PHARMACOLOGICAL CONTROL OF ADRENOCORTICAL AND GONADAL SECRETIONS[1,2]

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In his excellent review, Endocrine Pharmacology, last year, Munson (230) selected certain topics of special interest to him, and the present authors have followed this pattern. Inasmuch as the adrenal cortex secretes many hormones and the gonads produce sperm and ova in addition to their endocrine secretions, the field surveyed is not a narrow one, and complete coverage would be impossible.

Several reviews and monographs have appeared to which the reader's attention should be called. Gaunt *et al.* (108) have ably reviewed the field of endocrine pharmacology. Several aspects of adrenocortical function have been dealt with in detail in a Symposium of the English Biochemical Society (51) and in a monograph *The Adrenal Cortex*, edited by the International Academy of Pathology (228). A valuable compendium, *Hormones in Human Plasma*, has been contributed by 33 authors (5). A symposium entitled *Control of Ovulation* (312) covers both basic and clinical aspects of the subject, and a series of reviews on human ovulation are appearing in *Fertility and Sterility* (185). Neuroendocrine relationships in general have been reviewed by Bajusz (13).

PHARMACOLOGICAL AGENTS EXERTING THEIR INFLUENCE ENTIRELY VIA THE CENTRAL NERVOUS SYSTEM

Adrenal Secretion

A few additions have been made to the list of sedatives, tranquilizers, and anesthetics which have an effect on the release of ACTH. Kivalo & Rinne (179) found that perphenazine (Trilafon), injected 30 min previous to examination inhibited the adrenal ascorbic acid depletion caused by neurogenic

[1] The survey of the literature pertaining to this review was concluded in June, 1961.

[2] The following abbreviations will be used: CRF (corticotropin-releasing factor); FSH (follicle-stimulating hormone); ICSH (interstitial-cell-stimulating hormone); LH (luteinizing hormone); LTH (luteotropic hormone); MSH (melanophore-stimulating hormone); STH (somatotropic hormone); TSH (thyrotropic hormone); $NADPH_2$ [nicotinamide-adenine dinucliolide phosphate, reduced from (TPNH)]; NAD (nicotinamide-adenine dinucliotide).

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stress. Neither adrenal nor hypophyseal responsiveness was impaired. Daily administration of perphenazine could block the stress response for only five days. Kothari *et al.* (184) reported on the effect of two phenothiazines and two hydrazines in schizophrenic patients. The treatment prevented the usual drop in eosinophil count after heat stress (hot shower), but not the effect of ACTH. Ganong & Betz (103) found no inhibitive effect, on the elevation of 17-hydroxycorticoid levels in dogs following surgical trauma or immobilization, by acute or chronic treatment with chlorpromazine.

Slusher & Browning (292) employed an elegant method for taking small blood samples in rats without stressing the animals materially. They collected blood samples several times a day from an indwelling catheter (jugular vein) and, via the same route, replaced the blood with blood from hypophysectomized donor rats. They found the resting levels of free corticosterone in plasma to be elevated during the afternoon. Injection of morphine (1 mg/100 g body weight) via the catheter at nine a.m. resulted in a significant increase in corticosterone levels; however, the daily peak at five p.m. appeared to be depressed. At this time, an emotional stress could induce a second rise in corticoid titer. Apparently at this low dosage, the drug depressed the diurnal rise in the resting levels but not the stress response.

Intravenous injection of lysergic acid diethylamide [LSD] did not significantly change the adrenal vein output of 17-hydroxycorticoid or catecholamines when administered to anesthetized dogs 6 hr after adrenal vein cannulation, nor does the drug affect the sensitivity of the pituitary-adrenal axis to stress (105). It is, however, possible that pentobarbital anesthesia interferes with the central action of lysergic acid diethylamide [cf. (291)]

In an attempt to analyze the question of whether the inhibitory action of morphine on ACTH secretion is exerted at a central neural or at the pituitary level, Burdette *et al.* (42) injected nalorphine 10 seconds after morphine into rats under pentobarbital anesthesia; they then assessed the adrenal ascorbic acid response to histamine injected 10 min later. The antagonistic effect of nalorphine on the morphine-histamine interaction was shown to appear at the same dose level as that at which nalorphine antagonized the psychic effect of morphine. The results strongly suggest that morphine interferes with central nervous mechanisms concerned with the control of ACTH release. On the other hand, prolonged treatment with morphine may decrease the adrenal responsiveness to ACTH. Paroli & Malchiori (245) showed that the adrenal glands from morphine-treated rats (2 mg/100 g body weight for 5 to 30 days) produce less corticosteroids *in vitro* during both preincubation and incubation with ACTH as the duration of morphine administration increased. Thirty days of morphine treatment caused a 70 per cent decrease of the *in vitro* corticosteroid production. Moreover, the addition of morphine to the medium inhibited 3-β-ol dehydrogenase activity of adrenal homogenate *in vitro*.

The effect of ether and pentobarbital on pituitary ACTH content 10 min after injection was studied by Hedner & Rerup (129) in three species. Pento-

barbital increased the ACTH content, whereas ether caused its rapid depletion.

Reserpine appears to induce adrenal hypertrophy and other signs of hyperfunction in several species including man, but these changes disappear with chronic treatment (171). In the monkey, Rosenthal & Mason (264) observed a higher urinary excretion of 17-hydroxycorticoids during chronic administration of reserpine. During this period, the percentage of unconjugated 17-hydroxycorticoids in plasma is much higher than normal. Since this is never encountered after administration of ACTH, it is unlikely that elevated pituitary activity would account for this increase, so perhaps metabolic side effects of reserpine are involved.

The current concept of the mechanism of action of tranquilizers on the pituitary is that they directly or indirectly depress hypothalamic function, and that they interfere in this way with pituitary function. The group led by Brodie, however, is inclined to believe that a quite different explanation is possible. They found that reserpine as well as cold exposure deplete the hypophysis of ACTH to considerable extent (38). Both treatments prevent the adrenal response to stress when more than 60 per cent of the pituitary ACTH has disappeared. They conclude that this depletion may account for the failure of stressful stimuli to provoke adrenal activation. Another interesting fact is that the depletion of ACTH seems to bear an obligatory relationship to the degree of sedation exerted (182, 203).

These findings corroborate those of Kitay *et al.* (177), who previously showed that either reserpine or epinephrine treatment induces sustained hypersecretion combined with a significant depletion of ACTH. Whether the "inhibitory" effect of sedative drugs is actually caused by mere depletion and exhaustion of the hypophysis has not yet been decided. The gland should still contain enough ACTH to provoke a maximal adrenal response; hence, blockade at the central nervous level is not excluded unless it can be shown that direct stimulation of the pituitary under these circumstances is without effect. At any rate, these studies call for further examination of the inhibitory mechanism of these drugs.

