# ADRENAL CORTICAL FUNCTION AND WHITE BLOOD CELLS IN VARIOUS CLINICAL STATES

BY

HARRY DANIEL BAERNSTEIN JR

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In 1855, Thomas Addison (7) gave to medicine a classical description "On the Constitutional and Local Effects of Disease of the Supra-renal Capsules." With his work we can trace the beginning of physiological thought concerning this endocrine gland. Increasing emphasis has been placed upon the role of the adrenal cortex in the body economy. The idea of a relatively constant internal environment, or "homeostasis" so characteristic of mammals takes on added meaning as we learn more about the biochemistry of the adrenal cortex: the mechanisms which increase its activity, the chemical substances which it produces and pours into the blood stream, and the widespread biochemical and biophysical changes which these produce. Experiments on animals have frequently produced results which struck a responsive chord in the mind of a clinician; perhaps more different diseases have been thought to owe their pathogenesis in part to a disturbance of adrenal cortical function than to that of any other organ of the body.

This student of medicine came to the clinical years with very little understanding of the role of the adrenal cortex. When the adrenal gland was mentioned, it called to mind epinephrine, and mobilization of liver glycogen in the alarmed animal. The adrenal cortex remained a sort of never-never-land occupied by some minor changes in salt metabolism, and perhaps in sugar and protein metabolism. The fact had never been brought home to him that the animal deprived of

its adrenal medullae, while less able to stand cold, can live, if the external environment is kept favorable, while the totally adrenalectomized animal forfeits its very life, without constant attention to its internal environment as well; no amount of epinephrine can be injected which will prevent the rapid downhill course of its predominately biochemical disorder.

This review represents an effort by its author better to understand the present day knowledge about the adrenal cortex. Advances in the biochemistry of the gland are dealt with briefly; however, one relatively more recent more recent mode of study, the investigation of white blood cells, is dwelt upon more at length. It is hoped that the author may some day be concerned in some phase of investigation of the adrenal cortex. Until that time, he hopes to be able to recognize, in the patients he sees, any alterations in cortical function.

## I. Physiology of the Adrenal Cortex

A. General Considerations. The adrenals, or in better form, the suprarenals, are two ductless glands of mesodermal and neural origin, which lie near the upper poles of the kidneys. It would be out of place to describe in detail their gross anatomy, histology, comparative anatomy, and morphogenesis; these have been ably dealt with in the standard texts. The inner portions, or medullae, are part of the chromaffin tissue of the body, and, upon stimulation by pre-ganglionic sympathetic fibers, produce epinephrine. The outer, mesodermal portions, the adrenal cortices, have no significant efferent nerve connections, and, under the hormonal influence of the anterior lobe of the hypophysis, they secrete "cortin", a group of steroid hormones with effects upon nearly every system of the body. The cortical hormones are essential to life.

The adrenal cortex is the "organ of homeostasis." Its loss is most acutely felt when the organism is put under some strain by its external environment, and when it must readjust to keep its internal environment, the blood and the tissue fluid, in "homeostasis", or operating condition.

The adrenalectomized animal takes a downhill course to an early demise. His path is marked by tachycardia, thirst, oliguria, emesis, diarrhea, asthenia, anorexia, hypotension amounting to shock, coma, and death. Gland extracts put off his death by preventing the most rapidly fatal of the biochemical changes.

Figure 1 is intended as an outline of the pituitary-adrenal cortical mechanism. No discussion will be attempted in the text, since standard works in endocrinology can present this material at length, and with better result. Experiments upon which this scheme is based may nearly all be classified under four headings: (1) The effect of ablation of the adrenal cortex (2) the effect of adrenal extracts, fractions, and purified and chemically characterized compounds from the cortex (3) the effect of pituitary fractions (4) the study of the steroid end products excreted in the urine.

Gland extraction is not an ideal way to study the secretions of the gland, since only small amounts of the hormones are present in active form, and chemical changes may easily be induced during the extraction. The gland is somewhat like "the goose that laid the golden eggs," in that it secretes in life more hormone, in a short time, than can ever be extracted from it after death.

The physiologic actions of purified and characterized compounds also are hard to interpret. Each steriod has a major effect, and several minor effects. Selye (5) has suggested an arrangement of steroid hormones in a table, not reproduced here, reminiscent of the periodic table of the elements originated by Mendelejeff. Selye's table graphically shows the overlapping of the actions of these hormones, and expresses the empiric fact that each steroid has a maximum in one particular effect, and decreasing potency as measured by other effects.

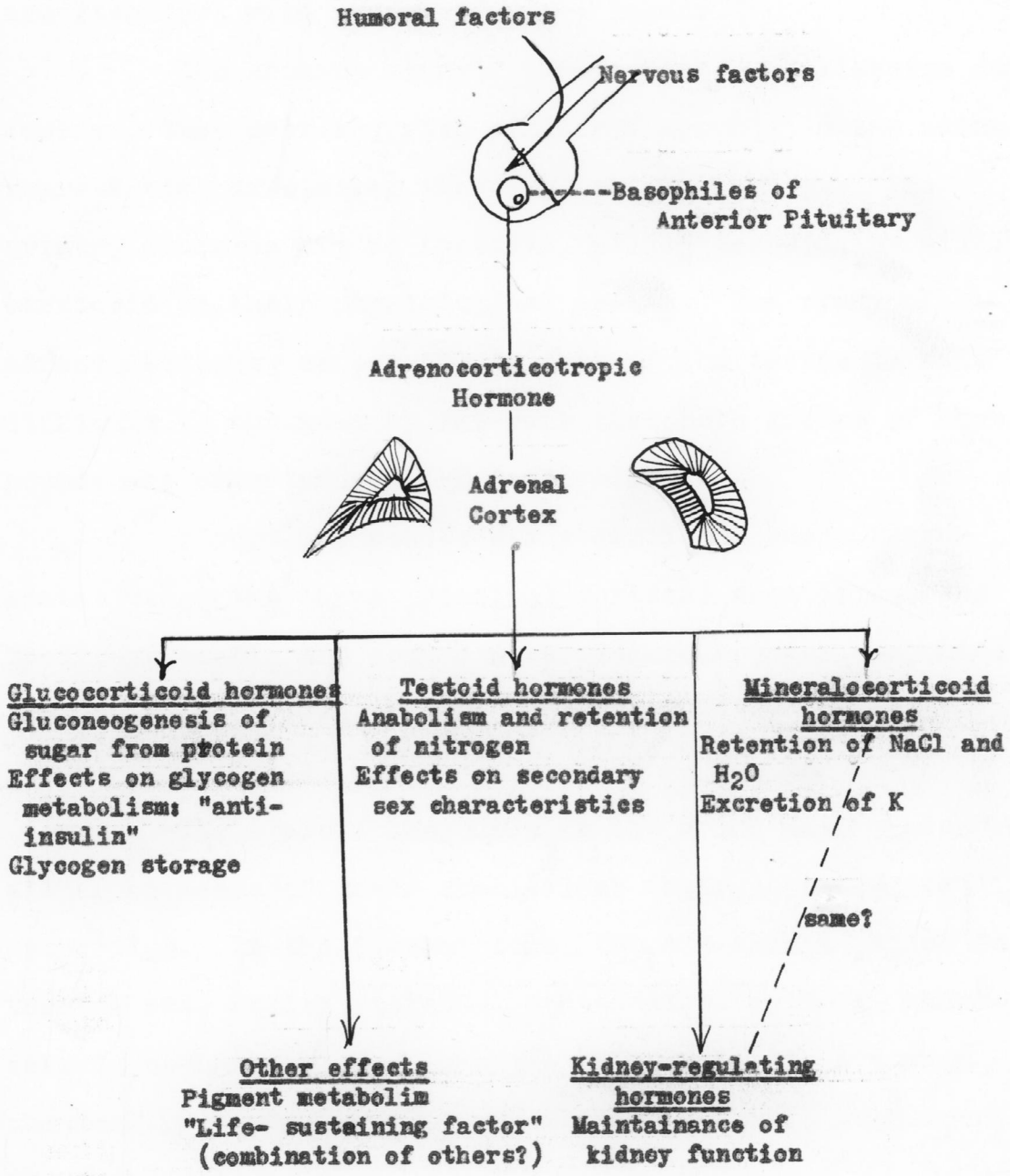

Humoral factors
Nervous factors
Basophiles of Anterior Pituitary
Adrenocorticotropic Hormone
Adrenal Cortex
Glucocorticoid hormones
Gluconeogenesis of sugar from protein
Effects on glycogen metabolism: "anti-insulin"
Glycogen storage
Testoid hormones
Anabolism and retention of nitrogen
Effects on secondary sex characteristics
Mineralocorticoid hormones
Retention of NaCl and $H_2O$
Excretion of K
same?
Other effects
Pigment metabolim
"Life- sustaining factor" (combination of others?)
Kidney-regulating hormones
Maintainance of kidney function

