EXCERPTS FROM THE PHARMACOLOGY OF HORMONES AND RELATED SUBSTANCES[1,2]

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Pharmacology is the *umuarama*[4] of biologists. It is connected to other basic and applied sciences by so many alleys and avenues that it was a challenge to this cicerone to select proper scenery for such a brief promenade. Like an ink drop on a blotter pharmacology has extended from Dorpat to Strassburg, Baltimore, Edinburgh and Ann Arbor (1, 2); from Montpellier and Paris to Rio de Janeiro; from Leipzig to Boston and São Paulo (3, 4).

The growth of scientific literature is so fast and impressive that it exerts a continuous impact on the specialist. It seems like an endless crop that we must harvest with more and more speed and without enough storehouse. Our apologies go to those colleagues whose work we apparently overlooked in the selections that follow.

Obvious limitations have restricted this review to the following topics: Melanotropins & Pineal; Hypothalamic Factors; New Factors or Agents; Gonadotropins; Plants and Endocrines.

Melanotropins and Pineal

Color display of certain poikilothermic animals depends on pigment granules within the chromatophoric cells of their skin. Melanocytes are a particular example of these cells, their melanin granules being expanded or retracted under the influence of hormones and pharmacological agents. When the animal is placed in a dark background the melanin granules are

[1] The survey of the literature pertaining to this review, for the most part, covers the period from July 1961 to April 1963.

[2] The following abbreviations will be used:

ACTH (adrenocorticotropic hormone); ADH (antidiuretic hormone); CRF (corticotropin releasing factor); EDTA (ethylenediamine tetracetate); EPS (exophthalmos-producing-substance); FSH (follicle stimulating hormone); GH (growth hormone, somatotropin); GRF (gonadotropin releasing factor); HCG (human chorionic gonadotropin); HMG (human menopausal gonadotropin); HPG (human pituitary gonadotropin); ICSH (interstitial cell stimulating hormone); IRP (international reference preparation); LATS (long acting thyroid stimulator); LH (luteinizing hormone, ICSH); LTH (luteotropin, prolactin); MSH (melanocyte stimulating hormone); PMSG (pregnant mare serum gonadotropin); TRF (thyrotropin releasing factor); TSH (thyrotropic stimulating hormone).

[3] I wish to thank my colleagues Aron Jurkiewicz, L. Miller de Paiva, Wilson Gouvêa and Zuleika P. Picarelli for their help in reviewing the available literature, and E. L. A. Carlini and A. C. M. Paiva for their cooperation and criticism.

[4] Place where friends meet together. A Brazilian Indian neologism from *umuã* friend or companion and *rétama* place or region.

dispersed with consequent darkening of the skin. In a clear environment, or following hypophysectomy, there is a marked lightening of the animal as a consequence of concentration of the granules around the nucleus of the melanophores. Melanotropins and melatonin are examples of antagonizing humoral factors which respectively expand and retract those granules. This very interesting subject of practical implications was well described years ago by Van Dyke (5), recently reviewed by Li (6), Lerner & Lee (7), and dealt with in book form by Waring (8).

Surely the dramatic progress in this field was attained because of reliable bioassay methods. Minute amounts of hormones, including melatonin preparations, can be tested by employing isolated pieces of frog skin. The melanophoric response as a function of the dosage can be measured, for example, with a photo-electric reflection meter as described by Mori & Lerner (9). According to these authors it is possible to detect with this bioassay method, amounts of melatonin as low as 10^{-11}g.

Two melanotropins have been identified in pituitary extracts from several species; α-MSH was found to be a tridecapeptide with the same sequence in pig, beef, horse, monkey and sheep: β-MSH contains 18 or 22 amino acid residues in sequences which show minor differences according to species (7).

Polypeptides have been synthetized which exhibited MSH activity. The melanocyte dispersing response of closely related natural and synthetic peptides was studied by Pickering & Li (10). All the preparations used were related to the first 19 amino acids of the ACTH sequence. The pentapeptide His-Phe-Arg-Try-Gly, or sequence 6–10 of ACTH, was the smallest chain with melanophoric property. The α-MSH, a tridecapeptide from equine origin, was the most active preparation found: it darkens the hypophysectomized *Rana pipiens* in a dose as low as 0.01 μg. While exhibiting one tenth of this activity α_p ACTH practically doubled the duration of the response. Previous heating of these peptides with NaOH solution led to a potentiation and prolongation of the melanocyte response. An hexapeptide was shown by Hofmann et al. (11) to exhibit a potency of about 3.7×10^8 units/g.

The action of melanotropins in mammals has been described. Snell (12) has studied the influence of pig β-MSH on the melanocytes and melanin in the skin of twelve adult normal male and castrate female guinea pigs. Subcutaneous administration of the hormone, roughly 800 IU for a month in each animal, provoked in only one male guinea pig a slight increase in the pigmentation of the skin of the anterior abdominal wall and areola. On the other hand, in man synthetic MSH peptides are effective agents for darkening the skin (11).

Some problems in this field are still awaiting forthcoming research. The activity of these humoral factors upon other chromatophores, besides melanophores, has been neglected; the mode of action of these MSH still remains obscure.

Extra-melanophoric activity of β-MSH.—Krivoy & Guillemin (13) ob-

served an increase, for long periods of time, of evoked monosynaptic potentials in the spinal cord of cats intravenously injected with 0.25–2 μg/kg body weight of highly purified β-MSH. Krivoy et al. (14) have studied the effect of β-MSH on the electrical discharges of the knife fish, *G. eigenmannia*. The presence of 0.1–0.2 μg/ml of β-MSH in the aquarium water produced a decrease in the frequency of spontaneous modifications of the amplitude of discharges.

Drugs and melanotropins.—Reserpine, when injected into the dorsal lymph sac of *Rana* frogs at dose levels of 0.05–0.25 mg. was shown to provoke melanophore dispersion in the skin; this does not occur *in vitro* (15). It was suggested that this effect is due to an increased secretion of endogenous MSH. The same effect was observed with *Bufo* toads but not with *Hyla* frogs. Reserpine suppresses FSH, LH, TSH and ACTH but increases LTH, ADH and MSH secretion.

Scott (16) has studied the effect of some psychic energizers on melanocytes in *Rana pipiens*. The administration of amphetamine, ritalin, catron, phenelzine, iproniazid and other energizers resulted in supression of the melanocyte response to conditions that would normally lead to pigment dispersion. However the same drugs were unable to reverse the degree of dispersion and bring about concentration of the pigment in the cell as does epinephrine. Their action appears to be on the central nervous system.