Gonadal Secretion

Earlier studies on the effects of such nerve-blocking agents as atropine, morphine and barbiturates on the release of ovulating hormone (chiefly LH) have been reviewed by Everett (90) and Sawyer (274). Recent investigations reveal that morphine also blocks the release of gonadotropin in the male rat [Hohlweg, Knappe & Dorner (149)] and prevents the appearance of castration changes in either male or female rat pituitaries (75). Morphine differentially elevates the FSH content of the rat pituitary and increases the number of "purple gonadotropic cells" [Rennels (260)]. It also blocks the release of TSH (270) and prevents the development of "thyroidectomy cells" in the hypophysis (149). Barbiturates also block castration-changes in the rat pituitary (180), and the estrogen-induced release of pituitary LH in the

pregnant rat (255). Presl's interpretation of a prolonged pituitary-stimulation process (210) appears to have ignored the evidence for a diurnal rhythm in the LH release mechanism revealed in the earlier barbiturate experiments of Everett & Sawyer (89).

Chlorpromazine and reserpine block the release of pituitary gonadotropin, suppressing ovulation or estrogen production, or both, in rats (19, 170, 212), monkeys (88) and humans (213, 302, 315). The lactating or reserpine-treated female rat appears to convert androgen to estrogen, perhaps as a result of the influence of lactogenic hormone [Alloiteau (3)]. Reported failure of chlorpromazine to affect the pituitary-gonad axis in rats (33) is probably attributable to inadequate dosage. Treatment with 5-hydroxytryptamine (serotonin) and monoamine oxidase inhibitors such as iproniazid retards sexual development in female mice [Robson & Botros (261)] and rats (286), and interrupts pregnancy in mice and rabbits (254). Little effect was noted in male mice and rats (261, 186), but temporary testicular atrophy from serotonin treatment, followed by hypertrophy at the end of treatment in adult rat, has recently been reported (30). Meprobamate (Miltown) does not by itself block ovulation in the rat, but it synergizes with the anticholinergic drug, tridihexethyl chloride (Pathilon) to exert this effect (110). Many of these agents induce pseudopregnancy in the rat, an indication that luteotropic hormone has been released (110, 20, 299). Chlordiazepoxide (Librium) has been reported not to affect pituitary gonad function in the rat (32). In addition to these endocrine influences, tranquilizers effect the release of two other hormones which appear to be chronically held in check by the central nervous system: lactogenic hormone (220, 273, 304, 307), and melanophore-dispersing hormone (172, 282). Confirmation of the suggestion that the drugs exert these effects by inhibiting a chronic inhibitory condition is supplied by evidence that hypothalamic lesions activate the release of lactogen in the rabbit (128, 161), and luteotropin in the rat (213).

HUMORAL CONTROL OF PITUITARY SECRETION

Corticotropin-Releasing Factor

The methods for assay of substances with CRF-activity have been extensively reviewed by Munson (230). Recently, De Wied (62) published an improved assay method which should meet all the requirements for a CRF assay. On the day following the placement of an extensive lesion in the tuberal part of the hypothalamus of rats, the left adrenal gland is removed under ether anesthesia and the assay material is injected intravenously. The right adrenal is removed after decapitation 15 min later. The corticoid production *in vitro* of the left adrenal serves as an index for the "effectiveness" of the lesion; that of the right adrenal reflects the CRF activity of the injected material. With this technique, Pitressin, purified lysine vasopressin, and a hypothalamic extract appeared to be active, whereas no noticeable effect was apparent from saline, histamine, nicotine, synthetic oxytocin, or a frontal lobe extract. None of these compounds in the dosages used was effective in hypophysectomized rats. The method has the advantage over

pharmacological blockade in that pituitary sensitivity is not decreased and that the blockade cannot be overcome by increasing doses of powerful stressor agents [cf. (277)].

A report in abstract form by Martini and associates (207) indicates that dexamethasone can be used as a tool for assay of CRF. They inject 25 μg of dexamethasone 4 hr prior to the intravenous injection of the assay material under ether anesthesia, and collect blood 30 min later for the determination of the plasma level of free corticosterone. The assay is claimed to be highly specific and very sensitive.

The isolation of principles possessing CRF activity is still under way in several laboratories. Guillemin's group reported that two factors have been isolated, one related to vasopressin and called β-CRF, and another one close to α-MSH, called α-CRF. The β-CRF is the more potent substance and is considered to be the true corticotropin-releasing factor; α-CRF has some inherent ACTH activity and might be a precursor of ACTH. This substance may be identical with Privat de Garilhe's factor (122, 276). More recently, Schally *et al.* (278) reported that a new factor possessing CRF activity, called α_2-CRF, could be isolated, which is also closely related to α-MSH. The CRF activity, if assayed in the pentobarbital-morphine blocked rat, is slight, but about 2.5 times higher than that of α-MSH itself.

Privat de Garilhe *et al.* (256) reported on further studies on the extraction of hog posterior pituitary powder; they added zone electrophoresis according to Porath, and obtained a factor closely related to α-MSH. Some synthetic polypeptides, notably a hepta- and a decapeptide (162), showed pronounced CRF activity when assayed *in vitro* according to the method devised by Saffra.

The effects of extracts from several parts of the dog hypothalamus on the 17-hydroxycorticoid level in the plasma of intact trained dogs were studied by Brizzee & Eik-Nes (36); only extracts from the supra-optic and anterior median eminence region caused a significant elevation. It would be interesting to know if these effects can be ascribed to CRF activity; the possibility that brain stem substances like histamine and epinephrine are involved, has nor yet been excluded.

Recently, Linquette *et al.* (196) described the effect of a substance with remarkable ACTH-releasing activity in humans. Following the method of Privat de Garilhe for purification by dialysis and column chromatography, they isolated a polypeptide fraction from acetone-dried posterior lobe powder which contains 15 amino acids, does not cause a rise in blood pressure when injected into the dog at a dose level of 0.6 mg/kg, and does not show antidiuretic effects at dosage levels that induce adrenal activation. The fraction causes a rise in urinary 17-hydroxycorticoids in normal men and in patients with moderate adrenal insufficiency, but no rise in Addisonian and patients with pituitary insufficiency. When given as an intravenous infusion, the lowest effective dose level is 10 μg.

It is to be expected that several naturally occurring polypeptides showing CRF activity will be isolated and identified, and that synthetic polypeptides

with even stronger activities will be made. However, still to be achieved is the task of demonstrating that any of these principles plays the physiological role of neurohumor. Of the storage, production, and release of these substances in the intact animal we still know virtually nothing, except for vasopressin.

Vasopressin.—There is still much controversial opinion expressed on the role of vasopressin in the control of ACTH secretion, although current consensus seems to be that this hormone is not CRF, but shares CRF activity [cf. (104, 183)]. A thorough and valuable review of the role of vasopressin in ACTH-release was given recently by Nichols (235). In rats blocked with Nembutal and morphine, synthetic lysine vasopressin has been reported to elevate the plasma corticosterone levels in doses ranging from 17 mU of pressor activity upward (314). Martini *et al.* (207) determined plasma corticosterone levels in rats blocked with dexamethasone after injection of several posterior lobe principles. Pitressin, lysine-vasopressin, and phenylalanyl-lysine vasopressin were active at dose levels ranging from 20 to 80 mU of pressor activity, whereas 320 to 1280 mU were needed to cause adrenal activation if Pitocin, synthetic oxytocin, or valine-oxytocin were given.

