cyclically perfused rat liver. Ann. N. Y. Acad. Sci. **82**: 329–337, 1959.

189. MUELLER, G. C.: Some biochemical studies on the mechanism of action of estrogens. Cancer, N. Y. **10**: 716–720, 1957.

190. NEUMAN, W. F. AND NEUMAN, M. W.: The chemical dynamics of bone mineral. Univ. of Chicago Press, Chicago, 1958.

191. NICHOLSON, T. F.: The mode and site of the renal action of parathyroid extract in the dog. Canad. J. Biochem. Physiol. **37**: 113–117, 1959.

192. NOALL, M. W. AND KIPNIS, D. M.: The cellular penetration of amino acids in rat tissues studied with α-aminoisobutyric acid. Fed. Proc. **18**: 294, 1959.

193. NOALL, M. W., RIGGS, T. R., WALKER, L. M. AND CHRISTENSEN, H. N.: Endocrine control of amino acid transfer. Science **126**: 1002–1005, 1957.

194. NORMAN, D., MENOZZI, P., REID, D., LESTER, G. AND HECHTER, O.: Action of insulin on sugar permeability in rat diaphragm muscle. J. gen. Physiol. **42**: 1277–1299, 1959.

195. PARK, C. R.: The effect of insulin and alloxan diabetes on the transport of glucose and other sugars into cells of muscle and brain. The Hypophysial Growth Hormone, Nature and Actions. p. 394. Blakiston, McGraw-Hill, New York, 1955.

196. PARK, C. R., JOHNSON, L. H., WRIGHT, J. H., JR. AND BATSEL, H.: Effect of insulin on transport of several hexoses and pentoses into cells of muscle and brain. Amer. J. Physiol. **191**: 13–18, 1957.

197. PARK, C. R., POST, R. L., KALMAN, C. F., WRIGHT, J. H., JR., JOHNSON, L. H. AND MORGAN, H. E.: The transport of glucose and other sugars across cell membranes and the effect of insulin. In: Internal Secretions of the Pancreas. Ciba Foundation Colloquia Endocrinology **9**: 240–265, 1956.

198. PARK, C. R., REINWEIN, D., HENDERSON, M. J., CADENAS, E. AND MORGAN, H. E.: The action of insulin on the transport of glucose through the cell membrane. Amer. J. Med. **26**: 674–684, 1959.

199. PARK, J. H., MERIWETHER, B. P. AND PARK, C. R.: The uncoupling of oxidative phosphorylation by thyroxine and tri-iodothyronine. Biochim. biophys. Acta **28**: 662–663, 1958.

200. PERKINS, H. R. AND DIXON, T. F.: Parathyroid and bone citrogenase. Science **118**: 139–140, 1953.

201. PÉRON, F. G. AND KORITZ, S. B.: On the exogenous requirements for the action of ACTH *in vitro* on rat adrenal glands. J. biol. Chem. **233**: 256–259, 1958.

202. PERSKE, W. F., KVAM, D. C. AND PARKS, R. E., JR.: Hepatic phosphorylase and epinephrine hyperglycemia. Biochem. Pharmacol. **1**: 141–151, 1958.

203. PETERS, R. A.: Hormones and the cytoskeleton. Nature, Lond. **177**: 426, 1956.

204. PHILLIPS, A. H. AND LANGDON, R. G.: The influence of thyroxine and other hormones on hepatic TPN cytochrome reductase activity. Biochim. biophys. Acta **19**: 380–382, 1956.

205. POPJÁK, G. AND TIETZ, A.: Coenzyme A dependent reactions in a soluble enzyme preparation from rat mammary gland. Biochem. J. **57**: xiv, 1954.

206. POTTER, V. R.: The present status of the deletion hypothesis. U. Mich. Med. Bull. **23**: 401–412, 1957.

207. PRICE, W. H., CORI, C. F. AND COLOWICK, S. P.: The effect of anterior pituitary extract and of insulin on the hexokinase reaction. J. biol. Chem. 160: 633–634, 1945.

