REVIEW ARTICLE

THE PITUITARY-ADRENAL RELATIONSHIP

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Introduction

The success which has accompanied the recent clinical use of adrenocorticotrophic hormone and cortisone has stimulated an immense amount of research on the physiology of the pituitary-adrenal relationship. Physiologists have devoted much attention to a study of the mechanism by which the adrenocorticotrophic activity of the pituitary gland is controlled, and pharmacologists have concerned themselves with the development of convenient methods for the biological standardisation of adrenocorticotrophic hormone. It is with these two aspects of the study of the adrenocorticotrophic hormone that this review is concerned.

Although some understanding of the function of the adrenal glands began in the latter half of the nineteenth century, it was not until comparatively recently that the influence of the pituitary gland on the adrenal cortex was appreciated. The existence of a pituitary adrenocorticotrophic hormone became evident in 1930 when Smith[1] showed, in rats, that removal of the pituitary gland resulted in atrophy of the adrenal cortex. Smith[1] found that the adrenal cortical atrophy, which normally followed hypophysectomy, could be prevented by daily transplants of anterior pituitary tissue. Evans[2] obtained similar results with aqueous alkaline extracts of anterior pituitary glands, and Collip, Anderson and Thomson[3] demonstrated the same effects with purified protein preparations obtained from pituitary tissue.

The Nature of Adrenocorticotrophic Hormone

In 1943, Li, Evans and Simpson[4] obtained a protein with considerable adrenocorticotrophic activity from the pituitary glands of sheep, and, simultaneously, Sayers, White and Long[5] reported the isolation of a similar preparation from pig pituitaries. Both groups of workers considered that they had prepared pure adrenocorticotrophic hormone. The two preparations exhibited similar chemical, physical and physiological properties, and it was estimated that their molecular weights were about 20,000.

Li *et al.*[4] found that their protein preparation was stable to heat in acid or neutral solution but not in an alkaline medium. They showed that its activity was destroyed by trypsin but not by pepsin, and they found that 36 per cent. of the protein could be digested without the loss of biological activity. Thus, it became evident that the molecule of the active principle was smaller than was originally believed. Li[6] reported that protein adrenocorticotrophic hormone could be broken down by heat and strong acid, and that a peptide, with a molecular weight of 1200 and possessing

considerable adrenocorticotrophic activity, could be isolated from the products of the reaction.

Cortis-Jones, Crooke, Henly, Morris and Morris[7] prepared adrenocorticotrophic hormone concentrates from the pituitary glands of cattle. They found that their preparations could pass through cellophane membranes impermeable to molecules whose molecular weights were greater than 13,000. Morris and Morris[8] reported the isolation of a polypeptide with 10 times the biological activity of the original protein preparations of Li *et al.*[4] and Sayers *et al.*[5]

It appeared, therefore, that the biological activity of the original protein preparations of adrenocorticotrophic hormone resided in a peptide fraction of the molecule. However, Lesh, Fisher, Bunding, Kocsis, Walnszek, White and Hays[9] obtained an adrenocorticotrophic hormone preparation of which the activity was 10 to 15 times as great as that of the Morris and Morris[8] preparation, and of which the molecular weight appeared to be between 2500 and 10,000. Astwood, Raben and Payne[10] showed that active material could be separated from the protein hormone by adsorption on oxy-cellulose. The fact that highly active preparations could be obtained from protein adrenocorticotrophic hormone by processes which did not involve hydrolysis has also been shown by Dixon, Moore, Stack-Dunne and Young.[11] Recent workers have generally based the assessment of the biological activity of their preparations on the ability of adrenocorticotrophic hormone to cause adrenal ascorbic acid depletion in hypophysectomised rats. Stack-Dunne and Young[12] reported the presence of two factors in adrenocorticotrophic hormone. One factor increased the weight of the adrenal glands of hypophysectomised rats and the other caused adrenal ascorbic acid depletion.

Stack-Dunne and Young[12] considered that the ascorbic acid factor was probably a basic peptide which was associated in the pituitary gland with a slightly acidic protein.