There are several indications that removal of the posterior lobe abolishes the adrenal response only to certain types of stress. Nowell (239) found that the adrenal ascorbic acid response to hypertonic saline and cold was inhibited by posterior lobectomy in male rats, while bell ringing induced a normal depletion. On the other hand, Smelik (294) reported that posterior lobectomy did not interfere with traumatic stress, but completely abolished the ascorbic acid depletion seen in intact female rats after the application of an emotional stimulus. These results supported the idea that posterior lobe ACTH might be involved in emotional stress (224, 262). However, another interpretation was offered by similar experiments, subsequently performed in the same laboratory by De Wied (68), using plasma corticosterone levels as an index. He found that in posterior lobectomized male rats, systemic stimuli induced normal adrenal activation, whereas the response to neurogenic stress was greatly reduced. Moreover, treatment with Pitressin tannate in such a dose that the water intake was normalized, removed this inhibition. Depletion of the posterior lobe antidiuretic hormone content by dehydration had effects similar to posterior lobectomy. De Wied's conclusion is that vasopressin may be involved in the adrenal response to emotional stress, but not to systemic stress.

These findings may account for some of the objections raised against the identification of vasopressin and CRF (see 104, 235). The main argument has been that under several circumstances there is a dissociation between the release of vasopressin and ACTH: preventing release of the one does not necessarily interfere with the release of the other. It is conceivable that inhibition of vasopressin release does not exclude the release of another CRF, depending on the type of stress applied. On the other hand, the fact that antidiuresis is not always accompanied by ACTH release may be explained by assuming that the amount of vasopressin necessary to evoke ACTH secretion is much greater than that required to induce antidiuresis. This

amount rather seems to be of the magnitude provoking a rise in blood pressure (69).

Hind brain factor.—The work done by Egdahl on the effect of brain removal on adrenal response to stress has now been published in detail (78, 79, 80). Partial constriction of the thoracic vena cava in both intact dogs anesthetized with pentobarbital, and dogs with brain removed down to the pons, produced a release of ACTH. Removal of the cerebral cortex down to the hippocampus resulted in high resting levels of 17-hydroxycorticoids which were suppressed by pentobarbital and elevated by sciatic nerve stimulation and ACTH. Since barbiturates are known to depress the reticular formation, stimulation of which may activate the hypothalamic ACTH-controlling center, Egdahl's speculation is that removal of the cortex results in a potentiation of the tonic influence exerted by the reticular formation on the hypothalamus. In agreement with this hypothesis is his recent abstract indicating that pentobarbital does not suppress the corticoid level in dogs with complete brain removal (81). These experiments do not offer further evidence for the existence of a hind brain factor, neither do they give another explanation for his original findings. The possibility that ACTH had been released by epinephrine liberated as a consequence of a widespread sympathetic nervous excitation, has not been excluded in his original experiments [(78), cf. (37)]. They certainly demonstrate that attention should be paid to the probability that the organism may have an astonishing capacity to reach the same goal by many different means. Whereas this can often be shown under abnormal circumstances, it will be extremely difficult to determine which mechanisms actually play a role in the intact animal.

Gonadotropin-Releasing Factor

The existence of (an) hypothalamic-hypophyseal gonadotropin-releasing factor(s) has been assumed for many years. Notable recent evidence for humoral control via the pituitary portal system has been the finding by Winer & Everett (90) in female rats, that retransplanting the almost non-functional hypophysis from the kidney capsule back into the sella turcica restores gonadal function when the portal vessels regenerate. More recently, Smith (297) has observed the return of normal sex function in hypophysectomized female (295) and male (296) rats after the "take" of a delayed pituitary homotransplant into the region of the median eminence.

Two laboratories have been actively testing hypothalamic extracts for gonadotropin-releasing factor activity. Harris *et al.* (43, 127) have made microinjections of extracts into the rabbit pituitary and have successfully induced ovulation. Winer has had similar success injecting extracts into the hypophysis of the proestrous pentobarbital-blocked rat (127). The nature of the active principle is unknown but it appears not be be epinephrine, histamine, serotonin, substance P, or neurohypophyseal oxytocin or vasopressin. McCann *et al.* (217) have made hypothalamic extracts which induced LH release as evidenced by the depletion of ovarian ascorbic acid. Vasopressin is effective but its action is exerted directly on the ovary (216).

Pituitary stalk-median eminence extracts elevate the plasma LH content of the estrogenized ovariectomized rat (215). The hypophysectomized, pituitary-transplanted rat has been proposed as an assay animal for gonadotropin-releasing factor (12).

Inasmuch as oxytocin was ineffective in causing release of gonadrotropin in either of these systems it seems likely that the polypeptide was exerting some other effect in the experiments which suggested that it might be the gonadotropin-releasing factor (6, 126, 181, 208, 287). An action on the brain which registers as changes in the electroencephalogram (EEG) (93, 165) or changes in an EEG threshold (166) may explain its indirect influence on adenohypophyseal function.

Recent reports (8, 137) confirm earlier observations that the male pituitary, separated from its median eminence source of portal blood, may receive enough GRF via the systemic circulation to maintain spermatogenesis.

EFFECTS OF HORMONES

Pituitary-Adrenal Axis

Glucocorticoids.—An increase in the blood level of adrenocortical hormones, or the injection of cortical principles, inhibits the release and probably the production of ACTH. The exact mechanism of this action is still unknown. Most studies favor the concept that the depressant effect is on the central nervous system, presumably the hypothalamus, but there are several indications that the site of action is the pituitary gland itself. Martini *et al.* (209) reviewed the effects of corticoid administration; they reported that adrenal sensitivity to ACTH and the hypophyseal response to Pitressin are diminished in rats pretreated with high doses of hydrocortisone. Hodges & Vernikos (144) studied the effect of hydrocortisone on the ACTH levels in blood and pituitary of adrenalectomized

venously 2.5 min after ether anesthesia caused a rapid lowering of the elevated ACTH blood levels. Small amounts of hydrocortisone (0.1 mg) prevented the usual persisting rise in ACTH blood level that follows adrenalectomy, but they did not abolish the burst of ACTH secretion attributable to stress. They conclude that the synthesis rather than the release of ACTH may be depressed by corticoids. In accordance with this view is the preliminary report that in adrenalectomized animals, stress induces a sharp rise in pituitary ACTH content within a few minutes, followed by a depletion, whereas there is no change in ACTH content under stressful conditions in intact rats (205). Apparently the presence of corticosteroids stabilizes the ACTH production.

A depressed pituitary level of ACTH (and also of STH, LTH, and MSH) was noted after chronic treatment with corticoids by Sulman & Steiner (303). Hess *et al.* (135) found pituitary RNA levels increased after adrenalectomy and decreased RNA after administration of corticoids. The RNA presumably is correlated with ACTH synthesis (134, 136).

Barrett (22) compared the inhibitory potency of cortisone and hydrocortisone in acutely stressed rats on the elevation of blood ACTH levels.

Hydrocortisone appeared to be more potent; the peak in blocking action of both hormones was reached 16 hr after subcutaneous injection.