FIGURE 1

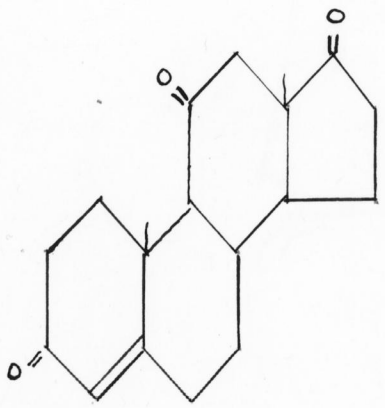
Some compounds isolated from the adrenal cortex are identical with others from the gonads.

The urinary steroid end-products are likewise confusing. They overlap, with similar compounds in the urine representing dissimilar steroids from the cortex. The urinary steroids may be inactive, mildly testoid, or mildly corticoid in their physiological action. The study of adrenal activity as opposed to that of the testis is made difficult in the male by the fact that both groups of compounds are excreted as 17-ketosteroids.

Figure 2 presents six steroid compounds representative of the three principal cortical activities, and their systematic and common nomenclature. These compounds are those most often referred to in literature concerning the adrenal cortex.

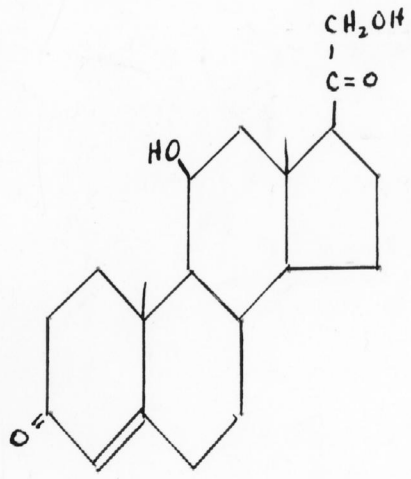
The steroid compounds in the urine may be measured either by means of their chemical or their physiological properties. At the present time, the bio-assays, which determine only active steroids, and chemical methods, which take in both active and inactive compounds with a common chemical property, are to be used in a mutually supplementary manner (17).

B. Physiologic Variations in the Activity of the Pituitary-Adrenal-Cortical Mechanism. Even in everyday life, without any particular stress, the pituitary-adrenal cortical mechanism undergoes cyclic changes. The standard texts devote little space to these changes, because they are



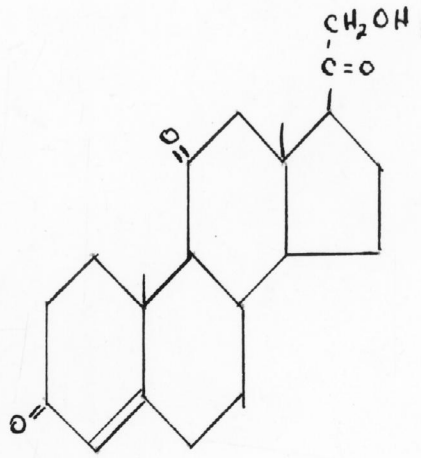
$\Delta^4$ androstene-3,11,17-trione
(Adrenosterone)

TESTOID



17($\beta$) [1-keto-2-hydroxyethyl] -
$\Delta^4$ androstene-3-one-11($\beta$)-ol
(Corticosterone)

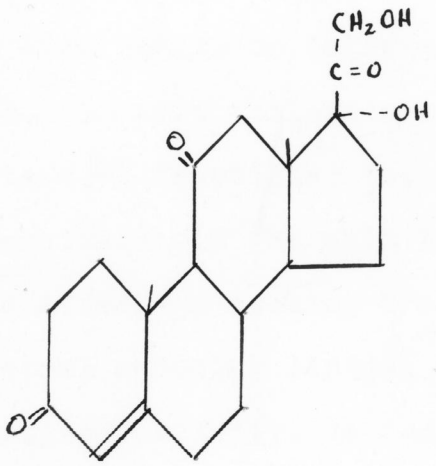
GLUCO*CORTICOID



17($\beta$) [1-keto-2-hydroxyethyl] -
$\Delta^4$ androstene-3,11-dione
(11-dehydrocorticosterone)

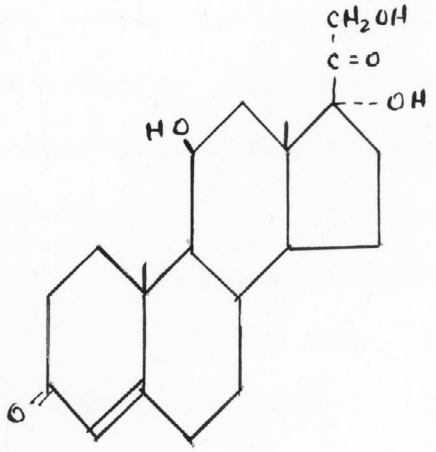
GLUCO-CORTICOID

**FIGURE 2**



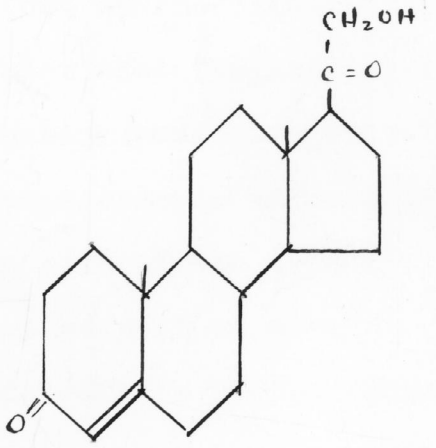

17(β?)[1-keto-2-hydroxyethyl]-
$\Delta^4$ androstene-3,11-dione-17(∝)-ol
(17-hydroxy-11-dehydrocortico-
sterone or "Compound E")

GLUCO-CORTICOID




17(β)[1-keto-2-hydroxyethyl]-
$\Delta^4$ androstene-3-one-11,17(∝)-diol
(17-hydroxycorticosterone or
"Compound F")

GLUCO*CORTICOID




17(β)[1-keto-2-hydroxyethyl]-
$\Delta^4$ androstene-3-one
(Desoxycorticosterone or "DOCA")

MINERALO-CORTICOID

relatively less studied than the pathological variations. In experiments on internes in a hospital, Pincus et al (65, 67) made collections of urine in night, morning, and afternoon fractions; the urine during sleep contained least 17-ketosteroid per unit time, the morning fraction most, and the afternoon-evening fraction an intermediate amount. The neutral reducing lipids, also thought to be reflective of cortical activity, varied in a similar manner, but the pattern of excretion of the two groups of compounds did not have statistically significant similarities, according to the authors. For this reason they could not state that the adrenal cortex was responsible for both the curves of variation.

Young animals probably have somewhat less adrenal cortical function than older animals; there may be more testoid function in their adrenals.

The menstrual cycle in man probably exerts some effect on the pituitary-adrenal cortical mechanism. Selye (5) states that the urinary steroids reflecting adrenal cortical function remain relatively constant; however, he also states that estrus in animals is accompanied by some increase in the size of the cortex. Any clinical signs or symptoms that suggest an adrenal cortical imbalance may be due to side effects of the female sex hormones.