208. PRICE, W. H., SLEIN, M. W., COLOWICK, S. P. AND CORI, G. T.: Effect of adrenal cortical extract on the hexokinase reaction. Fed. Proc. 5: 150, 1946.

209. RABINOVITCH, M., JUNQUIERA, L. C. U. AND ROTHSCHILD, H. A.: Influence of testosterone on nucleic acid phosphorus of rat seminal vesicle. Science 114: 551–552, 1951.

210. RAISZ, L., AU, W. AND TEPPERMAN, J.: To be published.

211. RALL, T. W. AND SUTHERLAND, E. W.: Formation of a cyclic adenine ribonucleotide by tissue particles. J. biol. Chem. 232: 1065–1076, 1958.

212. RALL, T. W., SUTHERLAND, E. W. AND BERTHET, J.: The relationship of epinephrine and glucagon to liver phosphorylase. IV. The effect of epinephrine and glucagon on the reactivation of phosphorylase in liver homogenates. J. biol. Chem. 224: 463–475, 1957.

213. RALL, T. W., SUTHERLAND, E. W. AND WOSILAIT, W. D.: The relationship of epinephrine and glucagon to liver phosphorylase. III. Reactivation of liver phosphorylase in slices and in extracts. J. biol. Chem. 218: 483–495, 1956.

214. RAMEY, E. R., BERNSTEIN, H. AND GOLDSTEIN, M. S.: Effect of sympatholytic blocking agents on increased oxygen consumption following administration of thyroxine. Fed. Proc. 14: 118, 1955.

215. RANDLE, P. J. AND SMITH, G. H.: Regulation of glucose uptake by muscle. I. The effect of insulin, anaerobiosis and cell poisons on the uptake of glucose and release of potassium by isolated rat diaphragm. Biochem. J. 70: 490–500, 1958.

216. RANDLE, P. J. AND SMITH, G. H.: The effects of insulin, anaerobiosis and cell poisons on the penetration of isolated rat diaphragm by sugars. Biochem. J. 70: 501–508, 1958.

217. RASMUSSEN, H.: The influence of parathyroid function upon the transport of calcium in isolated sacs of rat small intestine. Endocrinology 65: 517–159, 1959.

218. RASMUSSEN, H. AND CRAIG, L. C.: Purification of parathyroid hormone by countercurrent distribution. J. Amer. chem. Soc. 81: 5003, 1959.

219. RENOLD, A. E., HASTINGS, A. B. AND NESBETT, F. B.: Studies on carbohydrate metabolism in rat liver slices. III. Utilization of glucose and fructose by liver from normal and diabetic animals. J. biol. Chem. 209: 687–696, 1954.

220. RENOLD, A. E., HASTINGS, A. B., NESBETT, F. B. AND ASHMORE, J.: Studies on carbohydrate metabolism in rat liver slices. IV. Biochemical sequence of events after insulin administration. J. biol. Chem. 213: 135–146, 1955.

221. ROBBINS, P. W., TRAUT, R. R. AND LIPMANN, F.: Glycogen synthesis from glucose, glucose-6-phosphate, and uridine diphosphate glucose in muscle preparations. Proc. nat. Acad. Sci., Wash. 45: 6–12, 1959.

222. ROBERTS, S. AND SZEGO, C. M.: The influence of steroids on uterine respiration and glycolysis. J. biol. Chem. 201: 21–30, 1953.

223. ROSS, E. J.: The influence of insulin on the permeability of the blood aqueous barrier to glucose. J. Physiol. 116: 414–423, 1952.

224. ROSS, E. J.: The "permeability" hypothesis of the action of insulin. Medicine 35: 355–388, 1956.

225. RYAN, K. J. AND ENGEL, L. L.: Hydroxylation of steroids at carbon 21. J. biol. Chem. 225: 103–114, 1957.

226. SANGER, F.: Chemistry of insulin; determination of the structure of insulin opens the way to greater understanding of life processes. Science 129: 1340–1344, 1959.