The role of the feedback mechanism has been reviewed recently by Ganong & Forsham (104). They concluded that, whereas there is good evidence to indicate that an increase in corticoid levels inhibits ACTH secretion, a decline in circulating corticoids has not been found to initiate hypersecretion of ACTH. The available evidence is indeed against this proposition. It is however, possible that the central mechanism for ACTH release is triggered, not by an actual, but by a virtual drop in corticoid levels. Yates *et al.* (324, 325) designed experiments to support the hypothesis that in case of stress this virtual drop in corticosteroid titers is effected by a reset of a central feedback controller on a higher setpoint, thus inducing release of ACTH until the desired new level of corticoids has been reached. Although it is difficult to see how this mechanism would operate in adrenalectomized animals, the idea of a controller with a variable setpoint is certainly consistent with the mechanism of a true homeostatic (cybernetic) system.

Two recent reports in summarized form deal with the site of action of corticoids. Yoshida & Sayers (326) observed that daily injection of corticosterone reduces the ACTH content of pituitary grafts in rats, which suggests a direct action on the pituitary. On the other hand, injection of dexamethasone directly into the pituitary *in situ* did not exert a more pronounced blockade than did systemic injection (169). There is a good possibility that physiologically elevated levels of corticoids raise the threshold of brain stem structures concerned with the control of ACTH secretion, whereas an overdose of cortical hormones will extend the inhibiting action to the pituitary itself (or at least the hypothalamo-hypophyseal system), and even to the adrenal gland.

Several reports indicate that corticoids may inhibit the production of corticosteroids directly. Péron *et al.* (249) found that the elevation of adrenal corticoid content by ACTH was less pronounced if 5 mg of corticosterone was injected simultaneously. Similar experiments with adrenal glands *in vitro* were inconclusive (but see below). Hilton and associates studied the effect of exogenous hydrocortisone in *in vivo*-isolated adrenals of hypophysectomized dogs. They infused arterial blood from hypophysectomized donor dogs, to which ACTH, hydrocortisone, or corticosterone was added. The hydrocortisone output of the perfused adrenal was decreased after the infusion of 200 mg per cent hydrocortisone, but was unaltered by an equal level of corticosterone (29). Hydrocortisone was also reported by Saffran & Vogt (269) to reduce the adrenal secretory capacity.

A Japanese group studied the influence of steroids on the corticosterone production of adrenals *in vitro*. Hydrocortisone and prednisolone in small quantities (10 μg) added to the medium prevented the corticosterone production induced by 1 mU of ACTH. Tetrahydrocortisol, methyltestosterone, and estradiol-17β had no effect. No inhibition took place if the glucocorticoids were given 30 min after the addition of ACTH, or if glucose-6-phosphate and TPN were added. The inference was that presumably glucorcorti-

coids block the phosphorus metabolism by inhibition of phosphorylase activity [Fukui *et al.* (102)]. These interesting results suggest that moderate amounts of corticoids may indeed have some direct effect on the adrenal gland; confirmatory work both *in vitro* and *in vivo* should be done before this evidence is definitely accepted.

Epinephrine.—Little interest has been paid lately to the action of adrenal medullary hormones on ACTH secretion, although epinephrine is one of the most potent endogenous stimulators of ACTH release. Norepinephrine is much less active in this respect, and has been reported to cause adrenal ascorbic acid depletion without concomitant cholesterol depletion. Epinephrine and isopropyl-epinephrine deplete both compounds. Levorotatory isomers of catecholamines are more active than are dextrorotatory isomers (117).

Intramuscular infusion extending over 1 hr, of as little as 0.6 μg of epinephrine produces a pronounced ascorbic acid depletion, which is completely prevented by previous removal of the posterior lobe of the hypophysis (173). However, the rise in plasma corticosterone levels after injection of epinephrine seems not be be inhibited by posterior lobectomy (94).

Thyroid hormones.—Thyroid hormones are known to increase the adrenal weight, whereas hypothyroidism has the opposite effect [cf. Saffran & Saffran (268)]. Melby *et al.* (222) analyzed the effect of several thyroxine analogues on the production and catabolism of hydrocortisone in dogs subjected to adrenal vein cannulation. They observed an increase in hydrocortisone output after an intravenous infusion of triiodothyroacetic acid and triiodothyronine, but not after thyroxine. Hypophysectomy prevented this effect. Triiodothyronine treatment increased significantly the disappearance rate of injected hydrocortisone. They assumed that the accelerated catabolism of corticoids is caused by an increase in $NADPH_2$ in the liver, and may act as a stimulus on the pituitary to release ACTH. This would be in accordance with earlier observations (39). The pituitary ACTH level, after treatment with thiouracil for a three-week period, was assessed by Lazo-Wasem (192); in spite of pituitary enlargement, the content appeared to be very low.

It seems unlikely that the effects of goitrogens on the pituitary-adrenal axis can be sufficiently explained by their action on the thyroid gland alone [cf. (218, 219)].

Gonadal hormones.—A rapidly growing literature is concerned with the influence of sex, pregnancy, and treatment with gonadal hormones, on the adrenocortical secretions. A sex difference in adrenal weight and reaction patterns has been observed for a long time. Barrett (21) reported that ACTH levels in female rates are higher than those in males, because of cyclical variations. In females, poststress ACTH levels are much higher during proestrus and oestrus than during metoestrus and dioestrus. Intact and sham-castrate female rats show a maximal plasma corticosterone level which is three times higher than that in males; castration brings this level down to the values observed in males (57). Kitay (176) reported that ether and ACTH induce a more elevated and persistent rise in plasma corticosterone in female

rats; the corticosterone concentration in adrenal vein blood is 2.5 times that in males. The catabolism of corticosterone in liver slices from female rats is much more rapid than that in male liver. Pituitary ACTH content is also much higher in females (57). Implantation of 5 mg. of stilbestrol increases pituitary ACTH levels only in female rats and also elevates resting levels of plasma corticosterone. On the other hand, stilbestrol inhibits the *in vitro* production of corticosterone in adrenals from female rats, but enhances it in adrenals from males (175).

Estrogens are known to increase the corticoid-binding capacity of plasma. The binding protein has been called transcortin (271, 272, 290). Peterson *et al.* (250) found very high plasma levels of hydrocortisone in humans after treatment with synthetic ethinyl estradiol. However, this was not associated with a more intense feedback action or with an eosinopenia. Apparently, the corticoid is not present in active form, but is largely protein-bound. Similar conclusions were reached by Herrmann *et al.* (133) in a study involving estrogen treatment in patients. It is known that the plasma-binding capacity is very limited for, whereas under resting conditions almost all the corticoids are bound, any elevation in corticoid levels results in the appearance of free compounds. Mills *et al.* (226) analyzed the free: bound ratio of hydrocortisone in patients after treatment with estrogens, and found that the binding capacity had increased about three times. The hydrocortisone-binding protein appeared to be electrophoretically in the same position as the thyroxine-binding protein. Preliminary reports on the isolation and chemical properties of transcortin have been presented (87, 283).

The work in this field may offer an explanation for some of the observed effects of female sex hormones on the adrenal function. A higher binding capacity in females might cause a lower level of free corticoids, and subsequently less inhibition of the ACTH production. The striking hypertrophy of adrenal glands transplanted into the hepatic portal circulation of female rats or rats treated with estrogens, observed by Bernstein (24), could perhaps be explained in this way.