As β-MSH potentiates spinal reflexes, whereas chlorpromazine depresses them, Krivoy & Guillemin (13) tried to investigate whether β-MSH could antagonize the action of chlorpromazine on the positive intermediary potential of cat spinal cord. The administration of chlorpromazine to spinal cats, in doses of 4–10 mg/kg, resulted in inhibition of the positive intermediary potential. The subsequent administration of β-MSH in doses of 0.14–0.25 mg /kg resulted in restoration of the positive intermediary potential towards normal. This would be in agreement with the hypothesis that chlorpromazine acts to lower the central excitatory state by modifying the activity of β-MSH in the nervous system.

Pineal.—Osima & Bianchini (17) have tried to demonstrate the endocrine activity of the epiphysis which would indeed produce: Lerner's melatonin to contract melanophores, Farrell's adrenoglomerulotropin to influence aldosterone secretion and a third hormone, *anestrine*, to prolong rat diestrus, being therefore an antiestrogenic substance.

Melatonin (N-acetyl-5-methoxytryptamine) provokes a maximal melanophoric retraction in *Scardinius erythrophthalmus* specially if a broad expansion is previously induced by caffeine (18). Its fate and degradation in animals have been studied by Kopin et al. (19).

Arguments in favor of adrenoglomerulotropin being a true diencephalic hormone were presented by Farrell & Taylor (20) while Ullrich & Marsh (21), in their review on water and electrolyte metabolism, are prone to admit its minor role in corticosteroidogenesis. Pineal extracts appear to increase the carboxylic acid esterase activity of the glomerulosa cells of rat adrenals (22).

From bovine pineal extracts Milcu and associates (23) have isolated a polypeptide with pressor and oxytocic properties which they identified as arginine-vasotocin or a very closely related substance. The hypothalamo-hypophyseal system, which produces arginine or lysine-vasopressin and oxytocin, and the epithalamo-habenulo-pineal system, which secretes arginine-vasotocin, would be two physiologically antagonistic systems in mammals, a contention that is far from being demonstrated. What relations could exist between this arginine-vasotocin and angiotensin II, now involved as the trophic hormone in aldosterone secretion (24), is a matter worth investigation.

Hypothalamic Factors

The fall of the pituitary gland from the regency of the endocrine system has directed the attention of investigators to the hypothalamic nuclei as a source of important humoral substances which regulate anterior lobe functions (25). This problem, of a well recognized complexity, has been approached by different methods outlined as follows: (*a*) production of thermal or electrolytic lesions (26, 27) or by electrical stimulation (28, 29, 30) in restricted hypothalamic areas using electrodes stereotaxically placed; (*b*) hypothalamic lesions provoked by systemic injections of drugs such as aurothioglucose (31, 32); (*c*) injections of minute amounts of mediators either into the third and lateral ventricles (33, 34) or directly within the hypothalamus, tuber or pituitary (35, 36, 37); (*d*) stereotaxic implantation of hormone micro-pellets e.g. testosterone (38, 39) and hydrocortisone (40); (*e*) intrahypothalamic and intrapituitary thyroid and ovary grafts as in the experiments of Bogdanove & Crabill (41); (*f*) indirect stimulation of the hypothalamic region through intracerebral injections of hypertonic sodium chloride solution (42) or local application in the cerebral cortex of a 5 percent epinephrinesolution (43); (*g*) assays of hypothalamic extracts from various sources for their content in humoral factors (44), enzymes (45, 46) and pharmacologically active substances (47, 48). Recently the following hypothalamic factors have attracted the attention of investigators.

Corticotropin-releasing factor (*CRF*).—This factor has been the subject of continous research in the last two years (49). The liberation of ACTH under different circumstances is followed by increased activity of the adrenal cortex. This release of ACTH depending on the integrity of the hypothalamus hypophysial axis and on blood content of corticosteroids, illustrates an important feedback mechanism of homeostasis in vertebrates. However, it is not well established whether CRF is a particular principle different either from pitressin or from such suggested central mediators as acetylcholine, catecholamines, serotonin and histamine.

Leeman, Glenister & Yates (50) have recently shown the occurrence of CRF in a bovine hypothalamic extract. They tested the ability of the extract to liberate ACTH when injected intravenously into male rats. Increased cor-

ticosteroid output and ascorbic acid depletion of the adrenal glands were obtained also by administration of histamine and pitressin. Previous treatment with pentobarbital and morphine, however, inhibited the ACTH release response to these two agents but not to the extract.

Another approach to the problem of CRF individuality was used by Grindeland et al. (51), who investigated the release of ACTH by cells of a particular kind of adenohypophyseal tumor (MET-F4) implanted in the rat hindleg. Intravenous injection of vasopressin to these animals increased the output of corticosteroids by the adrenal glands even after hypophysectomy. Studies have been conducted in animals presenting lesions at the level of the median eminence of the hypothalamus which is considered the site of CRF production. Those lesions provoked by aurothioglucose are, of course, of a particular interest to the pharmacologist. Unfortunately the hypothalamic lesions in mice (31) and rats (32) provoked by this compound are not enough to abolish completely the ACTH release induced by physical and chemical agents. Davidson & Feldman (40), on the other hand, have shown that ACTH release after unilateral adrenalectomy in rats was not observed if hydrocortisone microcrystals were stereotaxically placed at the level of the median eminence. Control experiments by implanting cholesterol at the same site or of estradiol or hydrocortisone within the adenohypophysis did not prevent ACTH release.

To study adrenocortical function after separation of the hypophysis from hypothalamic connections, Kovács and associates (52) have carefully observed adult rats suffering from diabetes insipidus as a consequence of electrolytic destruction of the pituitary stalk. Control and lesioned animals, killed at the eighth day of the experiment, had their adrenal cortex activity evaluated by several parameters. No significant difference was found in the mean weights and microscopic characteristics of the adrenal tissue between the two groups. However, if the right adrenal was removed one hour after the left one, a decrease in the latter's content of ascorbic acid was observed in the control animals but not in the operated rats. These findings suggest that ACTH, which is accepted as responsible for the depletion of adrenal ascorbic acid, was produced in the controls because of the integrity of the hypothalamic-hypophyseal axis. A lower degree of adrenal activity in animals with lesioned pituitary stalk is also indicated by a decrease in corticosterone output both *in vivo* and *in vitro*.

Critchlow et al. (53) have treated hypophysectomized female rats, with their own pituitary implanted beneath the renal capsule, with lysine-vasopressin, melanotropin and an hypothalamic extract. The adrenal corticosteroids and ACTH content of the implanted hypophysis were significantly higher after injections of hypothalamic extracts.