227. SAYERS, G.: The adrenal cortex and homeostasis. Physiol. Rev. 30: 241–320, 1950.

228. SCAIFE, J. F. AND MIGICOVSKY, B. B.: Effect of alloxan, insulin, and thyroxine on cholesterol and fatty acid synthesis by rat liver homogenates. Canad. J. Biochem. Physiol. 35: 15–23, 1957.

229. SEARLE, G. T. AND CHAIKOFF, I. L.: Inhibitory action of hyperglycemia on delivery of glucose to the bloodstream by liver of the normal dog. Amer. J. Physiol. 170: 456–460, 1952.

230. SEUBERT, W., GREULL, G. AND LYNEN, F.: Die Synthese der Fettsäuren mit gereinigten Enzymen des Fettsäurecyclus. Angew. Chem. 69: 359–361, 1957.

231. SHAPIRO, B. AND WERTHEIMER, E. J.: The synthesis of fatty acids in adipose tissue in vitro. J. biol. Chem. 173: 725–728, 1948.

232. SHAW, W. N., DITURI, F. AND GURIN, S.: Lipogenesis in particle-free extracts of rat liver. II. Experimental Diabetes. J. biol. Chem. 226: 417–425, 1957.

233. SHAW, W. N. AND STADIE, W. C.: Coexistence of insulin-responsive and insulin nonresponsive glycolytic systems in rat diaphragm. J. biol. Chem. 227: 115–134, 1957.

234. SHAW, W. N. AND STADIE, W. C.: Two identical Embden-Meyerhof enzyme systems in normal rat diaphragms differing in cytological location and response to insulin. J. biol. Chem. 234: 2491–2496, 1959.

235. SHAW, W. N. AND WINEGRAD, A. I.: Interrelationship of glucose and lipid metabolism. Diabetes 8: 68–69, 1959.

236. SHELESNYAK, M. C.: Histamine releasing activity of natural estrogens. Proc. Soc. exp. Biol., N. Y. 100: 739–741, 1959.

237. SHOEMAKER, W. C., MAHLER, R. AND ASHMORE, J.: The effect of hepatic glucose metabolism in the unanesthetized dog. Metabolism 8: 494–511, 1959.

238. SHORR, E. AND BARKER, S. B.: In vitro action of insulin on minced avian and mammalian muscle. Biochem. J. 33: 1798–1809, 1939.

239. SIEKEVITZ, P.: On the meaning of intracellular structure for metabolic regulation. In: Ciba Foundation Symposium on the Regulation of Cell Metabolism, pp. 17–45. Little, Brown and Co., Boston, 1959.

240. SINEX, F. M., MACMULLEN, J. AND HASTINGS, A. B.: The effect of insulin on the incorporation of C¹⁴ into the protein of rat diaphragm. J. biol. Chem. 198: 615–619, 1952.

241. SIPERSTEIN, M. D.: Interrelationships of glucose and lipid metabolism. Amer. J. Med. 26: 685-702, 1959.

242. SIPERSTEIN, M. D. AND FAGAN, V. M.: Role of glycolysis in fatty acid and cholesterol synthesis in normal and diabetic rats. Science 126: 1012-1013, 1957.

243. SIPERSTEIN, M. D. AND FAGAN, M.: Studies on the relationship between glucose oxidation and intermediary metabolism. II. The role of glucose oxidation in lipogenesis in diabetic rat liver. J. clin. Invest. 37: 1196-1201, 1958.

244. SOFFER, L. J., DANTES, D. A., GROSSMAN, E. B., SOBOTKA, H. AND JACOBS, M. D.: Ultrafiltrable magnesium in hyperthyroidism. J. clin. Invest. 18: 597-601, 1939.

245. SPAZIANI, E. AND SZEGO, C. M.: The influence of estradiol and cortisone on uterine histamine of the ovariectomized rat. Endocrinology 63: 669-678, 1958.