Another effect of estrogens has been found on corticoid catabolism. The disappearance rate of hydrocortisone (246) and corticosterone (251) is much slower after estrogen treatment.

During pregnancy, the blood levels of corticosteroids are elevated, presumably by alterations in their catabolism. Frantz *et al.* (101) reported an increase in the urinary level of 6-β-hydroxycortisol during pregnancy, which suggests an altered cortisol metabolism. The same compound was found in higher quantities in the urine of men treated with diethylstilbestrol (197).

Several progestational compounds have been shown to exert an inhibiting effect on the adrenal cortex. Holub *et al.* (151), confirming Edgren *et al.* (77), found that 6-methyl-17-acetoxy-progesterone causes adrenal atrophy in the rat, and diminishes the ACTH content of the pituitary gland. Since the effect on adrenal weight was not abolished by hypophysectomy, the mechanism cannot be explained completely by an action at the pituitary level only. Another compound, 6α-methyl-17α-hydroxyprogesterone causes pituitary,

ovarian, and adrenal atrophy, presumably by acting at the hypothalamic level (199).

Chorionic gonadotropin does not stimulate the adrenal cortex (and particularly the adrenal androgen production) directly. No effect could be observed in castrate and hypophysectomized-castrate male rats, indicating that the effect is via the testes [(70), cf. (189)].

Vasopressin.—Yoshida & Sayers (326) found that subcutaneous injection of Pitressin tannate enhances the ACTH content of pituitary grafts in rats. Hilton *et al.* (142.143) demonstrated a direct stimulating effect of vasopressin on steroid production by the adrenal cortex. Direct arterial perfusion of the adrenal gland of hypophysectomized dogs with synthetic lysine-, arginine- and acetylarginine-vasopressin resulted in increased hydrocortisone secretion. The effective dose range for arginine-vasopressin was 10 to 400 mU/min. The effect is transient, and could not be maintained by prolonged perfusion, in contrast to the sustained rise after ACTH. It is difficult to estimate whether the minimal dose levels are within the physiological range; systemic injections of vasopressin preparations in moderate doses fail to show any direct effect on the adrenal gland (67).

Pituitary-Gonad Axis

Pituitary gonadotropins.—Several studies have been made of the pituitary content of gonadotropins under experimental conditions, mostly related to the reprodutive cycle in the female. New techniques of assaying interstitial cell-stimulating hormone, employing radioisotopes, were reported (85, 124). In the sheep pituitary, the FSH level was found to be highest during anestrus, and LH reached a peak ten days after the onset of estrus (156). Clegg & Ganong (52) reported that ventral hypothalamic lesions involving the pituitary stalk depressed the content of both FSH and LH in the ewe pituitary. Sheep pituitary ICSH has several protein components (159). The proestrous rat has been found to lose pituitary LH between ten a.m. and five p.m., a change which does not occur on other days of the cycle (281). On a protein-deficient diet, the rat pituitary FSH content was raised (298). The production and release of LH is inhibited during lactation in both intact and ovariectomized rats (214, 244, 265) and mice (267).

Effects of estrogens and androgens on gonadotropin secretion.—Removal of the "feedback" influence of target organ steroids by castration elevates pituitary ICSH (LH) content as well as FSH titers (56, 60, 138, 214, 243, 244, 305). In the male dog, basal hypothalamic posterior median eminence lesions depress the pituitary production and postcastration elevation of both gonadotropins [Davidson *et al.* (95)]. In the rat with postcastration gonadotropin levels, testosterone differentially depresses LH (263) and elevates FSH content (241). Median eminence lesions which induce constant diestrus prevent the LH rise following ovariectomy, while anterior hypothalamic "constant estrus" lesions do not [Taleisnik & McCann (305). The latter region contains an "FSH-inhibitory center" [Donovan (71)], and localized lesions in the anterior hypothalamus prevent the inhibitory effect of estrogen

[Flerko (95, 96)] and androgen (98), on pituitary FSH production. In such rats, unilateral ovariectomy often leads to ovulation in the remaining ovary, perhaps by lowering the amount of circulating estrogen and permitting LH synthesis and release (97).

New direct evidence confirms earlier proposals that the gonadal steroid "feedback" action is exerted on the hypothalamus rather than the hypophysis. Flerko & Szentagothai (99) inhibited gonadotropic function in the rat by implanting ovarian tissue into the anterior hypothalamus. Hohlweg & Daume (145) induced testicular atrophy in rats by injecting 1/125 the effective systemic dose of estrogen directly into the brain. Davidson & Sawyer (62, 63) have recently induced ovarian atrophy in the rabbit and testicular atrophy in the dog by implanting tiny amounts of solid estradiol benzoate and testosterone propionate, respectively, into the posterior median eminence-basal hypothalamic region. Control implantations in the hypophysis gave negative results.

In an important series of studies, Barraclough (16, 17) has found that a single injection of testosterone to infant (2 to 5 days old) female rats will make them permanently sterile. They can be ovulated by exogenous gonadotropin or by electrical stimulation of the hypothalamus following treatment with progesterone (18). Progesterone is ineffective in inducing spontaneous ovulation, but it interrupts the constant cornification of the vaginal smear and permits storage of enough pituitary LH to induce ovulation in response to the hypothalamic stimulus (116). In a somewhat similar study, Segal & Johnson (284) reported that the hypophysis of anovulatory rats contained adequate gonadotropin and that it was capable of complete function if transplanted contiguous to the hypothalamus of a control female rat.

With its estrogen removed from the circulation by the liver, a rabbit ovary transplanted to the spleen ovulates and shows evidence of hypersecretion of gonadotropins (186). Paesi & Rees (240, 258) have warned that in rats a reflex discharge of ICSH at castration may interfere with subsequent reaction to sex steroids. Estrogen inhibits both production and release of FSH while testosterone interferes only with its release (259). Miroestrol, a new nonsteroid estrogen, has been shown to be highly effective in inhibiting pituitary activity (158). Single high dosages of stilbestrol induce pseudopregnancy in the rat, the "Hohlweg effect" (147). A phenanthrene derivative, RO-27239, has been found to be effective in blocking gonadotropic function in the rat by virtue of its androgenic activity (72, 74).

Miscellaneous effects of other hormones on gonadotropic secretion.—Thyroidectomy in female rabbits leads to the production of cystic ovaries with hypertrophy of follicular tissue, and a high content of mucopolysaccharides (306). In rats, thyroxine, at a daily dosage of 25 μg, inhibits release of gonadotropin (146). Hydrocortisone counteracts the inhibitory effect of estrogen on pituitary gonadotropin in the ovariectomized rat (206). In the castrate rat, pregnancy is maintained by treatment with amphenone, apparently via its effect on the adrenal glands (310). Pineal extracts have been reported to block rat ovarian weight increase in response to light (323) and to interrupt

the persistent estrous state of older female rats, but the mechanisms are poorly understood (223).

EFFECTS OF THE TROPIC HORMONES AT THE TARGET ORGANS

Adrenals

Since several reviews have recently dealt with adrenal metabolism (73, 100, 118), reference is made only to some of the more recent findings concerning the effect of ACTH on the biosynthesis of corticoids.