The available data seem to support the conclusion that a new principle is indeed formed at the median eminence level of the hypothalamus to release corticotropin from adenohypophyseal cells. Interesting enough is the fact that

a heptapeptide, with an amino acid sequence partially contained in both α-MSH and ACTH, had an *in vitro* biological activity similar to that of CRF (54, 55).

Thyrotropin-releasing factor (TRF).—The relationship between hypothalamus and thyroid has been presented in a thoughtful monograph by Blanquet & Faure (56). A new principle was postulated as necessary for the control of adenohypophysis and thyroid. The existence of Shibusava's TRF in canine hypothalamus has been challenged by Reichlin et al. (57) although an active material which liberates TSH from rat pituitary *in vitro* was prepared by Schreiber & associates (58). This factor, probably not identical with histamine, epinephrine, norepinephrine, oxytocin, vasopressin acetylcholine or serotonin, was obtained from acetic acid extracts of bovine hypothalami after electrophoresis and filtration on sephadex column. The active preparation, which also activates pituitary phosphatase *in vitro*, contained a small peptide of at least nine given amino acids. The hypothalamic influence over the pituitary thyrotropic function does not appear to be mediated through the control of hypophyseal blood flow (59).

Gonadotropin-releasing factors (GRF).—Evidence for an hypothalamic control of the gonadotropic functions of the anterior lobe of the hypophysis has been accumulated for many years (60). Permanent estrus and sterility in adult rats as a consequence of only one subcutaneous injection of testosterone propionate at early life have been explored in connection with this nervous control. According to Gorski & Barraclough (61) the steroid would lead to a neuronal disturbance of the pre-optic and suprachiasmatic areas of the hypothalamus thus interfering with the secretion or release of luteinizing hormone. Unilaterally castrate female rats, with or without constant vaginal estrus, killed 20 days after operation, exhibited the same degree of ovarian compensatory hypertrophy while only a slight increase was detected if the animals were previously submitted to hypothalamic lesions. Disturbance of the cyclic pituitary secretion of FSH and LH has been induced in rats by Desclin et al. (62) as a consequence of electrolytic lesions located in the anterior hypothalamus. Some aspects of this hormonal imbalance have been presented by Tadewaki (63). It is worth reckoning that secretion and release of gonadotropins have been known for some time to be under the command of nervous centers. Martins et al. (64) have emphasized the cyclic character of this mechanism for the female and the acyclic one for the male as a very early imprinted pattern in the hypothalamic area.

Nikitovitch-Winer (35) has assayed in pentobarbital-blocked proestrus rats the hypothalamic substance(s) capable of releasing the ovulating hormone. Through a small cannula stereotaxically placed into the pars distalis, 28 animals were injected with control substances and 34 with an extract from the median eminence. Within the first group only one rat ovulated whereas in the second group, 24 animals presented ova on examination of the oviducts. Intrapituitary infusions of epinephrine, histamine and arginine-vasopressin were without action. McCann (65) and Ramirez & McCann (66) have as-

sayed this LH-releasing factor from the median eminence, using the ovarian ascorbic acid depletion method, to evaluate LH content of pituitary and blood plasma. The presence of a LH-releasing factor in sheep hypothalamus was confirmed; localization of this GRF in only this area of the brain is as yet uncertain (67).

McCormack & Meyer (68) have conducted experiments based on the ovulatory response of immature rats following only one subcutaneous injection of pregnant mare serum (PMSG) which are of particular interest in this regard. Female, 21-day-old rats were obtained from a Holtzman colony, where they had been exposed to nine hours of light each day, and from a Sprague-Dawley colony, where the animals were reared in continuous illumination all day. The same proportion of rats from both colonies ovulated on the third day after an injection of PMSG, although the average number of ova was consistently lower in the Sprague-Dawley strain. The frequency of ovulation decreased significantly if the animals were pretreated with sodium barbital (167 to 250 mg/kg i.p.) at 1:30 p.m. the second day, that is 18.30 to 23.30 hours before autopsy. If barbital was administered at 5:30 p.m. then the frequency of ovulation was practically the same as in the saline injected controls. This means that ovulation in immature rats after only one PMSG injection occurred on the third day between 1:30 and 5:30 p.m. The existence of this critical period points to a precise mechanism for the release of the ovulating hormone from the anterior lobe. We do not know yet whether in the immature rat, as a biological clock, the mechanism would already be set to ring for ovulation at that time.

Taleisnik et al. (42) were able to stimulate the hypothalamus of female rats through intracerebral injection of 2 μl of hypertonic saline. The observed depletion of the ovarian ascorbic acid was taken as indicative of a LH release by the adenohypophysis. The needle per se, particularly when placed at the parietal zone, produced the same result although the ovarian response could be prevented by adding procaine at 5 percent concentration to the hypertonic sodium chloride solution. Rats with constant estrus, produced either by suprachiasmatic lesion or by early treatment with testosterone, did not exhibit that response. Probably the impulses originated at the cortical site of injection would reach the hypothalamus to promote the LH discharge. Subcutaneous injection of estradiol benzoate or progesterone completely blocked those impulses.

The finding of gonadotropic secretion imbalance as a consequence of either surgical or steroid lesions of the hypothalamus suggests a resemblance between these two processes. Davidson & Sawyer (38) had shown that in dogs testosterone implanted at the median eminence level led to gonadal atrophy accompanied by a decreased volume of ejaculate or even aspermia; control experiments of hormone implantation within the thalamus or pituitary gave negative results. Thus it appears that the hypothalamic median eminence is the critical region where testosterone exerts its negative feedback upon the gonadotropic function of the anterior lobe. Lisk (39) has extended these

experiments by observing rats for 30 days after implants of minute amounts of solid testosterone in different regions of the diencephalon. Marked decreases in the weights of the gonads and accessory organs were also observed when the hormone was placed at the level of the arcuate-mammilary region. Impairment of the gonadotropin production by the pituitary was more pronounced in the female than in the male animals, thus keeping consonance with the characteristic cyclic pattern of the female hypothalamus mentioned above.

Lipschutz & Cerisola (69) have described ovarian tumors in two strains of mice as a result of disturbed secretion of gonadotropins brought about by castration and intrasplenic, intrahepatic or intrarenal ovarian grafts. Ovarian tumorigenesis would be initiated, as Lipschutz says, through "a kind of biological chain-reaction on the ovarian-hypophysial-hypothalamic level." At the onset minor ovarian changes take place because of the abnormal site of the organ, then hormonal imbalance occurs and finally, in the course of time, neoplastic initiation arises.