Pregnancy exerts a long-term, slowly-increasing stimulation on the pituitary-adrenal-cortical mechanism. Sharp interest has been focussed upon the toxemias of pregnancy, especially eclampsia, in the past few years, in an effort to find out if some form of adrenal malfunction is not causative.

In the female, post-menopausal changes occur in the adrenal cortical function, probably in the direction of increase.

C. Pathologic Variations in the Activity of the Pituitary-Adrenal Cortical Mechanism. One form of pathologic change in cortical function probably affects more people than all the others together; this is the general adaptation syndrome. The recognition of this syndrome cannot be credited to any one man or laboratory; however, Selye's (4) monograph on this subject will make him long remembered in physiology and medicine. Figure 3 is a diagram from this work.

Many different stimuli of injurious nature have been found to produce the same generalized effect on the animal. These "non-specific systemic reactions of the body which ensue upon long-continued exposure to stress", as opposed to the specific, local reactions, constitute the general adaptation syndrome. The syndrome is characterized by stages of "shock", "Counter-shock" merging into a stage of resistance, and a stage of exhaustion ("secondary shock").

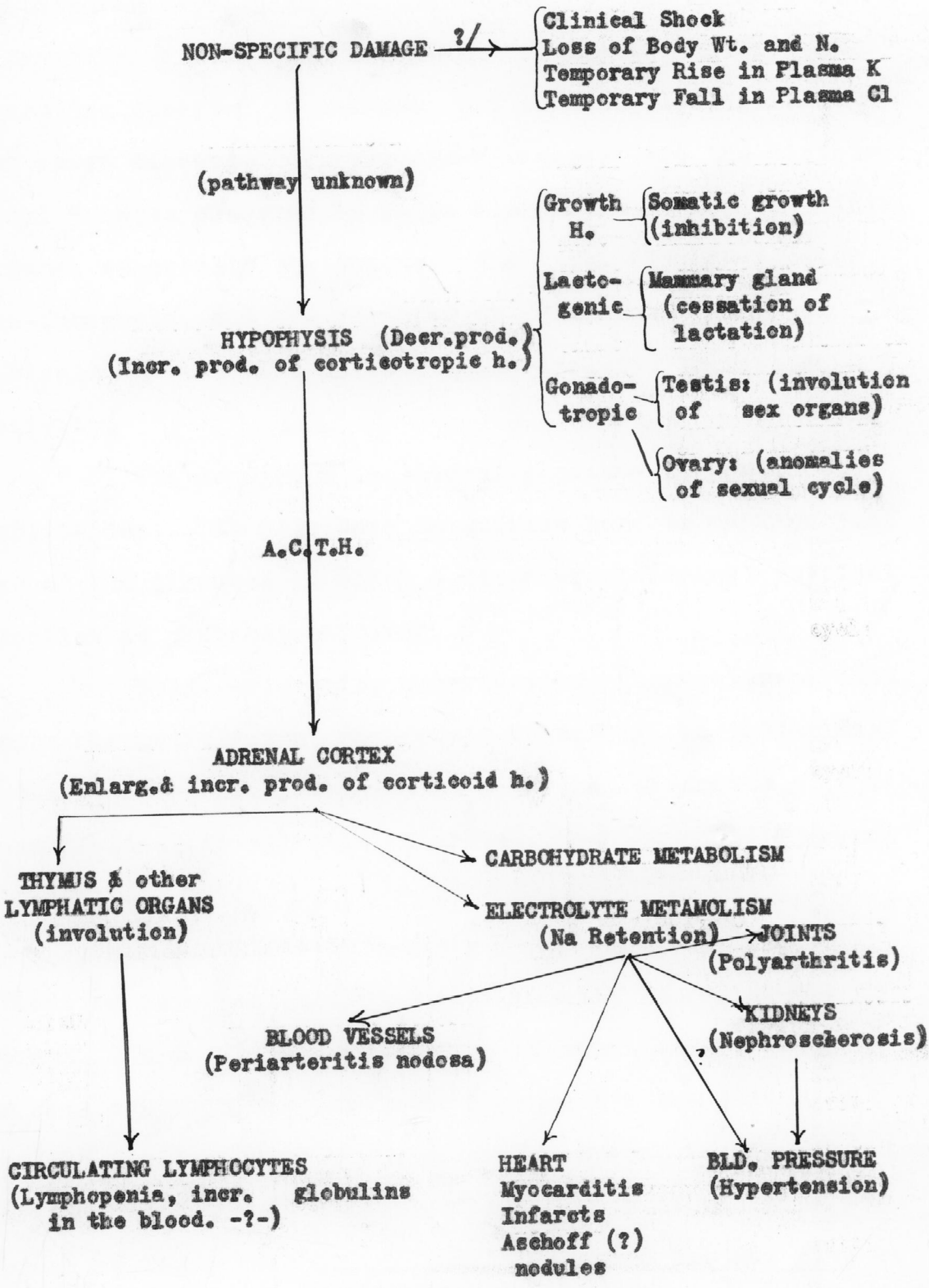



FIGURE 3 (After Selye[4])

The diagram expresses, for the most part, the changes characteristic of resistance, and many of the interesting side reactions observed in animals, and of possible significance for human disease. Of particular interest are the histological changes observed by Selye and others in the lymphatic organs, especially the thymus. The thymo-(lympho-?)cytes dis-integrate, and are engulfed by macrophages, and the reticulo-endo-thelial cells display markedly increased activity.

The monograph is literally packed with clinical implications. It will have to suffice here to mention just a few of the diseases in which a disorder of adrenal cortical function is probably involved.

Nephrosclerosis, hypertension, glomerulonephritis, acute rheumatic fever, rheumatoid arthritis, periarteritis nodosa, Waterhouse-Friedrichson syndrome, eclampsia, appendicitis, tonsillitis, diabetes, Cushing's and Simmonds' diseases.

One notices in Selye's monograph that little attention is given to the adrenal medulla in resistance to stress. It seems to one observer (6) that however logical or reasonable it may have been to consider the adrenal medulla a major organ of response to stress, it has never been established as indispensible. Experiments of the type performed by Ingle (48) on the medullectomized rat may give an accurate picture of the relative importance of the two organs.

He found that a rat with all its functional medullary tissue removed was much more resistant to stress than a totally adrenalectomized one, but much less resistant than a normal one. Adrenalin increased the resistance of the medullectomized animal but cortical extract did not.

That steroid excretion experiments tend to support this concept of adrenal overactivity in the adaptation syndrome is borne out by such papers as those of Shipley et al (78,79) Talbott et al (87) and Forbes et al. (39).

An interesting sidelight which has received little attention is the possible endocrine function of the spleen (91) and its role in the production of diminished coagulation time and capillary fragility (81). However, Downs (31) in his review, makes no mention of such a function.

Primary Hypocorticoid states. Classical Addison's disease may be called gradually developing primary adrenal cortical failure, due to a destructive local process. Tuberculosis is still the most common type of destruction, but has decreased in its relative importance in recent years. One also sees primary atrophy, with fibrosis, and with changes suggestive of chronic inflammation. Since it follows chronic intoxications and infections, the presumption is that it represents a state of exhaustion of the adrenal cortex. Metastatic carcinpma of both adrenals, an occasional bilateral, non-secreting, primary carcinoma, or an adrenal amyloidosis, also give rise to the same picture, Trauma, thrombosis,

hemorrhage, or septic embolism would be more likely to produce rapidly developing adrenal failure, as in the Waterhouse-Friedrichson syndrome, where the hyperemia of the gland following upon sepsis predisposes to the lodgement ofseptic emboli.