The hypothesis emerging from the work of Haynes, Hechter, Péron, Schönbaum, and others, in which the conversion of cholesterol into progesterone is considered to be the primary site of action of ACTH, has received much confirmation. Adrenocorticotropin promotes this conversion by accumulating adenosine-3′5′-monophosphate, increasing phosphorylase activity necessary for the glucose metabolism which leads (in the presence of the appropriate dehydrogenase systems) to the production of reduced ricotenomide-adenine dinucleotide phosphate. Since reduced form $NADPH_2$ is abundantly present in the adrenal gland and is involved in the conversion of ($NADPH_2$) progesterone into hydrocortisone and corticosterone, the action of ACTH seems to result in making $NADPH_2$ available for the conversion of cholesterol into progesterone. This might be a matter of changes in permeability of the mitochondria. If the glucose-6-phosphate dehydrogenase system is extramitochondrial, the $NADPH_2$ produced is not readily available for the biosynthesis of progesterone, which occurs in the mitochondria. Work done by Koritz & Péron, and by Schönbaum, suggests that sugar permeability may be one of the most important factors affected by ACTH (266, 280). In this connection it is of interest that more recent work of Hechter's group indicated that ACTH increases the entry of sugar into the adrenal cells. Eichhorn *et al.* (82) observed that the nonutilizable pentose, D-xylose, is found in the intracellular water in much higher quantities after the injection of ACTH and after stress. However, they subsequently demonstrated that D-xylose enters the adrenal cells easily in the absence of ACTH when the adrenals are incubated *in vitro* (114). Apparently, the permeability to sugars is not a limiting factor *in vitro*, which fact prompted new speculations on the mechanism of action of ACTH.

Greenberg & Glick (120) confirmed that ACTH increases the activity of 6-phospho-gluconate and glucose-6-phosphate dehydrogenase systems. They also observed an ACTH-induced shift of activity maxima from the zona fasciculata to the border of the reticularis, indicating that the latter zone is the main site of steroid formation. Work on the effect of cold-induced stimulation of the adrenal cortex by Cohen (53) was also consistent with the Haynes theory. Another substantiation of this theory was provided by the study made by Hilton *et al.* (141, 142) in dogs with *in vivo*-isolated adrenal glands and with slices of dog, rat, and guinea pig adrenals *in vitro*. They found an increase in corticoid production by adenosine monophosphate *in vivo* as well as *in vitro*, but not in adrenomedullary secretion *in vivo*. Glucagon, which

is known to cause a selective increase in adenosine monophosphate in liver slices, did not have an effect on its accumulation in adrenals.

Eichhorn *et al.* (83) also observed an enhanced accumulation of the amino acid analogue, α-aminoisobutyrate in adrenals and in diaphragms after the administration of ACTH or insulin. Accumulation was diminished after hypophysectomy.

In a study on the effect of calcium and glucose on adrenal metabolism, Birmingham *et al.* (27) found that calcium increases the potassium concentration of adrenals *in vitro* and potentiates the effect of adenosine monophosphate, whereas glucose has no effect. Neither calcium nor glucose was needed for the *in vitro* production of corticoids initiated by ACTH. It is likely that the effect of ACTH on permeability is not concerned with glucose itself, but with an intermediate metabolite. Péron & Koritz (248) found that the stimulating effect of calcium in adrenal homogenates appears to be located in the large particles of the cells. At which step in the biosynthesis calcium is required is still a matter of conjecture.

Bakker & De Wied (11) investigated adrenal sensitivity to ACTH *in vitro*. The corticosterone production is enhanced by preincubation and by previous heating (60°C); presumably these procedures remove the influence of an ACTH-inactivating enzyme leaking from the cut surface of the adrenal. Adrenals from stressed rats were less sensitive to ACTH, which may be the result of the presence of endogenous ACTH.

The production of corticosterone and aldosterone *in vitro* is stimulated in the same proportions by incubation with anterior and with posterior lobes of rat hypophyses [Mialhe-Voloss & Beaulieu (225)], suggesting that the posterior lobe contains ACTH.

Adrenal ascorbic acid.—The role of ascorbic acid in adrenal metabolism is still obscure. The presence of the substance markedly inhibits cholesterol conversion into progesterone. This might be related to the fact that ascorbic acid is rapidly removed from the adrenal following activation by ACTH.

Ascorbic acid depletion is more closely related to corticoid release than to corticoid synthesis, since Reck & Fortier (257) found that unilateral adrenalectomy induces a rapid increase in adrenal corticosterone content and a slower change in ascorbic acid content and plasma levels of corticosterone, Koritz & Péron (182) investigated the effect of ascorbate on nicotinamide-adenine dinucleotide (NAD), which has been shown to inhibit corticoid production, presumably by oxidation of $NADPH_2$. They observed that ascorbic acid removes the inhibition of NAD, and suggested that prevention of this inhibition might be a functional aspect of ascorbic acid.

Slusher & Roberts (293) found that adrenal ascorbic acid response and corticoid release (measured in adrenal vein effluent) are equally sensitive to ACTH. The minimal effective dose of intravenous ACTH in 2-day hypophysectomized male rats was 0.37 mU. These data corroborate the findings of Lipscomb & Nelson (198).

Schapiro *et al.* (279) found in neonatal rats a good correlation between the onset of responsiveness of adrenal ascorbic acid depletion and of increase in

adrenal corticosterone content to stress. The response to stress begins to appear at the age of eight days; however, during the first week there is a response to ACTH and to vasopressin (Pitressin and synthetic lysine-vasopressin), indicating that maturation of the central nervous system is the limiting factor involved in the unresponsiveness to stress in neonatal rats.

In this connection it is of interest that, according to Adolph (1), neonatal rats do not respond to water load with an increase in diuresis.

Gonads

In a valuable recent paper Woods & Simpson (318) have shown conclusively that ICSH is the important principle in maintaining or restoring both tubules and interstitial cells in the rat testis after hypophysectomy. Follicle-stimulating hormone FSH synergizes with ICSH as do also LTH and growth hormone, but without ICSH all of these are powerless to maintain the testis. Growth hormone has also been seen to synergize with LH in the hypophysectomized female rat (119). Mason & Samuels (210) observed that the dog testis *in vivo* responded rapidly to gonadotropic stimulation by synthesizing testosterone and pouring it into the spermatic vein. The rat anterior pituitary contains an "antagonist" which, on intraperitoneal injection, reduces the ovarian response to simultaneously administered gonadotropins (317). The gonadotropic potency of the mouse pituitary is elevated at the expense of the ovary by irradiation and treatment with gonadotropic antiserum (86).

Testosterone pellets implanted directly into the rat testis retard the regression of the gonad which follows systemic treatment with estrogen (148) or hypophysectomy (157). Small doses of cadmium salts sterilize the male rat by causing acute destruction of the testis (123, 242). The testis is protected by the simultaneous injection of a large dosage of zinc. Cadmium depresses the uptake of Zn^{65} by the testis and dorsolateral prostate, a process controlled by ICSH (123). Much larger dosages of cadmium also damage the ovary (163). Estrogen has again been shown to exert a direct effect on the ovary of the immature rat (34, 58).