Drugs and the hypothalamo-hypophyseal axis.—Briggs & Munson (70) have already shown that pretreatment of rats with morphine plus sodium pentobarbital exerts a pharmacological disruption of the hypothalamo-hypophyseal axis. Marks & Vernikos-Danellis (71) made the interesting observation that, in rats, the acute liberation of ACTH by the adenohypophysis can be blocked by D-1-ethionine, probably through a competitive mechanism with methionine. Paget et al. (72) obtained striking changes in the reproductive system of rats, dogs and monkeys by administration of dithiocarbamoylhydrazines. One of these, 1α-methylallylthiocarbamoyl-2-methyl-thiocarbamoylhydrazine, was shown to be a potent suppressor of gonadotropic secretion or release. It prevented the appearance of pituitary castration cells and did not antagonize the gonadal response to gonadotropins and that of the accessory genitals to estradiol or testosterone. Its site of action is probably on the central nervous system. For obvious reasons new inhibitors of the gonadotropic function of the anterior lobe, besides steroids, were met with unusual interest and will probably be commented on in another section of this volume.

New Factors or Agents

Exophthalmos-producing substance (EPS).—Chromatographic purification of an EPS from crude bovine thyrotropin was achieved by Brunish et al. (73). Its biological assay is based on a proptosis fish response, one milligram of a chosen TSH preparation being taken as the unit. It remains to be resolved if this effect actually depends on a new hormone or if it is only a collateral activity of the thyrotropic preparation administered. In any case, it is interesting to note that these substances may have, as a main site of action, either the retrobulbar structures or the thyroid gland. Pituitary stalk section and cauterization of the pars distalis in a patient with severe exophthalmos

were followed by a dramatic improvement of the ocular symptoms accompanied by a decreased EPS activity of the serum (74).

Long-acting thyroid stimulator (LATS).—A LATS which circulates in the serum of thyrotoxic individuals was assayed by Adams (75). It would increase thyroid weight of hypophysectomized mice, being, as he believes, neither a TSH releaser nor similar to this hormone. Pimstone et al. (76) have made parallel assays of TSH, LATS and EPS in plasma of normal individuals and patients. From their results it seems that LATS correlates more closely than EPS with exophthalmos.

Fat-mobilizing factors.—A particular fraction from hog pituitary which mobilizes free fatty acids into rabbit's serum, labelled "Fraction H" by Rudman et al. (77) was considered and compared by Friesen, Barrett & Astwood. (78). These authors prefer to designate the active peptides that they have obtained as peptide I and II, instead of fat mobilizing factors I and II. Both substances liberate free fatty acids from rabbit mesenteric adipose tissue while provoking weak lipemic response after subcutaneous administration to human subjects.

Calcitonin—Perfusion experiments of isolated dog thyro-parathyroid glands with blood containing different amounts of calcium afforded to Copp et al. (79) the opportunity to study *calcitonin*, a new hormone which induces hypocalcemia. In their experiments the level of calcium of the perfusing blood was raised 2 mg percent by addition of $CaCl_2$ or lowered with EDTA. In the first instance calcitonin was liberated. The blood calcium level of a young dog, fed a low calcium diet for at least four days before the test, was employed to assay the perfusates. According to these authors parathormone and calcitonin integrate the humoral feedback mechanism for maintenance of normal blood calcium in a fashion similar to insulin and glucagon in the maintenance of the blood sugar level. This subject was dealt with in the excellent review by Munson and associates who point out that an eventual relationship between calcitonin and parotin, a protein from bovine parotid glands which also lowers blood calcium, has not yet been investigated (80).

Gonadotropins[5]

Gonadotropins are hormones of a mucoprotein nature produced either by the anterior lobe of the hypophysis or by the chorionic placental villosities (82, 83). Both classes are represented by circulating or serum gonadotropins and by excreted or urinary gonadotropins. Follicle stimulating hormone (FSH), luteinizing or interstitial cell stimulating hormone (LH or ICSH) and prolactin or luteotropin (LTH) are the main physiological entities which usually receive the designation of gonadotropins. Pregnant mare serum (PMSG), human chorionic (HCG) and human menopausal (HMG) gonado-

[5] We accept the arguments of Stewart & Li (81) for using *gonadotropins* and not *gonadotrophins*.

tropins are available preparations which evoke physiological effects rather similar to the combined actions of those main principles (82). Arguments are in favor of FSH, ICSH and LTH as being characteristic individualities even despite the fact that they were not yet obtained as chemically pure substances. Difficulties also arise when we know that their composition and immunological properties depend on the source used for their isolation (84 to 87). Recently Squire, Li & Andersen (88) described an ICSH obtained and purified from human hypophyses. Li (89) also gives details about the purification of lactogenic hormone. Reichert (90) took advantage of the low FSH content of bovine pituitaries to prepare and purify the ICSH from that source. A high degree of purification of HCG was achieved by Got & Bourrillon (91).

Gonadotropins act directly upon the gonads of both sexes and indirectly upon the male and female genitals through the release of gonadal steroids. Gametogenesis is maintained or repaired in either sex by FSH; ICSH stimulates ovarian interstitial tissue, ovulation, corpora lutea formation and production of androgens by Leydig cells; secretion of progesterone and lactogenesis are under the control of LTH. This rather schematic picture presents *nuances* according to the animal species and physiological conditions. As we know from general pharmacology, the biological actions of gonadotropins depend on: (*a*) *individual characteristics*, such as species, strain, male or female, immature or adult, pregnant or nonpregnant, normal or hypophysectomized: (*b*) *inherent conditions of the hormonal preparation* used, v.g. source, degree of purity, dosage, route of administration, schedule of treatment, biotransformations, antagonistic or synergistic agents; (*c*) *environment:* season, temperature, illumination and even the time of injection! All these circumstances, which are obvious to the specialist (92), ought to be considered particularly when one tries to investigate the type and strength of a given gonadotropin. To find its potency, for instance, one needs first to adopt a reliable method for measurement and further to compare, under strictly the same experimental conditions, the accepted end point with that exhibited by a standard preparation. Fortunately enough, as a consequence of joint effort of internationally recognized institutions, new standard reference gonadotropins are now available for comparative investigations.