246. STADIE, W. C.: Current concepts of the action of insulin. Physiol. Rev. 34: 52-100, 1954.

247. STADIE, W. C.: Current views on the mechanisms of insulin action. Amer. J. Med. 19: 257-273, 1955.

248. STADIE, W. C.: Recent advances in insulin research. Diabetes 5: 263-275, 1956.

249. STADIE, W. C.: Newer concepts of the action of insulin. Amer. J. clin. Nutr. 5: 393-403, 1957.

250. STADIE, W. C. AND HAUGAARD, N.: The hexokinase reaction in tissue extracts from normal and diabetic rats. J. Biol. Chem. 177: 311-324, 1949.

251. STADIE, W. C., HAUGAARD, N. AND HILLS, A. G.: The effect of insulin and adrenal cortical extract on the hexokinase reaction in extracts of muscle from depancreatized cats. J. biol. Chem. 184: 617-626, 1950.

252. STEIN, A. M. AND KAPLAN, N. O.: Relationship of 3-α-hydroxysteroid dehydrogenase to pyridine nucleotide transhydrogenase. Science 129: 1611-1612, 1959.

253. STETTEN, D., JR. AND BOXER, G. E.: Studies in carbohydrate metabolism III. Metabolic defect in alloxan diabetes. J. biol. Chem. 156: 271-278, 1944.

254. SUTHERLAND, E. W. AND CORI, C. F.: Influence of insulin preparations on glycogenolysis in liver slices. J. biol. Chem. 172: 737-750, 1948.

255. SUTHERLAND, E. W. AND CORI, C. F.: Effect of hyperglycemic-glycogenolytic factor and epinephrine on liver phosphorylase. J. biol. Chem. 188: 531-543, 1951.

256. SUTHERLAND, E. W. AND RALL, T. W.: Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles. J. biol. Chem. 232: 1077-1091, 1958.

257. SUTHERLAND, E. W. AND WOSILAIT, W. D.: The relationship of epinephrine and glucagon to liver phosphorylase. I. Liver phosphorylase; preparation and properties. J. biol. Chem. 218: 459-468, 1956.

258. SWANN, M. M.: The control of cell division: A review. II. Special mechanisms. Cancer Res. 18: 1118-1160, 1958.

259. SYMINGTON, T. AND DAVIDSON, J. N.: The effect of exogenous adrenocorticotropic hormone (ACTH) and conditions of stress on the chemical composition of the human adrenal gland. Scottish med. J. 1: 15-31, 1956.

260. SZEGO, C. M. AND ROBERTS, S.: Steroid action and interaction in uterine metabolism. Recent Progr. Hormone Res. 8: 419-469, 1953.

261. TALALAY, P., HURLOCK, B. AND WILLIAMS-ASHMAN, H. G.: On a coenzymatic function of estradiol-17-β. Proc. nat. Acad. Sci., Wash. 44: 862-884, 1958.

262. 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.

263. TALMAGE, R. V. AND ELLIOTT, J. R.: Changes in extracellular fluid levels of calcium, phosphate and citrate ions in nephrectomized rats following parathyroidectomy. Endocrinology 59: 27-33, 1956.

264. TALMAGE, R. V. AND ELLIOTT, J. R.: Influence of parathyroids on intestinal absorption of radio calcium and radiostrontium. Fed. Proc. 17: 160, 1958.

265. TALMAGE, R. V., KRAINTZ, F. W., FROST, R. C. AND KRAINTZ, L.: Evidence for a dual action of parathyroid extract in maintaining serum calcium and phosphate levels. Endocrinology 52: 318-323, 1953.

266. TELFER, M. A.: Influence of estradiol on nucleic acids, respiratory enzymes and the distribution of nitrogen in the rat uterus. Arch. Biochem. Biophys. 44: 111-119, 1953.

267. TENG, C. T., SINEX, F. M., DEANE, H. W. AND HASTINGS, A. B.: Factors influencing glycogen synthesis by rat slices in vitro. J. cell. comp. Physiol. 39: 73-88, 1952.