Much research has been conducted on the biosynthesis of progesterone, the nature of its metabolites, and the factors controlling its production (15). The ovary and blood of several species have revealed 20-hydroxypregn-4-ene-3-one (188, 288), and Short (289) has detected a wide variety of progesterone derivatives from the follicular fluid of the mare's ovary. *In vitro* production of progestins has been studied on slices of ovary and luteal tissue of the rat (193, 301), sheep (193), pig (76), and cow (204). Steroid synthesis is stimulated by equine gonadotropin, human chorionic gonadotropin, and luteinizing hormone. Luteinizing hormone but not FSH stimulates the uptake of glucose by rat luteal tissue (7). Gonadotropins or coital stimulation, but not lactogenic hormone, activates the release of progestins (largely 20-α-hydroxy-pregn-4-ene-3-one) *in vivo* from the rabbit ovary lacking corpora lutea [Hilliard *et al.* (139, 140)]. Progestin output begins within minutes of gonadotropic stimulation, more than 10 hr before ovulation. Preovulatory production of progesterone has been noted by Forbes and others in many

species, most recently in the mouse (125). Its function at this period is unknown but in the frog ovary, *in vivo* or *in vitro*, progesterone activates ovulation, and Wright (320, 321) has suggested that the steroid might serve an intermediary role between gonadotropin release and the ovulatory process.

ADRENAL CORTEX INHIBITORS

Knowledge about drugs which inhibit corticoid biosynthesis is rapidly growing, since a systematic search for these compounds has become a generalized practice in industrial laboratories. For general information see several recent reviews on this subject (2, 40, 49).

A series of chemically related compounds, which undoubtedly will be extended in due time, interfere with the adrenal metabolism in several ways. Dichlorodiphenyl dichloroethane-like compounds reduce adrenal cortical secretion, and render the adrenal gland unresponsive to ACTH [cf. (236)]. The most active component in the commercial product appears to be the *o'·p'*-isomer (237, 311). Nichols & Richardson (238) found that technical dichlorodiphenyldichloroethane prevents the rise in 17-hydroxycorticoids in dogs after infusion of ACTH, but not the increase in blood flow caused by ACTH.

Metopirone (Su-4885), a compound closely related to amphenone-B, inhibits primarily the 11-β- hydroxylation, thus preventing the formation of hydrocortisone and aldosterone. The consequent rise in ACTH production results in an excess secretion of other cortical compounds, normally secreted in very small amounts; these exert pronounced mineralocorticoid effects. Gold *et al.* (112, 113) used Metopirone as a tool in a standard test for pituitary function in man. In normal subjects the expected rise in ACTH secretion after infusion of 30 μg of Su-4885 results in a doubled excretion of urinary 17-ketogenic steroids, since the metabolic pathway is forced to go via 17-hydroxy-progesterone→androstenedione→11-β-hydroxy-androstenedione→17-keto-steroids. The urinary level of this end product can be used as a measure of pituitary function. Liddle (195) also used Su-4885 in function tests in patients. It has been suggested recently that it interferes primarily with glucose oxidation (14). Some new derivatives, which appear to block 17-β-hydroxylation, have been reported (50).

Goodman (115) found that ω-methyl-pantothenic acid reduces corticosterone secretion in the rat, but this inhibition can be overcome by chronic administration of ACTH. The drug may compete with pantothenic acid, since it is known that pantothenic-deficient rats produce less corticosterone.

Triparanol (MER-29) blocks cholesterol formation and consequently decreases corticosterone and aldosterone production. Melby *et al.* (221) found that adrenals from Triparanol-treated rats exhibit a decreased production of these corticoids *in vitro*, whether or not ACTH was added. Holloszy & Eisenstein (150) observed in rats treated with Triparanol that corticosterone secretion was depressed Lower levels of serum cholesterol were found by Carver *et al.* (47) in patients after Triparanol treatment. It seems that the principal site of action of this drug is at the last step in the biosynthesis of

cholesterol. It would interfere with the reduction of the side chain double bond of 24-dehydro-cholesterol (10).

Two triazine analogues decrease urinary corticoid excretion in the guinea pig considerably, but ACTH has an almost normal effect (54).

Diphenylhydantoin (Dilantin), an anticonvulsant, abolishes the adrenal ascorbic acid response to several kinds of stress and to vasopressin, according to Bonnycastle & Bradley (31). Prolonged administration even reduced the adrenal responsiveness to ACTH. This depressant action is not shared by a series of other anticonvulsants tested. A single dose of diphenylhydantoin does not seem to decrease the adrenal responsiveness to ACTH [cf. (187)], indicating that the immediate action is not at the adrenal level.

In contrast to other reports, Eisenstein & Strack (84) observed that sodium deficiency *in vitro* as well as *in vivo* reduces corticosterone production.

Asphyxia seems to inhibit corticosteroid production directly, since simultaneous ACTH infusion does not overcome the block in corticoid production (48).

ANTIFERTILITY AGENTS

Attempts to control the "population explosion" have made this area of research a most important one. Progress through 1960 has been reviewed by Nelson (231). Drugs under investigation block the release of pituitary gonadotropin, inhibit the effect of the released gonadotropin on the ovary, prevent implantation of the ovulated egg, or interfere with sepermatogenesis.

The antiovulatory effects of progestational steroids such as norethynodrel and norethindrone have proven successful both in experimental animals (211, 227, 252) and in women (263). These agents prevent the release of pituitary gonadotropin, and Sawyer & Kawakami (275) have presented evidence that they exert at least part of this effect by raising specific thresholds of activity in the central nervous system. 17-α-Acetoxy-progesterone delays estrus in the dog (41); 6-α-methyl-17-α-hydroxy-progesterone induces reversible atrophy in rat gonads of both sexes (199); these and other steriods act by inhibiting the release of pituitary gonadotropin (173).

Ethamoxytriphetol (MER-25), a new drug which "blocks the response of estrogen-target organs to the stimulating action of estrogens" (194), prevents implantation in the rat uterus (234). It is weakly antigonadotropic, and it synergizes with estrogen in this action (59). Clinically, ethamoxytriphetol has served the opposite purpose, that of inducing ovulation in cases of secondary amenorrhea (308) and the Stein-Leventhal syndrome (174). A related agent, chloramiphene, is a much more potent antigonadotropin (155). It blocks ovulation in the female rat, and in high dosage renders the male sterile (154). It appears to function by competing with or substituting for estrogen. Even in dosages which do not prevent ovulation, chloramiphene interrupts pregnancy in the rat, perhaps by its effect on the uterus (285). Fertility is rapidly restored following withdrawal of the drug.

The antifertility progestogens also block spermatogenesis in male rats

(232) and in man (9, 132). However, a new series of *bis*-(dichloro-acetyl)-diamines which arrest spermatogenesis reversibly without apparent effect on the Leydig, Sertoli, or spermatogoneal cells of the testis, hold even greater promise (25, 55, 253). Rather than blocking gonadotropin, these agents apparently depend on the concomitant action of the hormone to exert their effects on spermatogenesis (233), which is arrested at the secondary spermatocyte stage. The primary site of action of these agents appears to be the germinal epithelium. The *bis*-(dichloro-acetyl)-diamines are receiving careful screening on human patients (130, 131, 202), and they appear to be highly effective, safe, and almost completely reversible after treatment is withdrawn. Triethylenemelamine also interrupts spermatogenesis in the rat, by a direct action on the maturation cells in sperm development (300).