Biological assays.—The work of Woods & Simpson (93) on the maintenance and repair of the male reproductive tract of young hypophysectomized rats is a thoughtful contribution to the understanding of the main effects of gonadotropins and the influence of other pituitary hormones. In their biological assays of FSH and ICSH, for example, they used immature female hypophysectomized rats of the Long Evans strain and they defined the *rat unit* of FSH as the "minimal total dose among a series of graded doses injected subcutaneously once daily for three days which after 72 hours results in microscopically measurable follicular development with beginning antrum formation, an increase in follicular diameter from 375 μ to 450-500 μ in two-thirds of the rats tested." This minimal effective dose corresponds to ED67

from probital analysis (94). Taking as a reference standard the sheep FSH distributed by the National Institutes of Health (NIH-FSH-S1), they assayed four different FSH preparations obtained from extracts of sheep pituitaries by chromatography on DEAE-cellulose. The *rat unit* for their preparations averaged 2.8 μg whereas for the standard it corresponded to 55 μg. They have also defined their *rat unit* for the ICSH as "the minimal total dose injected intraperitoneally daily over a 3-day period which initiates repair of the deficient interstitial cells in the hypophysectomized female rat." The *rat unit* for their preparation of ICSH averaged 1.2 μg whereas for the reference standard (NIH-LH-S1) it corresponded to 5.0 μg. The contamination of their FSH preparations with ICSH may be inferred from the fact that 40 times the ED67 of FSH were necessary for the exhibition of one ED67 of ICSH. On the other hand, their ICSH preparations exhibited less than 0.02 percent of FSH and less than 0.1 percent of TSH, LTH and ACTH.

In repair experiments after post-hypophysectomy regression, ICSH failed to act upon the testes as a complete gonadotropin unless treatment with FSH was carried out concomitantly. Furthermore, comparable results upon the tubules were obtained by associating FSH with testosterone propionate. The role of ICSH in spermatogenesis would be explained, then, through the release of the male hormone by the Leydig cells. This synergistic effect of FSH plus ICSH or of FSH plus testosterone was still more manifest when GH and LTH were also administered. Addition or supplement of TSH or ACTH did not reinforce that synergism. It appears therefore that, at least for the male hypophysectomized rat, the mixture or complex FSH+ICSH+GH+LTH behaves as the true and complete *gonadotropic hormone.*

Reinitiation and restoration of spermatogenesis with testosterone propionate and other hormones after a long-term post-hypophysectomy regression period in rats were recently dealt with by Boccabella (95). Spermatogenesis was restored qualitatively but not quantitatively by testosterone. The addition of growth hormone and, to a lesser extent, of thyroxine led to a synergistic reinforcement of this inherent action of the male hormone upon the tubules.

Schmidt-Elmendorff, Loraine & Bell (96) detailed the six bioassay methods they have employed to study the activity of eight gonadotropins: two samples distributed by the National Institutes of Health (NIH-FSH-S1 and NIH-LH-S1), one human FSH, three samples of human menopausal gonadotropin (HMG-20A, HMG-IRP and Pergonal) and the international standards PMSG and HCG. The FSH activity of these different gonadotropic preparations was determined by the method previously described by Steelman & Pohley (97) based on the ovarian augmentation weight test in intact immature mice pretreated with large doses of HCG. The ovarian ascorbic acid depletion test in intact immature rats pretreated with PMSG and HCG (98) and the ventral prostatic weight test in hypophysectomized immature male rats (99) were the methods employed for detecting ICSH activity in those preparations. Indices of *total gonadotropic* activity were

inferred from the uterine or ovarian weight of immature mice and from the uterine weight of intact immature rats. In 58 out of 60 assays a four-point design with two equally spaced doses of the standard (S) and the unknown (U) was adopted and in 48 instances the HMG-IRP was the standard used. Indices of precision (λ), relative potencies (R.P.) and fiducial limits of error were given. The human FSH had the highest specific activity of any sample assayed and the NIH-LH-S1 was shown to be a highly specific preparation. The higher discrimination index (Gaddum's D.I.) of 8.03 was found between the human FSH and the HMG-IRP when both preparations were assayed by the ovarian augmentation test (FSH) against the ascorbic acid depletion or prostate weight methods (ICSH).

Taking advantage of the changes caused by urea in some protein molecules, Schmidt-Elmendorff, Loraine & Bell (100) have examined the gonadotropic activity remaining after incubation of gonadotropin preparations with 6 *M* urea at 24° C for 24 hours. This treatment, except in the case of the PMSG, led to a 95 percent reduction of the ICSH activity present prior to incubation with urea.

Butt & associates (101) studied the biological and immunological properties of four fractions obtained by electrophoresis in starch gel of a highly potent preparation of human follicle stimulating hormone. The techniques they employed to detect each one of the gonadotropic activities were the uterine weight of immature mice (an indirect test believed to measure the combined effect of ICSH and FSH), the ovarian weight augmentation test (FSH), and the ovarian ascorbic acid depletion method (ICSH). Two fractions were separated exhibiting mainly either FSH or ICSH activities and a synergistic action was observed particularly when 25 parts of fraction 1, mainly luteinizing, were added to 75 parts of fraction 4, mainly FSH. Also, after injecting NIH-FSH-S1 into immature female mice, Brown & Billewicz (102) concluded that mixtures containing LH and FSH in the ratio 1:4 were the most potent and produced considerably greater uterine responses than the same total amount of either of two standards administered separately.

Cunningham (103) described in detail a new assay method for detecting gonadotropic activity based on induction of ovulation in immature mice. The assay is based on the linear relationship obtained when the probits representing the percentage of ovulation are plotted against the log-dose. By using this procedure, in addition to the uterine weight method for *total gonadotropin*, the ovarian augmentation test for FSH and the ventral prostate weight method for ICSH activity, Butt et al. (104) determined the main type of gonadotropic activity of four fractions obtained by chromatography of crude acetone dried human pituitary extracts in carboxymethyl-cellulose and diethylaminoethyl-cellulose. Two of these fractions exhibited mainly ICSH activity, the other two having mainly an FSH type of action. According to these authors the ovarian augmentation test for FSH and the ventral prostate weight method for ICSH seem to be the best methods now available to differentiate the two types of gonadotropins. In fact, when the two methods

were applied to FSH and ICSH they afforded indices of discrimination far from unity. Concerning possible intrinsic differences among available human gonadotropins, Butt et al. (105) stated that the better preparations obtained from urine had only one tenth of the activity of the best pituitary fractions.