The Physiological Effects of Adrenocorticotrophic Hormone

The physiological actions depend upon its ability to stimulate the adrenal cortices to increase their output of steroid hormones. It has very little effect on adrenalectomised animals and almost all its physiological actions are mediated by the adrenal cortex.

Adrenocorticotrophic hormone produces changes in the adrenal cortex which are associated with an increased secretion of adrenocortical hormones. The elevated blood level of adrenocortical steroids is responsible for the production of effects which are widespread throughout the body and which include actions on protein, fat, carbohydrate and salt metabolism, growth, lymphoid tissue and many others. In this review it is proposed to consider mainly the *direct* effects of adrenocorticotrophic hormone on the adrenal cortex.

The adrenocortical and metabolic changes which follow the subjection of animals to various types of stress stimuli have occupied the attention of physiologists for many years. Only comparatively recently has it become

possible to explain some of the observations and to correlate the experimental findings with a knowlege of the pituitary-adrenal relationship.

The subjection of a normal animal to various types of harmful stimuli (e.g. cold, heat, hæmorrhage, trauma, injections of toxins or drugs) results in adrenal hypertrophy,[13] and depletion of the sudanophilic lipides,[14] cholesterol[15] and ascorbic acid[16] in the adrenal cortex. These adrenocortical changes in response to stress are not produced in hypophysectomised animals.[17] However, the injection of adrenocorticotrophic hormone causes the changes in both normal and hypophysectomised animals.[4,14,16,18,19] Sayers and Sayers[16] claimed that the injection of adrenocorticotrophic hormone into hypophysectomised rats produced changes in the adrenal glands identical with those which occur in the adrenals of normal animals subjected to stress. The same authors showed, in normal animals, that the increase in adrenal weight and depletion in adrenal cholesterol and ascorbic acid concentration caused by stress was proportional to the severity of the stress. Sayers, Sayers and Woodbury[20] found, in hypophysectomised animals, that the fall in adrenal ascorbic acid concentration produced by adrenocorticotrophic hormone was proportional to the amount of hormone injected. From these and other similar investigations it was concluded that stress results in increased pituitary adrenocorticotrophic activity, and that the increased secretion of adrenocorticotrophic hormone is responsible for the changes in the morphology, histology and chemistry of the adrenal glands. The changes in adrenal cholesterol and ascorbic acid concentrations are probably concerned with the synthesis and release of adrenocortical hormones.

Sayers[17] attempted to draw up a classification of adrenocortical responses to stress into various types. In general, all kinds of stress cause adrenal hypertrophy except when the stress stimulus is maintained for only a short period. Any stress except a very mild form (e.g. pregnancy or fasting) causes depletion of sudanophilic lipides, cholesterol and ascorbic acid in the adrenal glands. The sudanophilic lipide, cholesterol and ascorbic acid levels return to normal in time, except in prolonged intense forms of stress ending in death.

The important part played by the pituitary gland in the response of the organism to stress was indicated by the work of Tyslowitz and Astwood[21] and many others who showed that hypophysectomised animals, like adrenalectomised animals, were extremely sensitive to various types of harmful stimuli. Tyslowitz and Astwood[21] demonstrated that adrenocorticotrophic hormone and adrenocortical extracts increased the resistance of hypophysectomised rats to cold.

It has been firmly established that the adrenocortical response to stress is governed by the adrenocorticotrophic activity of the pituitary gland. However, the adrenal cortex cannot be entirely dependent upon the pituitary gland for its functional activity. Adrenalectomised animals die whereas hypophysectomised animals can survive. Although hypophysectomy results in adrenal atrophy, the cortices are apparently still capable of secreting a quantity of steroids sufficient to maintain life.