In Addison's disease, all three types of hormone secretion are reduced. Lack of the glycocorticoids is reflected by a flat oral or intravenous glucose curve, hypoglycemia, hypersensitivity to insulin (most prominent in diabetics who develop Addison's disease 15, 55), loss of muscle strength, low basal metabolism and body temperature. The lack of the mineralo-corticoids and kidney-regulatory factors is reflected in the hypotension, dehydration, lowered blood volume, loss of blood chloride and sodium, and rise in potassium and non-protein-nitrogen. Perhaps the gastrointestinal irritibility is also a function of the deprivation of the mineralo-corticoids. The testoid function is more difficult to evaluate. Low urinary 17-ketosteroids are almost always found in primary adrenal failure. The well-known pigmentary increases are as yet unexplained; when they are fully understood, we may also understand the pigmentation of pregnancy and of estrogen therapy.

The effective treatment of Addison's disease depends on restoration or circumvention of these cortical functions. Desoxycortico-sterone acetate tends to relieve the nausea and vomiting, low chloride, and high potassium,

and to increase the blood pressure to a level more nearly normal. However, it is usually necessary to supply extra salt as well. Potassium in the diet must not be excessive. Most of the adrenal cortical extracts available contain little of the glyco-corticoids; thus hypoglycemia is a constant threat to the Addisonian even if such extracts are being given. Extra sugar must be supplied. In adrenal crisis, it is necessary to give large amounts of intravenous fluids, salt, and glucose, besides specific corticoid substances.

Secondary Hypocorticoid States. Secondary to decreased pituitary adrenocorticotropic hormone production, we see hypoadrenal states often difficult to differentiate from Addison's disease. If the pituitary is unable to respond to stress, there is only minimal function of the adrenal cortices, in any case.

Such decreased production may be due to destructive lesions of the anterior pituitary: chromophobe adenoma, craniopharyngioma, other tumors, hemorrhage, inflammatory processes, granulomas, and atrophic states, the nature of which is not known. The degree of hypocorticoidism is less than that in complete adrenal ablation, probably because of a certain intrinsic secretory ability of the gland or perhaps because the other glands under the influence of the anterior pituitary are hyposecreting as well, and the need is not so acute.

Anorexia nervosa is an important form of hypopituitarism.

It is also possible to produce an unbalanced hypocorticoid state by administering one of the types of steroids. This tends to cause the secretion of the gland as a whole to diminish, probably by inhibiting adrenocorticotropin production.

Primary Hypercorticoid States. Hypercorticoid states arising in the cortex may be due to neoplasms of the cortex or of accessory, ectopic cortical tissue which secrete cortical hormones. These give rise to "Cushing's Syndrome", with evidence of overactivity of testoid hormone (virilism, acne, gain in weight and muscular power, mineralocorticoid hormone, enlargement of kidneys, hypertension, and sometimes low potassium, high sodium and chloride in the plasma,) and gluco-corticoid hormone (diabetes, with high insulin requirement, hyperglycemia). The striae and osteoporosis have an uncertain position.

The urinary 17-ketosteroids are increased tremendously, as is the testoid activity of the collected urinary steroids (94). Usually one particular type of 17-ketosteroid, the group with a 3-( $\beta$ ) - hydroxyl radical, is increased in cortical tumors, while there is no such increase in these compounds in primary pituitary hypercorticoidism. The gluco-corticoid urinary steroids, (47) and the formaldehyde-forming steroids (17) are increased.

Secondary Hypercorticoid States. Hypercorticoidism arising in the pituitary may be said to overlap the general adaptation syndrome. Basophile adenomas produce Cushing's Disease, which may be undifferentiable from Cushing's syndrome resulting from a functioning adrenal tumor. Usually, however, the testoid activity is less in the case of a basophile adenoma. Otherwise the two may be clinically and biochemically indistinguishable, except for the 3 (B) hydroxyl fraction of the 17-ketosteroids.

Whether hypertensive cardiovascular disease, nephrosclerosis, eclampsia, gout (44), rheumatic fever, rheumatoid arthritis (34), and the rest are disorderd of the pituitary stimulation of the cortex are fields of present research.

Sayers et al (75) have given us a table (Fig. 4) which expresses the pathogenesis of the secondary hypo-and hypercorticoid states.

Effect of Adrenal Cortical Secretion on the Lymphocyte. A large literature has arisen concerning the manner in which the secretion of the adrenal cortex affects lymphatic tissue, both the fixed tissue of the lymph nodes, thymus, and spleen, and the lymphocytes as they circulate in the blood. This literature has been in part reviewed by Valentine and his associates (92).

In situations where homeostasis is threatened, whether they arise as a result of trauma, infection, or change in the course of a disease, one finds that the number

| | TYPE I | TYPE II | TYPE III | TYPE IV | TYPE V |
|---|---|---|---|---|---|
| Pituitary | Sudden stimulation lasting a few hours | Very gradual increase in pituitary stimulation | Intense continuous stimulation | Dysfunction accompanied by hypersecretion | Dysfuntion accompanied by hyposecretion |
| Cortical hormone | Increased demand lasting a few hours | Slow gradual increase in demand for hormone | Demand continuous and great until death | No need for extra hormone | Demand not met by production |
| Adrenal cholesterol and sudanophilic material | Depleted within a few hours after stress. Returns to normal within a short period after stress. | No change tp slight change in concentration. | Markedly depleted | Deposition | Close to normal |
| Size of adrenals | No change or slight increased size of adrenals | Gradual hypertrophy | Marked hypertrophy | Hypertrophy | Atrophy |
| Clinical state | Temporary period of distress | Np noticeable change | Collapse ending in death | Evidence of excess hormone secretion | Poor resistance to stress |
| Examples | Exposure to cold for a few hours.<br><br>Exhaustive exercise<br><br>Single non-fatal dose of a drug which acts for a short period<br><br>Single injection of adrenocorticotropic hormone | Fasting, not prolonged<br><br>Change in climate from summer to winter<br><br>Gradual rise to high altitudes<br><br>Pregnancy<br><br>Change in composition of diet to higher protein content | Infectious diseases<br><br>Lethal doses of toxins<br><br>Burn "shock"<br><br>Hemorrhagic "shock"<br><br>Traumatic "shock"<br><br>Severe vitamin deficiencies<br><br>Starvation resulting in death | Hyperpituitarism<br><br>Prolonged administration of adrenotropic hormone | Hypopituitarism<br><br>Hypophysectomy |

of circulating lymphocytes in the blood decreases. This has long been known to occur in severe infections ( // ), where perhaps it has been overshadowed by the more specific neutrophilic leukocytosis. Probably it would be more often observed if one took the time to work out the absolute numbers (total white blood cells times percentage) of each kind of cell in the blood, as recommended by a standard manual of clinical pathology (90).

A wide variety of stressful stimuli have been shown to decrease the peripheral lymphocyte count: dry heat, sunlight, ex-rays (60) burns in man (76, 93) moderately severe infections in man (11), inanition in rats (80), x-rays in rats (28, 97) exposure to cold in rats (21), standardized pain stimulation in man (56), Surgical shock, muscular exercise, formalin and adrenalin injections in animals (41), psychomotor tests with or without anoxia, in man (46) injection of insulin (52).

That this lymphopenia is mediated though the adrenal gland is strongly indicated by experiments (28, 36, 97) in which the adrenals were removed bilaterally. Such experiments have been performed on mice, with x-ray as the stimulus (28, 97), and on rats with cold as the stimulus. Adrenalectomy abolishes the reaction. In humans, the reaction is lacking in Addison's disease (10), with the stimuli of infection and of adrenal crisis and shock. It is also disturbed in certain psychotic patients under much milder

forms of stress (66).

The reaction to x-ray seems to be of two types, depending upon the dosage; it seems apparent that two different effects are at work when large areas of the body are irradiated. Large doses of x-ray in rats and rabbits cause destruction of lymphocytes directly by radiation; this effect can be noted (28, 97) whether or not the adrenals are intact. It is of interest that the circulating lymphocytes survive if the adrenals have been removed, whereas pyknosis, and engulfment of the remnants by phagocytic cells occur in the lymph nodes and thymus (28). This subject has been reviewed by Dougherty and White (28). Lymphocytes in tissues can be caused to undergo degenerative changes by a wide variety of x-ray dosage. Lymphopenia is the early lymphocyte response to x-ray in the intact animal, but not in the adrenalectomized animal. In the latter, there are equally profound changes in the lymphoid tissue if it is directly exposed, but the circulating lymphocytes are spared. If lymphoid tissue is shielded, while the rest of an adrenalectomized animal is irradiated, the shielded tissue remains intact (8, 37). After exposure to x-ray, there may be a secondary rise in lymphocytes 24 hours later.