Taking advantage of the known effect of chloretone (1,1,1-trichloro-2-methyl-2-propanol) to elevate the ascorbic acid content of tissues, Ward et al. (106) studied the ovarian ascorbic acid depletion method in two groups of rats: one receiving a routine diet and the other the same diet containing 2 mg/g of chloretone. This treatment raised the ascorbic acid content of the ovaries by about 23 percent but did not affect the sensitivity or precision of the depletion response to ICSH. This ovarian ascorbic acid depletion effect of luteinizing hormone was histochemically studied by Goodstein & Sturgis (107) and its importance emphasized by Foreman (108). According to Sakiz & Guillemin (109) it is a highly convenient method to assess LH activity but extremely dependent upon the strain of rats used. As a regression exists between the weight of the ovaries and the ascorbic acid content of these organs the expression of LH activity in terms of absolute values or percentage of depletion could lead to false quantitative results. Because of this they propose adjustment of both parameters by covariance, thus increasing the reliability of the procedure. Guillemin & Sakiz (110) have also observed that four and a half hours after hypophysectomy the female rats did not respond any more to doses of LH that were active in intact animals. Prolactin was capable of restoring the ovarian responsiveness to LH after hypophysectomy.

Immature mice or rats of both sexes are commonly employed to assay gonadotropic preparations. The question arises whether the use of very young animals would prevent possible interactions between the exogenous hormone and the amount eventually produced by the animal's own pituitary while under treatment. Soper & Ladman (111) have employed groups of immature female rats, either intact or shortly after hypophysectomy, to evaluate their ovarian and uterine responses to PMSG. They have observed that even at the age of 28 to 30 days the female rat hypophysis produced enough gonadotropin to increase the response to a single injection of the hormone administered 24 hours before autopsy. Based on the target organ responses, it was also concluded that more than 10 IU of endogenous gonadotropin appear to circulate in the normal 30 to 33-day-old female rat. According to these findings hypophysectomized immature rats ought to be preferred for the bioassay of these hormones.

A new procedure for the bioassay of gonadotropins was recently introduced by Breneman et al. (112). It is based on the increased uptake of ^{32}P by chicken testes. Both NIH-FSH-S1 and NIH-LH-S1 increased that uptake, LH being at least ten times more effective than FSH. If both gonadotropins were used concomitantly, then an addition rather than a synergistic effect was observed in both the gonadal weight and the ^{32}P uptake indices. Uptake of ^{32}P by the pigeon crop sac glands was also suggested as a bioassay method for prolactin or luteotropin (113).

For the bioassay of prolactin (LTH) another procedure was proposed by Kovačić (114) based on the prolongation of the dioestrus interval of adult intact female mice. A positive response is observed when the treated animals present a dioestrus period of four to seven days instead of the normal two or three days. The percentage of positive responses transformed into probits enables one to calculate the relative potencies of two given samples. Three equally spaced doses respectively of the standard and of the unknown were daily injected subcutaneously for 4 days, using 20 animals for each dose. Although this method lacks specificity its degree of precision was reasonably good ($\lambda = 0.20$–0.25).

Anne Evans (115) described a new assay method for luteotropic activity based on the previously known effect of prolactin in enhancing the β-glucuronidase activity of testicular slices from rats and mice. Using phenolphthalein glucuronidate as substrate she has tested several substances, such as proteins and steroids, observing that only LTH significantly increased the enzyme activity. This assay is very precise ($\lambda = 0.05$–0.19) but its sensitivity is not better than that of the local crop sac method. The prolongation of the dioestrus period in mice and the increase of the β-glucuronidase activity of testicular slices were employed in the bioassay of four different prolactin preparations (116). Satisfactory results were obtained, as checked against the labeled potencies afforded by the pigeon crop sac method.

Absorption, excretion and uses.—Although gonadotropins are effective hormones only after parenteral administration, Rawstron (117) has shown, by an immunological technique, that PMSG may be absorbed through the sublingual mucous membrane of normal individuals. A human pituitary gonadotropic preparation (HPG), exhibiting mainly FSH activity, was injected intramuscularly in five male patients by Apostolakis et al. (118). The total dose of about 1500 U HMG used daily for three days was not enough to elevate the urinary output of 17-KS and 17-hydroxy-corticosteroids; recovery of the hormone in the urine was 10 to 15 percent of the amount administered. Parenterally absorbed gonadotropin, particularly HCG, is transported in the blood bound with the plasma β-globulin fraction (119). Kulagara & Pincus (120) have approached the problem of plasma level and renal excretion of gonadotropins by using a labeled preparation of HCG with fluorescent rhodamine. Single intravenous injection of this compound in rabbits (170 to 530 IU/kg) have shown that after an initial rapid disapperance of most of the hormone from circulation there is a phase of slow disappearance lasting 8 to 25 hours. Half lives of the circulating hormone detected by fluorometry compared reasonably well with those found with the bioassay procedure.

Debele-Maner & associates (121) have observed that normal women receiving ovine NIH-FSH-S1, intramuscularly twice a day for 3 or 4 days, in a total of 6 or 19 mg of the hormone, had an increased urinary estrogen output. Significant antihormone formation was not detected in such a short period of treatment. The activity of a gonadotropic preparation from human

pituitary, containing mainly FSH, was confirmed by Johannison et al. (122) in 5 amenorrheic women. Only one intramuscular injection of their preparation was enough to increase the urinary output of estrogens. Furthermore, if HCG was administered 48 hours later, then an additional increase of estrogens and the appearance of pregnandiol in the urine were observed.

Human chorionic gonadotropin (HCG) may serve to evaluate the functional status of the human testes. If daily injections of 10,000 IU of HCG for a three-day period do not increase the urinary phenolic fraction and 17-KS output, then a primary testicular deficiency may be diagnosed (123). Increased estrone output in men following HCG treatment was also suggested as a method for detecting hypogonadism (124).

Knobil & Josimovich (125) have observed that the rhesus monkey responded to injected gonadotropins from human, ovine or equine sources, although quantitative differences in the response to those heterologous preparations were observed An absolute species-specific antagonism does not seem to occur here as it does in the case of the growth hormone administration.

Synergism.—To the already mentioned examples of synergism between different gonadotropins (93, 124), Browning et al. (126) added their observations on the life of hyperemic corpora lutea in intraocular ovarian transplants in spayed mice. Functional ovarian grafts, appearing as behind a window, exhibited in each cycle a distinct hyperemia of the corpora lutea persisting for one or two days. The duration of this hyperemic phenomenon was not prolonged by FSH and/or ICSH treatments. After prolactin, however, it persisted as long as 8 days and even 17 days if ICSH and prolactin were given concomitantly.