268. TEPPERMAN, H. M. AND TEPPERMAN, J.: The hexosemonophosphate shunt and adaptive hyperlipogenesis. Diabetes 7: 478-485, 1958.

269. TEPPERMAN, J., BROBECK, J. R. AND LONG, C. N. H.: The effects of hypothalamic hyperphagia and of alterations in feeding habits on the metabolism of the albino rat. Yale J. Biol. Med. 15: 855-874, 1943.

270. TEPPERMAN, J. AND TEPPERMAN, H. M.: Effect of gonadotrophins on the cholesterol content of the testes of immature rats. Endocrinology 41: 187-195, 1947.

271. TEPPERMAN, J. AND TEPPERMAN, H. M.: Effects of purified gonadotrophins on the cholesterol content of the testes of immature rats. Endocrinology 50: 162-169, 1952.

272. TEPPERMAN, J. AND TEPPERMAN, H. M.: Effects of antecedent food intake pattern on hepatic lipogenesis. Amer. J. Physiol. 193: 55-64, 1958.

273. TEPPERMAN, J. AND TEPPERMAN, H. M.: Metabolism of glucose-1-C^{14} and glucose-6-C^{14} in liver slices of re-fed rats. Fed. Proc. 18: 157, 1959.

274. TEPPERMAN, J. AND TEPPERMAN, H. M.: Unpublished experiments.

275. TICHENER, E. B., GIBSON, D. M. AND WAKIL, S. J.: Requirements for fatty acid synthesis. Fed. Proc. 17: 322, 1958.

276. TIMIRAS, P. S. AND WOODBURY, D. M.: Effect of thyroid hormone on brain function and brain electrolyte distribution in rats. Endocrinology 58: 181-192, 1956.

277. VAN REEN, R. AND LOSA, F. L.: Organic composition of bone: localization of isocitric dehydrogenase in femurs. Nature, Lond. 181: 1543, 1958.

278. VAUGHAN, M., STEINBERG, D. AND SHAFRIR, E.: The effect of epinephrine, glucagon and adrenocorticotropic hormone (ACTH) on phosphorylase activity in adipose tissue. J. clin. Invest. 38: 1051–1052, 1959.

279. VILLAR-PALASI, C. AND LARNER, J.: A uridine coenzyme-linked pathway of glycogen synthesis in muscles. Biochem. biophys. Acta 30: 449, 1958.

280. VILLEE, C. A.: An estradiol-induced stimulation of citrate utilization by placenta. J. biol. Chem. 215: 171–182, 1955.

281. VILLEE, C. A.: Some current speculations on the action of estrogens. Perspectives in Biol. & Med. 2: 290–308, 1959.

282. VILLEE, C. A.: Estrogens and uterine enzymes. Ann. N. Y. Acad. Sci. 75: 524–534, 1959.

283. VILLEE, C. A. AND GORDON, E. E.: Further studies on the action of estradiol in vitro. J. biol. Chem. 216: 203–214, 1955.

284. VILLEE, C. A. AND HAGERMAN, D. D.: Effects of estradiol on the metabolism of human placenta in vitro. J. biol. Chem. 205: 873–882, 1953.

285. VILLEE, C. A. AND HAGERMAN, D. D.: Studies on the estradiol-sensitive isocitric dehydrogenase of human placenta. Proc. Int. Symp. Enzyme Chem., Japan, pp. 317–323, 1957.

286. VILLEE, C. A. AND HAGERMAN, D. D.: On the identity of the estrogen-sensitive enzyme of human placenta. J. biol. Chem. 233: 42–48, 1958.

287. VITALE, J. J., HEGSTED, D. M., NAKAMURA, M. AND CONNORS, P.: The effect of thyroxine on magnesium requirement. J. biol. Chem. 226: 597–601, 1957.

288. WAKIL, S. J.: A malonic acid derivative as an intermediate in fatty acid synthesis. J. Amer. chem. Soc. 80: 6465, 1958.