Further evidence to support the contention that the pituitary-adrenal-cortical mechanism is responsible for the lymphopenia and the degenerative changes in lymphoid tissue is given by experiments using adrenocorticotropic substances prepared from pituitary gland. As a rule, a great

effort has been made to distinguish any effect of any contaminating substances (posterior pituitary principles, foreign protein, etc.) in the protein preparations. Such experiments on mice (21,20), mice and rabbits (27), mice, rats and rabbits (96), mice, rabbits, rats, and humans (24), rats and dogs (72), and man (40, 45), with one exception (63), show that adrenocorticotropic hormone can produce a fall in the circulating lymphocytes within a few hours after injection. Such a fall does not occur in adrenalectomized animals or patients with Addison's disease.

Another link in this reaction has been partially elucidated. Adrenal cortical principles of the glucocorticoid type (Compound E (45), Compound F (10) ) in man, as well as larger quantities of cortical extract (21) in rats, are able to decrease the circulating lymphocytes in a similar manner.

Evidence of overproduction or overgrowth of lymphoid tissue when the adrenal gland is not functioning has also been demonstrated. Although Addison in his classical description did not find the lymphoid organs to be involved grossly in any way, observers in recent times have found enlargement of lymphoid tissues in patients dying of Addison's disease (32), and in many animals whose adrenals had beeb removed. The peripheral circulating lymphocytes are increased (12, 18, 32), The theory has been promulgated that the "status thymicolymphaticus" is in reality a form of adrenal insufficiency.

Long-continued stimulation of the adrenal cortex by adrenocorticotropic hormone in mice (98), and in humans with Cushing's disease, or with the hormone (18,28) results in a sustained lower level than normal.

Dougherty and White (21,22,23,25,26,28,97,99), in a series of articles, described experiments which they interpreted as evidence that the lymphocytes were the cells which released antibody and metabolic gammaglobulins to the circulation when needed. They believed that they could deminstrate rising titers of antibody after stimulation of the adrenal cortex or the destruction of lymphoid tissue, in the abscence of the adrenals, by x-ray, and also increased amounts of gamma globulin. They believed also that they had demonstrated that the lymphocyte contained antibodies in a concentrated form. This was a long-awaited finding, since lymphocytes have long been suspected of just such a role. However, contradictory reports have been published (13,35,47,92), and the clarifying and conclusive results which should give us the answer to this problem have still to be published.

The mechanism of depression of the circulating lymphocytes seen with adrenocortical stimulation is a problem in itself. Fundamental research is still necessary. Perhaps it would be worth while to outline the modes by which lymphocytes enter and leave the blood. Figure 5 is based on information from Drinker and Yoffey (32, P.208ff).

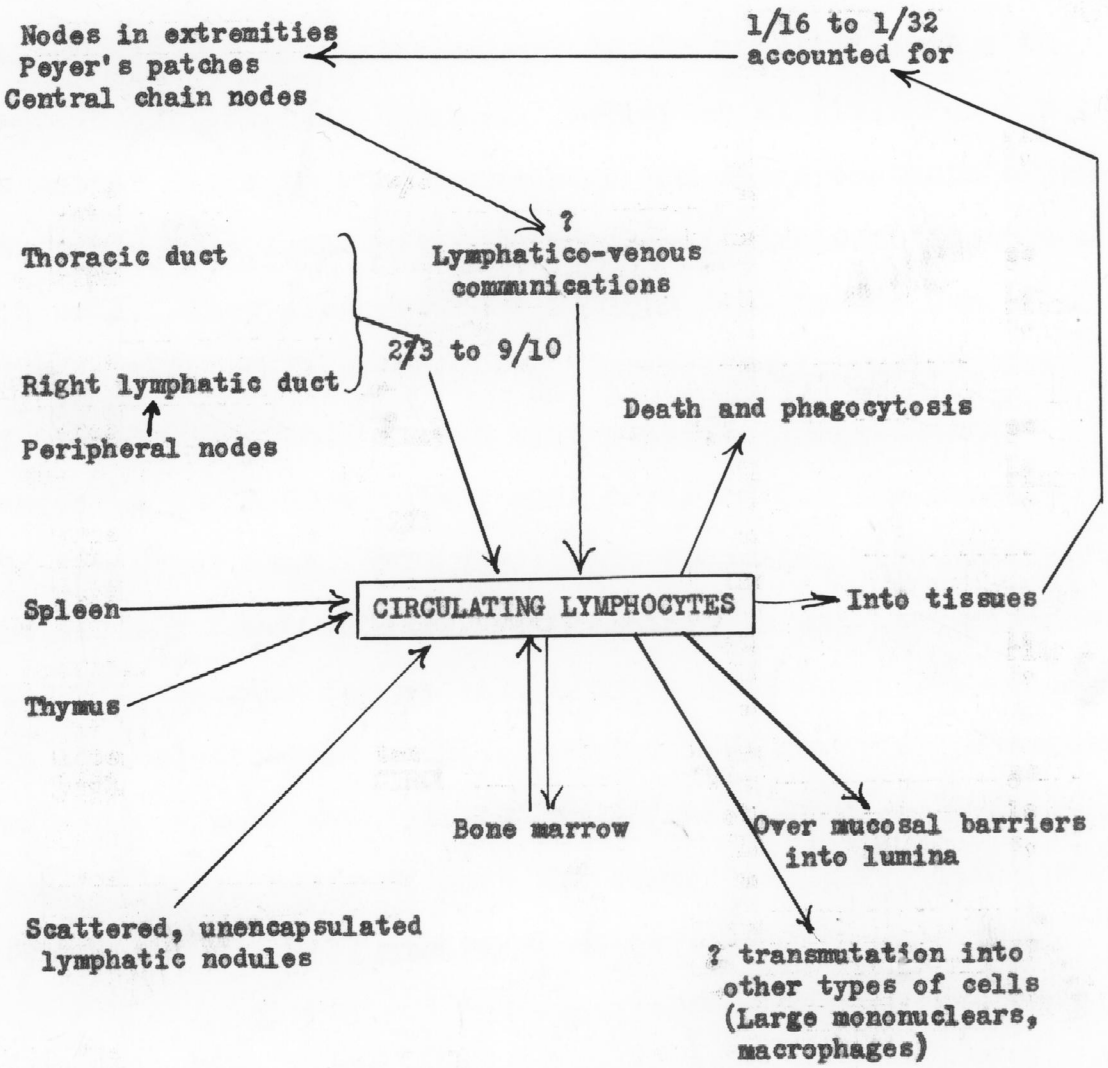

Nodes in extremities
Peyer's patches
Central chain nodes
1/16 to 1/32
accounted for
?
Thoracic duct
Lymphatico-venous
communications
2/3 to 9/10
Right lymphatic duct
Peripheral nodes
Death and phagocytosis
Spleen
CIRCULATING LYMPHOCYTES
Into tissues
Thymus
Bone marrow
Over mucosal barriers
into lumina
Scattered, unencapsulated
lymphatic nodules
? transmutation into
other types of cells
(Large mononuclears,
macrophages)

FIGURE 5

Reinhardt and Li (72.5) discovered 50% reductions in the cell count of the central lymph of the thoracic duct in normal and adrenalectomized female rats injected with adrenocorticotropic hormone. Yoffee et al (101) found substantial falls in the lymphocytes and other contents of the thoracic duct lymph after injection of adrenocorticotropin in cats. They also produced a minor fall by the use of "Eschatin". This preparation, interestingly enough, produced some lymphoid tissue hyperplasia. These changes appeared in 15-30 minutes, and persisted for the duration of the experiment, 4-10 hours. On the other hand, Valentine et al (92) found no significant change in cat thoracic duct lymph, either as far as volume or as cell count was concerned. In adrenalectomized animals treated with cortical preparations showed no significant variations in their thoracic duct lymph. Some believe, however, that the commercially available extracts are not very active gluco-corticoid preparations.