Lithosperm research continues to show that the plant product exerts its antifertility effect by inactivating gonadotropins rather than by affecting the pituitary or the target organ. It also inactivates TSH and LTH (168) and oxytocin (35). Rabbits fed on ladino clover became infertile, perhaps because of its high estrogen content (319). It was suggested that polyphloretin phosphate lowered fertility in female rats by inhibiting hyaluronidase in the ovarian follicles (316). Infertility may also be attributable to immunity reactions in the female to antigens present in sperm or seminal fluid (164).

ALDOSTERONE

Control of aldosterone secretion.—Farrell (91) has reviewed his work on the effect of extracts of pineal tissue on the production of aldosterone. The substance considerably increases aldosterone output in decerebrate dogs while not affecting hydrocortisone output significantly (92). Keller *et al.* (167) also reported that they had isolated an effective factor from pineal extracts which induced an increase in carboxyl esterase activity in the zona glomerulosa of treated intact rats.

On the other hand, Wurtman *et al.* (322) found no effect of pinealectomy or injection of pineal extracts on the zona glomerulosa, urinary potassium excretion, or selective saline intake. Davis *et al.* (64) observed that after midbrain transection in dogs, a considerable rise in aldosterone production occurs following bleeding or thoracic venal caval constriction, indicating that postulation of a regulatory center in the midbrain is not obligatory.

Bartter *et al.* (23) published in detail an earlier reported study on the effect of thyrocarotid arterial junction denervation on the hypersecretion of aldosterone following carotid artery constriction. Their conclusion that receptors in this area play a role in aldosterone regulation is not corroborated by Biglieri & Ganong (26) or by Carpenter *et al.* (45). The rise in aldosterone secretion after carotid constriction does not occur in hypophysectomized dogs. This failure is not caused by adrenal refractoriness since infusion with ACTH failed to restore the capacity for producing aldosterone under these conditions. It is apparent now that ACTH is capable of stimulating aldosterone secretion, but only in high amounts (106). The amount is approximately 100 times higher than that required for glucocorticoid production (106, 139).

To rule out the influence of ACTH, the most recent work has been done in hypophysectomized dogs.

Davis and associates showed that transplantation of both the kidney and the adrenal glands does not interfere with sodium retention and hypersecretion of aldosterone resulting from vena caval constriction (46). These results make a completely humoral control likely. In their search for the nature of the substance involved in aldosterone control they studied the role of the liver and the kidney. Bleeding increases aldosterone release significantly in intact, hypophysectomized, and decapitated dogs. Whereas in hepatectomized-hypophysectomized dogs a similar increase was seen after bleeding, the effect was completely inhibited in 19 out of 22 nephrectomized-hypophysectomized animals. In these animals, the initial aldosterone levels were very low and did not change after bleeding. A saline extract from the kidney produced a sevenfold increase in aldosterone production in these dogs; liver extracts were without effect (65, 66).

Ganong & Mulrow also studied the effect of kidney extracts in nephrectomized-hypophysectomized dogs. They found that nephrectomy prevents the hypersecretion after bleeding and, moreover, that an amount equivalent to at least 1/30 kidney increased aldosterone output considerably. Kidney extract from hypophysectomized dogs, however, failed to elevate aldosterone levels. The rise in aldosterone secretion caused by saline extract from one normal dog kidney was approximately sixfold; it was accompanied by a significant rise in blood pressure, but not by a change in the secretion rate of hydrocortisone (106, 107).

Gross (121) suggested earlier that the renin-hypertensin system might be involved in the control of aldosterone production. Impressive evidence is accumulating that this is indeed the case. In patients it has been observed that synthetic angiotensin increases aldosterone production (109, 190). Mulrow & Ganong (229) found that infusion of 3 μg of angiotensin II in nephrectomized-hypophysectomized dogs stimulates aldosterone output without increasing corticosterone secretion. In much higher amounts angiotensin II does have a stimulating effect on corticosterone and 17-hydroxycorticoid production. Carpenter *et al.* (44) also found in the same preparation that renin and angiotensin cause a rise in aldosterone production. Biron *et al.* (28) observed in human volunteers that infusion of valine-5-angiotensin II produced a 2 to 10-fold increase in aldosterone, whereas other pressor agents (glucose, norepinephrine, and phenylephrine) did not have an effect. The amount of angiotensin infused was 1.2–7.4/μg/min.

These exciting recent findings have provided a sound basis for the hypothesis that the kidney releases renin in response to any decrease in blood volume or pressure; renin acts on the renin-substrate protein in the blood to produce angiotensin I, of which a pair of amino acids subsequently is split off by the converting enzyme, yielding angiotensin II. This polypeptide would act directly on the adrenal cortex and would represent the actual trophic hormone for aldosterone production.

Aldosterone antagonists.—The subject has been reviewed recently by Munson (230), Gaunt *et al.* (108) and Albeaux *et al.* (2). Steroidal spirolactones compete with aldosterone on the renal tubular level, and do not interfere with aldosterone secretion. Kittinger *et al.* (178) showed that aldosterone production *in vitro* of adrenals from rats treated for several weeks with SC-8109 (3(3-oxo-17β-hydroxy-19-nor-4-androsten-17α-yl-1)propionic acid γ-lactone) actually is increased, whereas the corticosterone secretion is lowered. Kagawa (160) reported that a new, orally active spirolactone, aldactone (3-(3-oxo-7α-acetyl-17β-hydroxy-4-androsten-17α-yl)propionic acid-γ-lactone) is five times more potent than SC-8109. No androgenic, estrogenic, progestational, or cortisone-like side effects could be detected.

Reduction of the aldosterone output in dogs has been reported after treatment with acetyl strophanthidin (28).

Miscellaneous.—According to Gláz (111), deoxycorticosterone diminishes aldosterone production by adrenal glands *in vitro*, whereas the secretion of corticosterone is enhanced. Growth hormone has some effect on aldosterone secretion *in vivo*, but not *in vitro*. Monkey growth hormone injected into hypophysectomized rats increases aldosterone production of the adrenals from these rats *in vitro*. Whereas growth hormone added to the medium had no effect on aldosterone production, plasma from hypophysectomized rats treated with growth hormone did increase aldosterone secretion when added to the medium (201).

Layne *et al.* (191) found that during pregnancy the aldosterone turnover and plasma binding is not increased. According to Péron (247), 18-hydroxy corticosterone might be involved in aldosterone biosynthesis.

Anoxia, produced by the replacement of blood by plasma in dogs, led to an immediate increase in aldosterone secretion, according to Holzbauer & Vogt (152). Care had been taken not to alter the circulating blood volume. The effect was abolished by cutting the splachnic nerves. The same authors carefully studied the partition of corticosteroids in blood cells and plasma of several species (153). They found that aldosterone is often found in blood cells in much higher quantity than in plasma. The partition rate is very variable (0.125 to 4.7). Most of the corticosterone and hydrocortisone is found in the plasma. They conclude that it would be preferable to estimate aldosterone in whole blood rather than in plasma.

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