Synergism of the order of 50 times was obtained in hypophysectomized pigeons by Bates et al. (127) when ovine prolactin NIH-P-S3, bovine growth hormone, NIH-GH, L-thyroxine and prednisone were combined and, among other parameters, body weight, food intake, organ weights and intestinal length were estimated. Because of the old controversy about prolactin being a true growth hormone for pigeons it was rather surprising that LTH and GH in combination were less effective than each one separately. However, a synergism between LTH and GH was observed when thyroxine and prednisone were also administered.

Immunological properties.—It is well known that as a consequence of parenteral administration of heterologous protein hormones, including gonadotropins, antihormones may circulate in the treated organism. In fact, Midgley et al. (128) have studied the immunological characterization of HCG through the use of serum antigonadotropin. By sensitizing rabbits with an emulsified commercial preparation of HCG injected subcutaneously 5 times at the dose of 10,000 to 20,000 IU at intervals of 1 to 3 weeks, an antiserum was obtained from the animals 13 days after the last injection. If properly treated to eliminate nonspecific antibodies this antiserum presented properties which might have an application in the diagnosis of human

pregnancy. This possibility was investigated by Keele et al. (129) who made precipitin studies by adding dilutions of the hormone or of the human sera to the rabbit antisera, either in a liquid medium in Preer tubes or in a semi-solid gel agar medium as in the Ouchterlony technique. When applied to human pregnant sera from the 42nd to the 98th day of pregnancy both methods gave positive results. Furthermore, the antiserum was capable of antagonizing the HCG effects upon the prostate of treated immature male rats. Comparable studies were made by Franchimont (130) who used HCG and HPG as antigens.

Another contribution to the problem of antigenicity of gonadotropic preparations was presented by Contopoulos & Hayashida (131). Rabbits were immunized with rat pituitary homogenates by a series of injections extending over a period of 6 to 18 months. Immature hypophysectomized female rats were then treated with castrate rat serum to which was added either the antiserum or the normal serum from rabbits. Whereas the antiserum inhibited the action of the gonadotropin which circulates in castrated rats, the normal rabbit serum did not abolish the ovarian and uterine responses of the infantile treated animals. It was also observed that the antiserum blocked the rats' endogenous gonadotropins. The animals' condition after such a treatment was similar to that following hypophysectomy as far as the gonadotropic function is concerned. This antigonadotropic property of the antiserum could be explained: (*a*) by a blockade of the gonadotropic function of the hypophyseal anterior lobe; (*b*) by an inactivation or neutralization of the circulating hormone; or (*c*) through an unknown interference at the level of the target organs. The second mechanism appears to the authors as the most probable.

Rabbit immunization against a highly purified preparation of sheep ICSH was performed by Moudgal & Li (132) with interesting results. With the Ouchterlony agar diffusion technique a single precipitin band was obtained between the antigen β_s-ICSH and its rabbit antiserum. This antihormone was shown to prevent the ICSH activity of several gonadotropic preparations when concomitantly assayed by the ventral prostate weight method in immature hypophysectomized rats. It also inhibited the physiological action of the endogenous ICSH produced by the anterior lobe of normal rats. The antiserum did not prevent the ICSH activity of HCG and of chicken pituitaries, although it was effective in neutralizing the same activity of crude extracts from whale, pig and rat pituitaries. It was also capable of antagonizing the effect of human ICSH and PMSG.

As usual these results answered some questions by introducing new ones. Thus, further experiments were performed by the same group (133), now with rabbit antisera against ovine prolactin, to find whether the integrity of the lactogenic hormone molecule was essential to its immunological properties. They used, as antigens, highly purified "native" sheep prolactin (LTH) as well as LTH preparations modified by performic acid oxidation or partial hydrolysis with chymotrypsin. Rabbit blood samples collected from the first

bleeding seemed to behave more specifically for the native hormone whereas in subsequent bleedings antibodies against the modified antigens were also encountered. Thus the complete structure of the hormone would not be a prerequisite for specific antigenicity.

Rabbit immunization against ovine and human prolactins was also investigated by Levy & Sampliner (134), who estimated antibody concentrations by hemagglutination and agar diffusion techniques. Here a species-specificity was found and the authors pointed out that for a satisfactory immunoassay of human prolactin a highly purified preparation from human source is required as antigen.

In vitro *production and activity.*—Techniques have been recently described to study the hormonal production by isolated glandular tissue *in vitro* either in conditions of long term culture or in acute experiments. Nicoll & Meites (135) have observed, for instance, that rat anterior lobes in culture dishes produced and released to the synthetic medium, at least for three weeks, conspicuous amounts of prolactin. These results promptly assign, for this experimental condition, the independence of those physiological processes from posthypophyseal and hypothalamic regulation. The *in vitro* production of prolactin was augmented significantly when the donors were previously injected with estradiol benzoate (136). No influence was exerted by the addition to the medium of either oxytocin or pitressin (137). These results are of interest in view of the claims (138, 139) that oxytocin might be the hypothalamic mediator for the secretion of prolactin by the anterior lobe. Nicoll & Meites (140) also studied prolactin secretion *in vitro* and found that insulin was ineffective while sodium-L-thyroxine and L-triiodo-thyronine significantly enhanced prolactin secretion. Functional activity of the anterior lobe after prolonged cultivation and subsequent ocular transplantation in hypophysectomized female rats was investigated by Martinovitch et al. (141).

Mason, Marsh & Savard (142) have demonstrated the influence of gonadotropins on steroidogenesis by surviving slices of bovine corpora lutea. Glandular pieces were distributed in Warburg flasks and incubated for two or three hours at 37°, then the medium was processed in order to isolate progesterone. The synthesis of this hormone was found to be significantly stimulated by NIH-LH-S1 and NIH-FSH-S1 at a concentration of 0.2 μg/ml. Prolactin (NIH-P-S3) and peroxide-inactivated ICSH were ineffective. The rather surprising activity of FSH could be explained on the basis of LH contamination. It was also observed that this progesterone production *in vitro* could also be stimulated by reduced triphosphopyridine nucleotide generated from TPN and glucose-6-phosphate.