Dougherty and White state from unpublished work (21) that they found an increase in circulating lymphocytes and an enlargement of lymphoid tissues with the use of desoxycorticosterone.

Although it is an attractive theory, the pituitary-adrenal cortical "control" of lymphocytes in the blood and in lymphoid tissue is still not proven. Physiological changes in circulating lymphocytes have been studied by

Elmadjian and Pincus (37) in normal and psychotic subjects, which correlate with their (65,67) studies on urinary steroid. Malmo et al (56) showed that even such minor forms of stress as a painful stimulus, without actual injury, on a person's forehead, could excite the fall in lymphocytes.

Sabin et al (73) made supra-vital differential white counts and total white cell counts on normal persons at small intervals. They found that there were "rythmic fluctuations" in the total white count and in the vitality of cells. However, it was impossible to preserve the specimen long enough to count more than 100 cells by the differential method. Ponder, Saslow, and Schweizer (68) analysed the problem with care, and came to the conclusion that no significance could be laid upon this type of experiment.

In conclusion, we can only say that under experimental conditions, and in some clinical states, the glucocorticoid hormones of the adrenal cortex, under the regulation of the pituitary, cause degeneration of lymphoid tissue, and diminution of the number of circulating lymphocytes in the blood. When the natural secretion of the adrenal cortex is diminished, there is an inconstantly observed rise in lymphocytes and hyperplasia of lymphoid tissue. The control of lymphoid tissue production of lymphocytes and the control of circulating lymphocytes under day-to-day conditions, where homeostasis is not threatened, has not been proved to

be pituitary-adreno-cortical; however, there are indications in that direction.

Effect of Adrenal Cortical Secretion on the Eosinophile. The greater part of the literature on the eosinophiles and their variations in the blood stream concerns itself with relative and absolute increases in their number. Eosinophilia is characteristic of allergic, skin, and parasitic diseases, certain bone marrow disturbances, and many diseases of uncertain etiology, including periarteritis nodosa. However, little emphasis has been placed by present authors upon eosinopenia. It has been considered an unfavorable prognostic sign for the eosinophiles to be absent in an acute infection (11), or an injury or burn (76). Many early authors found a greatly lessened content of eosinophiles in the blood stream in any acute infection, with an increase to resting level and above with recovery. These observations have been made repeatedly with the ordinary differential blood count with determination of the total white cells, however, several good methods exist for counting the eosinophiles directly, thus eliminating one step in determination of the absolute number and permitting a greater accuracy (29, 69,102). The modified Dunger technique is being used most frequently at present.

Although it is not absolutely certain that the blood and tissue eosinophiles are the same, it is possible that certain falls in the lymphocyte count could be due to migration of these cells from the capillaries into the tissues. Histopathology tells us that there are often immense numbers

of these cells collected in a localized area in response to certain types of stimuli. At any rate, in studies concerning eosinophiles, it is important to state whether capillary or venous blood is being drawn.

Domarus (30) used a Dunger method along with the regular complete blood count in certain acute conditions, such as hemorrhage into the gastrointestinal tract and pernicious anemia in relapse. In every case, the eosinophile count was very low when the patient was at his worst, and it rose significantly with the first sign of improvement. A renewed hemorrhage would depress it again. In his cases of pernicious anemia, the eosinophiles began to be restored sooner than any other laboratory examination revealed any change. It paralleled subjective improvement, and overall clinical picture. The retculocyte count rose just a little later, and the red count and heoglobin later still. Under liver therapy the eosinophiles continued to rise above normal; one might explain this either as a non-specific bone-marrow stimulation, or as a sensitivity reaction to the foreign protein. Such experiments might be repeated at the present time, with vitamin $B_{12}$.

All have noted similar variations in the eosinophile count. Zappert (102) found low counts in the acme of the clinical course of such varied diseases as typhoid, malaria, rheumatic fever, measles, "pseudoleukemia", tuberculosis, poliomyelitis, nephrolithiasis, generalized

reactions to larte doses of tuberculin, acute yellow atrophy of the liver, and miliary tuberculosis. Staubli spoke of this as a "sign of great injury" to the organism. Van Duyn (93), spoke of the disappearance of eosinophiles as part of a degenerative white cell picture associated with toxemia from burns. Lowe (55.5) discovered a pronounced fall in eosinophiles 24-36 hours before the onset of malarial symptoms, in relapse or new infection. This was even more pronounced in patients who already had an eosinophilia on the basis of convalescence from malaria or infection with helminths. Randolph, and Randolph and Rawling (70,71) found a call in capillary eosinophiles associated with the onset of clinical symptoms, after test meals of various foods which patients were sensitive to, or test doses of sulfa drugs to which patients had had previous reaction. This sharp fall was followed by a rise above normal which the writers considered a somewhat better index of sensitivity. The eosinopenia did not occur if there was no clinical reaction.

The evidence that this fall in eosinophiles in various clinical states is mediated through the adrenal cortex and the pituitary is of the same type as was called upon in the case of the lymphocyte. Physiological variations during the day were observed by Domarus. He found that the eosinophile count taken in the morning as soon as the patient awoke, was higher than any later counts. The count leveled off soon, however. This is reminiscent of the

findings of Elmadjian and Pincus (37) with the lymphocytes and of Pincus et al (65,67) with urinary steroids. Domarus found no changes with eating, Englebreth and Videbaek, (38) none with the changing seasons.

Changes due to inanition have not been studied with any of the direct eosinophile methods, to my knowledge.

Neusser (62) described a sharp rise in eosinophiles just before and during the first day of the menses.

At the time of writing, workers are undoubtedly using eosinophile counts and other blood studies in combination with studies of the urinary steroids in various clinical states.

Adrenocorticotropin produces a sharp fall in the eosinophiles of normal subjects, and those with no clinical evidence of adrenal impairment, whereas in Addison's disease, the changes were either absent or very small. In the series studied (45) there was no overlapping. Administration of adrenocorticotropin for several days in normals produced continuous depression, with a rise above normal after the hormone was stopped. Compound F was not used in normal individuals but in Addisonians it produced a great lowering, while desoxycorticosterone was inactive in large doses.

Baez-Villasenor et al (10) found no significant difference in the eosinophile vales (by differential count) in 30 patients with Addison's disease without treatment, from a control group. However, in 14% of 63 patients getting replacement therapy there was an eosinophilia (over 5%).

These patients were usually getting desoxycorticosterone or "Eschatin".

Thorn et al (89) have devised a test of adreno-cortical function which depends upon the drop in eosinophiles to establish the functional integrity of the adrenal cortex.

In summary, it may be stated that under conditions of stress and of increased activity of the pituitary-adrenal cortical mechanism, the eosinophile count is depressed, whether it was normal or elevated beforehand. This effect is mediated by way of the gluco-corticoids of the adrenal cortex.

Changes in the Other White Blood Cells With Adrenal Cortical Activity. It seems well established that a polymorphonuclear increase almost always accompanies increased activity of the cortex; adrenalin can cause such a rise, (24,45) and moreover, stressful stimuli and adrenocorticotropin can produce some polymorphonuclear leucocytosis in the absence of the adrenals, although the response is not as great. Infectious processes have less ability to cause leukocytosis in Addisonians (10), and there is ordinarily some neutropenia (in conditions of ablation of the cortex (10,12,104). Van Duyn (93) states that toxemic burned patients have a degenerative white cell picture, with failure to develop a marked polymorhponuclear leukocytosis and shift to the left. It is probable that the adrenal cortex is one of the factors in the leucocytosis of stress, but there are many other factors.