289. WAKIL, S. J.: Discussion at Brook Lodge Symposium on Energy Balance, 1959, to be published. Amer. J. clin. Nutr., 1960.

290. WAKIL, S. J., PORTER, J. W. AND GIBSON, D. M.: Studies on the mechanism of fatty acid synthesis. V. Bicarbonate requirement for the synthesis of long chain fatty acids. Biochim. biophys. Acta 30: 376, 1958.

291. WEIL-MALHERBE, H.: The mechanism of action of insulin. Ergebn. Physiol. 48: 54–111, 1955.

292. WICK, A. N. AND DRURY, D. R.: Action of insulin on volume of distribution of galactose in the body. Amer. J. Physiol. 173: 229–232, 1953.

293. WICK, A. N., DRURY, D. R. AND MACKAY, E. M.: The disposition of glucose by the extrahepatic tissues. Ann. N. Y. Acad. Sci. 54: 684–692, 1951.

294. WINEGRAD, A. I. AND RENOLD, A. E.: Studies on rat adipose tissue in vitro. II. Effects of insulin on the metabolism of specifically labelled glucose. J. biol. Chem. 233: 273–276, 1958.

295. WINEGRAD, A. I., SHAW, W. N., LUKENS, F. D. W., STADIE, W. C. AND RENOLD, A. E.: Effects of growth hormone in vitro on the metabolism of glucose in rat adipose tissue. J. biol. Chem. 234: 1922–1928, 1959.

296. WOLFF, E. C. AND BALL, E. G.: The action of thyroxine on oxidation of succinate and malate. J. biol. Chem. 224: 1083–1098, 1957.

297. WOLFF, E. C. AND WOLFF, J.: The effect of thyroxine on isolated dehydrogenases. Biochim. biophys. Acta 26: 387–396, 1957.

298. WOOL, I. G. AND KRAHL, M. E.: An effect of insulin on peptide synthesis independent of glucose or amino acid transport. Nature, Lond. 183: 1399–1400, 1959.

299. WOOL, I. G. AND KRAHL, M. E.: Incorporation of C¹⁴-amino acids into protein of isolated diaphragms: an effect of insulin independent of glucose entry. Amer. J. Physiol. 196: 961–964, 1959.

300. WOSILAIT, W. D. AND SUTHERLAND, E. W.: The relationship of epinephrine and glucagon to liver phosphorylase. II. Enzymatic inactivation of liver phosphorylase. J. biol. Chem. 218: 469–481, 1956.

301. ZIEGLER, D. M., LINNANE, A. W., GREEN, D. E., DASS, C. M. S. AND RIS, H.: Studies on the electron transport system. XI. Correlation of morphology and enzymic properties of mitochondrial and sub-mitochondrial properties. Biochim. biophys. Acta 28: 524–538, 1958.

REFERENCES ADDED IN PROOF

302. CATRAVAS, G. N. AND ANKER, H. S.: On the regulation of fatty acid biosynthesis by lipogenesis. Proc. nat. Acad. Sci. 44: 1097–99, 1958.

303. GIBSON, D. M., HUBBARD, DOROTHY D., AND LOVE, W. C.: A primary defect in fatty acid biosynthesis in liver of the alloxan diabetic rat. Fed. Proc. 19: 227, 1960.

304. KRONFELD, D. S. AND KLEIBER, M.: Mammary ketogenesis in the cow. J. appl. Physiol. 14: 1033–35, 1959.

305. LEBOEUF, B. AND CAHILL, G. F., JR.: Regulation of fatty acid synthesis and direct oxidative pathway in adipose tissue. Fed. Proc. 19: 226, 1960.

306. MASORO, E. J.: An analysis of the depressed hepatic lipogenesis induced by cold stress. Fed. Proc. 19: 225, 1960.

307. TEPPERMAN, J., TEPPERMAN, HELEN M. AND DEWITT, JANET: The "repair" of lipogenesis on re-feeding: the early hours. Fed. Proc. 19: 226, 1960.