Armstrong & Greep (143) have studied the glucose metabolism of isolated ovarian tissue as influenced by the administration of pituitary hormones to the donors. Immature female rats were pretreated with PMSG and HCG and the glucose uptake of slices from their well luteinized ovaries was measured. This uptake was significantly higher when the donors had received an intravenous injection of a purified ICSH preparation. The effec-

tiveness of this gonadotropin to increase the rate of glucose uptake closely paralleled its ability to decrease the ascorbic acid concentration of the luteinized ovarian tissue. Injections of either prolactin or FSH were practically ineffective in this respect. The influence of ICSH on the ability of slices of rabbit testis to incorporate [1−^{14}C] acetate into [^{14}C] testosterone was analysed by Hall & Eik-Nes (144). The incorporation was increased either by ICSH and FSH added to the incubation medium or by ICSH and HCG administered to the donors. The response to ICSH *in vitro* was inhibited by chloramphenicol and puromycin. Obviously much is expected from this type of experiments as far as the elucidation of the mechanism of action of gonadotropins is concerned.

Steroid and gonadotropin secretion.—Parabiosis is a well known technique in classic experimental endocrinology. It has been employed particularly on problems of pituitary-gonad relationship (145). When a castrated rat is in parabiosis with an intact female, the latter receives from its castrated partner an increased amount of pituitary gonadotropin which stimulates the ovaries and leads to an excessive enlargement of the uterus. Testis implant or testosterone treatment of the castrated partner prevent the pituitary overproduction and release of gonadotropins. An experimental condition is thus available to test the ability of drugs, mainly of steroids, to check pituitary hyperactivity. By using such a method Kincl et al. (146) have studied a series of steroid compounds as inhibitors of the pituitary gonadotropic function. A relationship was first demonstrated between graded doses of testosterone propionate subcutaneously injected into the castrate and the decrease of the ovarian weight of the intact partner. Seminal vesicle, prostate and *levator ani* weights of the injected castrated parabiont were the parameters for estimation of the androgenic and myotropic actions of the compound used. Apparent dissociation between antigonadotropic and androgenic or anabolic activities was observed for a number of compounds, particularly 4-chloro-17α-methyl-19-nortestosterone and 2α-methyl-17β-hydroxy-19-norandrostan-3-one.

Two valuable papers by Miyake (147, 148) are careful contributions to this subject. Employing over 500 pairs of parabiotic rats and 1000 pairs of parabiotic mice he has studied the per cent ovarian growth as an index of antigonadotropic potency of a series of sexagens. Estrone administered orally or subcutaneously exhibited 100 or 1000 times respectively the antigonadotropic potency of testosterone. Known antiovulatory agents like norethynodrel and norethisterone exhibited roughly the same antigonadotropic potency as that of estrone in oral tests. To reinforce his opinion that estrogenicity is most likely responsible for antigonadotropic property, Miyake suggests that the antigonadotropic activity revealed by nonestrogenic steroids would actually depend on circulating estrogenic metabolites occurring after administration of androgenic, anabolic or gestagenic steroids.

Byler & Potts (149) have investigated, through another experimental approach, the influence of gonadal and adrenal cortical hormones on estrogen-

induced depletion of pituitary gonadotropin. Young mature male rats were subcutaneously injected for two weeks with the selected hormones and killed on the 14th day of experiment. The pituitaries were excised, weighed and homogenized with saline and the ventral prostate weight was taken as an index of androgenic activity of the compound administered. Immature hypophysectomized female rats were employed for assaying the gonadotropic content of the pituitary homogenates. Estradiol alone (0.4 mg/kg/day) depleted the anterior lobe of about 95 percent of its FSH content. This depletion was prevented by testosterone propionate or by progesterone but not by desoxycorticosterone acetate or cortisone acetate. Hormonal antagonism occurs therefore between estrogens and androgens in their action upon the pituitary gonadotropin content. Estrogens decrease and androgens increase the hypophysial stores of FSH. According to the same authors secretion of FSH would be regulated directly by circulating gonadal steroids whereas its release would depend on hypothalamic control.

Gans & Van Rees (150) have found that prolonged treatment of male and female castrated rats with estradiol benzoate exerted, according to the hormone dosage, different actions upon the pituitary production and release of ICSH. Small doses (0.1–0.2 μg) inhibited the release and high doses 0.5–2.0 μg) blocked the secretion of ICSH by the anterior lobe of the hypophysis.

Plants and Endocrines

The presence in plants of constituents possessing hormonal type of activity that interfers with endocrine functions in animals is obviously of high biological interest (151). Miroestrol, the estrogenic substance isolated from the tuberous roots of *Pueraria mirifica*, a leguminous plant growing in Thailand, was assayed by the immature mouse uterine weight and rat vaginal cornification tests. When administered subcutaneously it had about one third of the potency of stilbestrol (152, 153). It was reported as presenting a heterocyclic ring structure unrelated to steroids with the actual distance between the 3-OH and 18β-OH oxygen atoms nearly the same as that between the oxygen atoms of the 3-OH and 17β-OH in estradiol (154).

Wong & Flux (155) have investigated the estrogenic activity of the red clover *Trifolium pratense* isoflavones and some of their degradation products. Genistein (4′, 5, 7-trihydroxyisoflavone) would be transformed in the organism into a stilbene-like active compound less potent than stilbestrol itself. Demethylation would be necessary for the estrogenic activity of biochanin A (156). Formononetin and daidzein, a closely related isoflavone which appears as a glucoside in soybeans, *Glycina hispida*, are devoid of estrogenic activity. Soy flour diet, however, has been reported to be goitrogenic in rats (157). The antithyroid potency of barbarin, a phenylthiooxazolidone isolated from various species of *Barbarea*, was demonstrated by Greer & Whallon (158) through its inhibition of radioiodine uptake in rat and man.

Brahmachari & Augusti (159) have assayed the hypoglycemic action of

orally administered galenical preparations from *Allium cepa*, *Ficus bengalensis* and *F. religiosa* in rabbits with alloxan diabetes. The results indicate that the extracts of these plants may be useful substitutes for tolbutamide. The mechanism of action of these drugs appears to be the augmented output of endogenous insulin. The preliminary note by Rubenstein and associates (160) deals with the eventual importance of the manganese content of plants to explain their antidiabetic property, particularly in the case of alfalfa or lucerne, *Medicago sativa*.

Hypoglycin A, a nonapeptide isolated from the Jamaica fruits of *Blighia sapida*, when intravenously administered to dogs, cats, guinea pigs and hamsters, induces a prolonged and delayed hypoglycemia with depletion of liver glycogen in the last two species. It does not produce, like insulin, a change in the metabolism of epididymal fat pads of rats (161).

Keller & Romani (162) have reported the galactagogue property of an extract from *Bromosimum alicastrum*, which they believe to be mediated by an increased prolactin secretion by the adenohypophysis.

An enzyme, phyto-oxytocinase, which is remarkably stable and widely distributed among plants and which inactivates oxytocin, was described by Stemm (163).

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