A considerable amount of evidence is beginning to accumulate that adrenocorticotrophic hormone causes the adrenal cortex to secrete mainly 17-hydroxycorticosterone (compound F) with smaller quantities of corticosterone and 11-dehydro-17-hydroxycorticosterone (cortisone or compound E). Mason[22] isolated 17-hydroxycorticosterone from the urine of human subjects who had received injections of adrenocorticotrophic hormone or who had been subjected to the stress of surgery. Sprague, Hayles, Power, Mason and Bennett[23] prepared 17-hydroxycorticosterone from the urine of a patient with Cushing's syndrome. Cope, Boyson and McCrae[24] found, in normal human subjects, that increased adrenocorticotrophic activity caused increased excretion of cortisone and 17-hydroxycorticosterone. Stress caused no increased excretion of these steroids in a patient with panhypopituitarism. Zaffaroni, Burton and Keutmann[25] detected cortisone and 17-hydroxycorticosterone in small quantities in normal human urine and Schneider[26] isolated cortisone from the same source.

Methods are gradually being developed for the detection of adrenocortical steroids in adrenal effluent blood—a technique which was first developed by Vogt.[27] Nelson, Reich and Samuels[28] found that the principal steroid in the blood leaving the adrenal glands of dogs, which had received injections of adrenocorticotrophic hormone, was 17-hydroxycorticosterone. Bush[29,30] analysed adrenal effluents in various animal species by a paper partition chromatographic method. He found that stress or adrenocorticotrophic hormone resulted in a tremendous increase in the secretion of corticosterone and 17-hydroxycorticosterone. The ratio of these steriods was characteristic of the species studied and not changed by adrenocorticotrophic hormone.

The Assessment of Pituitary Adrenocorticotrophic Activity

Until comparatively recently, attempts to measure the concentration of adrenocorticotrophic hormone in blood have met with little success. Sayers, Burns, Tyler, Jager, Schwartz, Smith, Samuels and Davenport[31] showed that, following injection into human subjects, it disappeared very rapidly and very little was excreted. Greenspan, Li and Evans[32] demonstrated that, when injected intravenously into rats, it disappeared completely from the blood stream in 20 minutes. Taylor, Albert and Sprague[33] were unable to detect adrenocorticotrophic hormone in the blood of normal human subjects. Reiss, Badrick, Halkerston and Plaice[34] reported its extreme lability in blood *in vitro*, and Pincus[35] considered that it was destroyed rapidly by an enzyme present in blood. However, it should be mentioned that, recently, Geschwind and Li[36] found that it was *not* inactivated by rat plasma although it was rapidly destroyed by incubation with tissue slices and homogenates. On the other hand it was found to be unstable in rats' whole blood in this laboratory. Its instability in blood probably explains the failure of several workers to demonstrate its presence in normal serum.

Methods are gradually being developed for the direct detection of increased blood levels of adrenocorticotrophic hormone in various

experimental and clinical conditions. Direct estimations of the concentration in blood were first made by Golla and Reiss,[37] who found that extracts of pregnant mares' serum caused increases in the weight of the adrenal glands of hypophysectomised rats. Cooke, Graetzer and Reiss[38] demonstrated adrenocorticotrophic hormone activity in acetone precipitates of human plasma and Taylor *et al.*[33] detected increased levels in unfractionated serum from patients with Addison's disease. Bornstein and Trewhella[39] and Bornstein, Gray and Parrott[40] measured adrenocorticotrophic hormone activity in human plasma. Parrott[41] found elevated concentrations in human blood in Cushing's syndrome and pregnancy, and considerably decreased levels in the blood of patients suffering from Simmonds' disease.

At the present time, direct methods for the determination of the concentration in blood are not often used. More frequently indirect methods are employed as indices of the rate of secretion. The most commonly used methods depend upon (1) the effects which it produces on the weight and histology of the adrenal cortex and on the cholesterol and ascorbic acid content of the gland, (2) the fall in blood eosinophil and lymphocyte counts caused by increased secretion of adrenocortical hormones and (3) the increased urinary excretion of "corticoids" (adrenocortical steroids estimated biologically or adrenocortical-like steroids estimated chemically) caused by adrenocorticotrophic hormone.

The influence of adrenocorticotrophic hormone on the weight, histology and chemical constituents of the adrenal glands has already been considered. Fluctuations in adrenal ascorbic acid concentration are used most widely as indices of adrenocorticotrophic activity. However, there is a considerable amount of conflicting evidence concerning the part played by ascorbic acid in the synthesis of the hormones of the adrenal cortex. Sayers[17] maintains that changes in adrenal ascorbic acid concentration are reliable indices of adrenocortical, and hence of adrenocorticotrophic, activity in acute experiments in healthy rats.