Addison's disease is, as a rule, associated with a refractory normocytic, normochromic anemia. In crisis, of course, hemoconcentration appears, to confuse the picture. Increases in the cell count and hemoglobin of intact animals exposed to stress are probably mediated through the adrenal medulla.

The literature does not render up much material concerning the effect of cortical secretion on the monocytes and basophiles.

Special Tests of Adrenocortical Function.

A. Provocative test, with salt free diet. (Harrop, Weinstein, Soffer, and Tresher) (42).

This is a rigorous procedure for any patient who really has adrenal cortical insufficiency, in which a salt-free diet is used, producing clinical relapse of the Addisonian within several days. One death (Lilienfeld) (54) has been reported as recipitated by this procedure, and it is generally felt to be "too good" a test in the patient who actually has Addison's disease. At the present time, it is reserved as a last resort.

This is a test of mineralo-corticoid function.

B. Urinary excretion of electrolytes (Cutler, Power, and Wilder) (16).

Patients are taken off any extra salt or cortical hormone they may be getting on the day before the test. During the test, there is kept ready a venoclysis of 1000 cc, containing 50 gm. of glucose, 10 gm. of sodium chloride, 5 gm. of sodium citrate, and 20 cc. of an acqueous adrenal cortical extract. This is to be used in the event that adrenal crisis develops.

Diet during the test (a diet list is given in the article) is a low sodium, high potassium (diet, and contains about 0.95 gm. of chlorine, 0.59 gm. of sodium, 4.1 gm. of potassium. On the first afternoon of the test, the patient is given additional potassium in the form of the citrate, in a dosage of 42 mg. per pound of body weight.

On the second day, the fluid intake is 40 cc. per kilogram of body weight, and the dosage of potassium citrate is repeated in the morning. On the third day, the patient takes 20 cc. per kilogram before 12 noon. The urine is collected from 8 to 12 o'clock on the third day.

Chloride is determined in the urine by any standard method.

In Addisonians, the concentration is above 225 mg. per 100 cc., while in normals it is below 125 mg. per 100 cc. The test fails in renal disease.

This is also a test of mineralo-corticoid function.

C. Water excretion test (Kepler, Robinson, and Power) (49).

This is a simpler and safer test than those mentioned above.

The patient is given his regular diet the day before, but without added salt. He does not eat or drink after 6 pm. At 10:30 he voids, and the urine is discarded. His urine from 10:30 to 7:30 forms the night specimen. At 8:30 am. his bladder is again emptied, and specimens are collected at 9:30, 10:30, 11:30, and 12:30. At a convenient time between 11:30 and 12:30, a blood is drawn for uric acod determination if needed.

If the night specimen is less than any of the day specimens, the patient does not have addison's disease. If it is equal to or greater than any one of the day specimens, then the first part of the test is inconclusive and the following formula is solved.

$$A = \frac{\text{urea in urine (mg. \%)}}{\text{urea in plasma (mg. \%)}} \times \frac{\text{Chloride in plasma (mg.\%)}}{\text{Chloride in urine (mg.\%)}} \times \frac{\text{Vol. of day urine (cc.)}}{\text{Vol. of nt. urine (cc.)}}$$

The chemical determinations are run on the night urine, and the plasma, while the volume determinations are of the night urine and the largest of the day specimens. If the value of A is 30 or greater, then Addison's disease has been eliminated as a possible cause.

This is also a test of mineralo-corticoid function.

D. Glucose Tolerance.

Patients with Addison's disease have increased tolerance for glucose, because of deficience of the gluco-corticoid function. There may be a secondary hypoglycemia after the administration of glucose.

E. Urinary 17-ketosteroids.

Addisonians have quite low values for the 17-ketosteroids in the urine. In females, the values are in the neighborhood of 0-1 mg. on 24 hours, while in the male they may be 1-4 mg.

F. Eosinophile and Uric Acid Test (Thorn, Forsham, Prunty, and Hills) (89).

This recently promulgated test makes use of purified hog adrenocorticotropin, and unlike most of the other tests, it evaluates gluco-corticoid function. The eosinophiles are determined by a modified Dunger technique, and are expressed as percent of their level before the test

procedure. Uric acid excretion (88) is determined in relation to a relatively constant urinary constituent, creatinine, and thus the whole urine need not be accurately collected.

The test is carried on as follows: the patient is fasted from 8 pm. of the evening before. Water is given as desired. On the day of the test, blood is drawn at 8 and 12, urine collected from 6-8, 9-12. The pituitary substance is given intramuscularly, 25 mg., just after 8.

Adequate adrenal cortical reserve is present if there is a 50% or greater drop in eosinophiles, or a 50% or greater rise in the ration of uric acid to creatinine.

The uric acid excretion test may fail to give a true picture of adrenal reserve if 1) the cortex is already maximally stimulated, and uric acid is at its height 2) there is a decreased renal clearance, or an excessively high uric acid production, where excretion is also at a maximal level. The eosinophile count may fail to fall at the height of an allergic eosinophilia, because the cells are being poured out as fast as they can be removed from the circulation, and the cortex is already reacting maximally.

This test is positive also when the adrenal cortex is functionally exhausted.

IV. Eosinophile Counts in Two Patients With Psychoneurosis, and One With a Parasitic Disease. The method for counting eosinophiles is Thorn's modification (40) of Dunger's (29) direct method.

Oxalated venous blood is drawn up in a white cell pipette, to the 0.5 mark, and dilution (1:20) is made to the 11 mark with a special diluting fluid containing eosin. The pipette is shaken at once, for 30 seconds only, and then the counting chamber is filled. After waiting for 8 minutes, one may see clearly the eosinophiles, with their deeply stained red granules. The other white cells and the red cells disintegrate.

1. Balanced oxalate:

| | |
|---|---|
| Potassium oxalate | 0.8 |
| Ammonium oxalate | 1.2 |
| Dist. water q.s. ad | 100 |

0.5 cc.of this solution is placed in each of small, clean bottles. These are then placed in an oven, and the water is evaporated, leaving enough oxalate to prevent coagulation of 5 cc. of venous blood.

2. Diluting fluid:

| | |
|---|---|
| Acqueous eosin | 5.0 |
| Acetone | 5.0 |
| Dist. water q.s.ad | 100 * |

The counting chamber is one of greater depth and area than the regulation hemocytometer. It is a Fuchs-Rosenthal chamber, with a depth of 0.2 mm. and a ruled area of 16 square millimeters on each side. Such counting chambers are intended for use in spinal fluid cell counts.

The calculation is as follows:

$$\text{Eosinophiles per cu.mm.} = \frac{1}{16\text{sq.mm.} \times 0.2\text{mm}} \times \frac{20}{1} \times \text{Chamber count}$$

$$\text{or, chamber count} \quad \frac{100}{16}$$

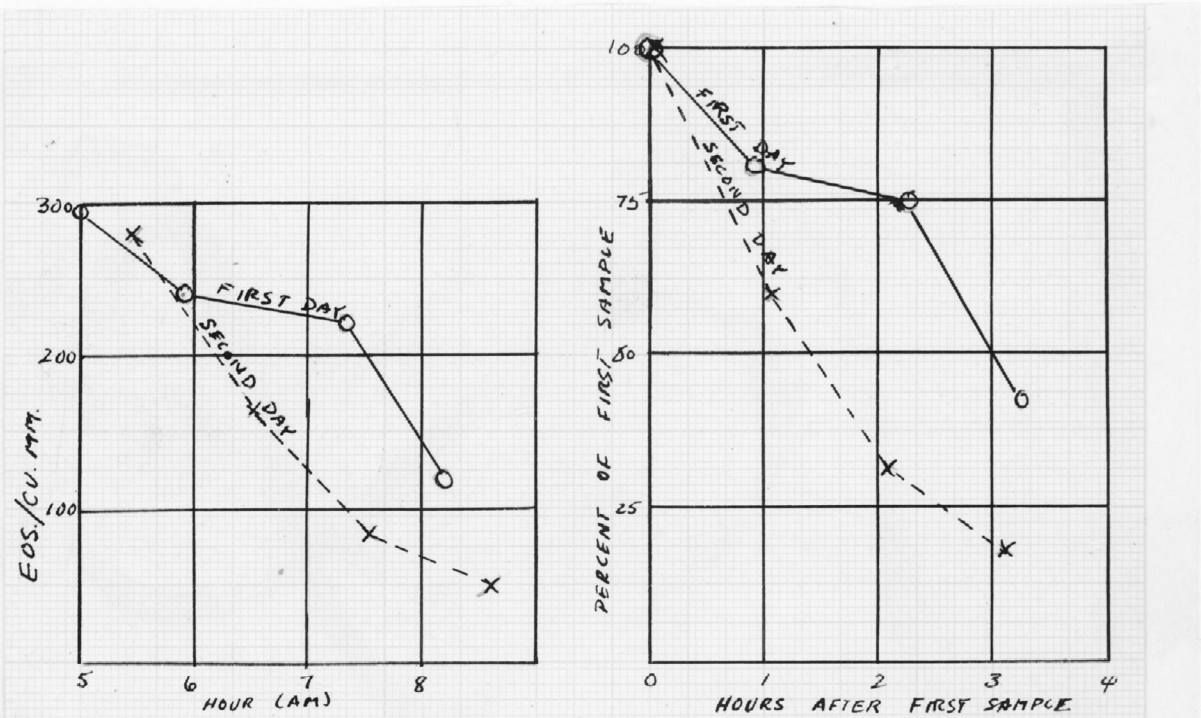
*An error in the figure here is found in the article (40) referred to above. A later publication (89) corrected this error.

M.B., 248962, a female aged 34. Weakness, difficulty in breathing, relieved by sedation. Physical exam revealed obesity, a slow, poorly articulated speech, scissors gait, B.P. 140/100, hyper-reflexia of lower limbs, Pos. Babinski and Chaddock reflexes. Urine, C.B.C., blood sugar, non-protein nitrogen within normal limits. Castric analysis: free acid 48°, total acid 69°. Cholesterol 364, 390 Wass. R. neg. Blood bromide normal. Upper G.I. series neg.
Diagnosis: Anxiety tension state, Little's disease.

| Control day 1-25-49 | Eos/cu.mm.* | Procedure day 1-26-49 | Eos/cu.mm.* |
|---|---|---|---|
| 5:00 am | 296 | 5:28 am | 276.5 |
| | | ** | |
| 5:55 | 239 | 6:30 am | 164.3 |
| 7:10 | 218 | 7:30 am | 89 |
| 8:10 | 120 | 8:30 | 48 |

The patient was awakened from sleep on each of the days. On the procedure day, she was given 0.5 cc. of 1:1000 epinephrine subcutaneously at 5:35 am. (**) There was a pronounced rise in pulse rate and strength, and the patient felt "shaky".

*Average of 4 to 8 determinations, 2 to 4 fillings of pipette.

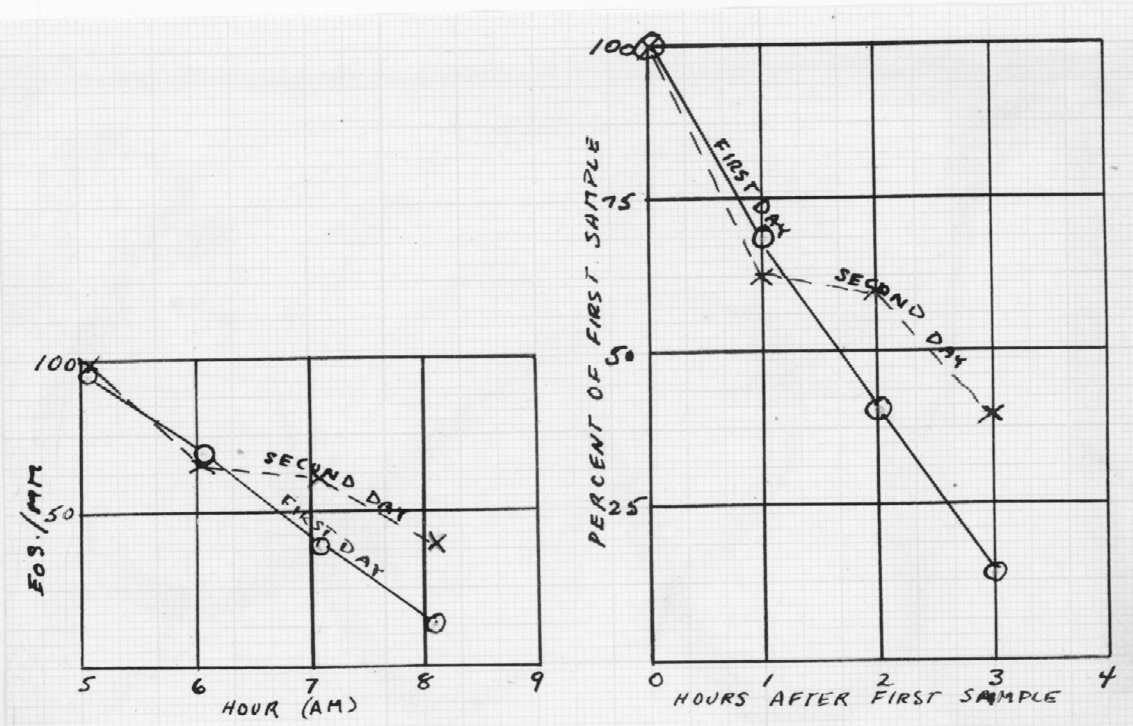


T. P. 262821, a female aged 19. Pain in left side, under surgical incisions, meno-metrorrhagia. Expressionless facies, flat personality, situational factors marked. I.Q. 91. No insight. Neg. physical, pelvic, urine, blood, blood sugar, non-protein nitrogen, cho esterol, sed rate, P.S.P., concentration test, Wass. R., sinus x-rays, barium enema, i.v. urogram, cystoscopy all within normal limits. Diagnosis: psychoneurosis.

| 1-26-49* | Eos./cu.mm. | 1-27-49 | Eos./cu.mm. |
|---|---|---|---|
| 5:04 am | 94 | 5:04 | 99 |
| | | ** | |
| 6:04 | 68 | 6:04 | 65 |
| 7:04 | 38 | 7:04 | 60 |
| 8:04 | 12.5 | 8:04 | 39 |

*1-26-49 was meant to be a control day on this patient. She was awakened from sleep at the time of the first sample. Shortly before the second sample was taken, however, she complained of nausea. Shortly after the last sample had been taken, she had a single episode of vomiting.

**At 5:10, the patient was caused to hyperventilate for 2 minutes, as a non-specific stimulus.



Private patient on whom the diagnosis of trichinosis was later established.

W. B. C. 14,800

| | | Absolute | |
|---|---|---|---|
| Poly's | 27.0 % | 4000 | |
| Non-fil | 1.75 | 259 | |
| Lymphs | 17.0 | 2520 | |
| Eos: non-fil | 5.0 | 740) | |
| 2 lobes | 37.0 | 5470( | 6735 |
| 3 or more | 6.25 | 925) | |
| Bas | 1.25 | 185 | |
| Mono | 3.5 | 518 | |
| Disintegrated | 1.25 | 185 | |

Direct eosinophile count: 7860.

## SUMMARY

The blood picture typical of increased adrenal-cortical function is a fall in lymphocytes, both relative and absolute, a great fall or disappearance of eosinophiles, and a rise in ~~eosinophiles~~ neutrophiles with a shift to the left. The first two of these changes are mediated by way of the sugar hormones of the cortex, while the latter is partly so.

In any patient who fails to show such a change in the presence of obvious clinical evidence of severe stress, one should suspect adrenal cortical insufficiency of some sort.

It is possible to use certain of the blood cell changes in conjunction with simple chemical determination to evaluate cortical function.

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APPROVED:

Edgar S Gordon

Associate Professor of Medicine

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