Dougherty and White[42] showed that the circulating lymphocytes were under the regulatory control of the adrenal cortex and that the lymphocytopenic response to a variety of stress stimuli was due to increased putuitary adrenocorticotrophic activity resulting in an increased secretion of adrenocortical hormones. They demonstrated that neither stress nor adrenocorticotrophic hormone caused lymphocytopenia in adrenalectomised animals.

The number of circulating eosinophils is also dependent upon the activity of the pituitary-adrenocortical system. This fact has been made the basis of a convenient method for assessing adrenocortical and pituitary adrenocorticotrophic activity. Forsham, Thorn, Prunty and Hills[43] showed that the administration of adrenocorticotrophic hormone to human subjects caused a prompt fall in the number of circulating eosinophils. The fall in the eosinophil count was twice as great as the simultaneous fall in the lymphocyte count. They considered that the eosinopenic response to adrenocorticotrophic hormone was less variable than the lymphocytopenic response. Stress has been found by many

workers to cause eosinopenia, and the literature concerning this effect of stress was reviewed by Hills, Forsham and Finch.[44]

The assessment of adrenocorticotrophic and adrenocortical activity by means of eosinophil and lymphocyte tests has been of great clinical advantage. In man the administration of adrenocorticotrophic hormone or the application of stress (e.g. surgery) is normally followed by a decrease in the lymphocyte and eosinophil counts. The absence of lymphocytopenic and eosinopenic responses is generally indicative of a failure of some part of the pituitary-adrenocortical system. In addition to their clinical applications, tests for pituitary adrenocorticotrophic activity based on eosinopenic and lymphocytopenic responses are frequently used in experimental work on laboratory animals. McDermott, Fry, Brobeck and Long[45] found that the results they obtained in tests using eosinopenic responses as indices of pituitary adrenocorticotrophic activity were in agreement with those obtained using changes in adrenal ascorbic acid concentration. These workers used eosinophil tests in their investigations on the part played by adrenaline in the production of pituitary adrenocorticotrophic activity in rats. Colfer, de Groot and Harris,[46] investigating the mechanism by which the secretion of adrenocorticotrophic hormone is controlled, used lymphocyte tests in their work on rabbits.

The presence of adrenocortical hormones ("corticosteroids") in human urine can be demonstrated by biological or chemical methods. The administration of adrenocorticotrophic hormone or the application of stress stimuli causes an increased secretion of adrenocortical hormones with the result that larger quantities appear in the urine. Thus the urinary output of corticoids is a measure of adrenocortical activity and hence of pituitary adrenocorticotrophic activity. Sayers[17] reviewed the various methods for the biological and chemical determination of urinary corticosteroids and illustrated the general correlation between adrenocorticotrophic activity and urinary corticosteroid excretion. He considered that the amount of corticosteroids, estimated biologically, in the urine gave a good index of adrenocorticotrophic activity. Chemical corticosteroid estimations provided a less satisfactory measure. Recently Cope *et al.*[24] found that there existed no parallelism between the urinary output of corticoids, estimated biologically, and corticoids, estimated chemically, in human subjects receiving adrenocorticotrophic hormone or ephedrine.

Increased pituitary adrenocorticotrophic activity also results in an increased urinary excretion of 17-ketosteroids. Sayers[17] listed his reasons for considering that 17-ketosteroid excretion was a very unreliable index of adrenocortical and adrenocorticotrophic activity. He stated that there existed no correlation between 17-ketosteroid excretion and adrenocorticotrophic activity as illustrated by other measures.

The Control of Pituitary Adrenocorticotrophic Activity

It has been firmly established that the secretion of the adrenal cortex in stress is almost entirely governed by the adrenocorticotrophic hormone of the anterior pituitary gland. However, the exact mechanism by which the secretion of adrenocorticotrophic hormone is produced is not yet fully

understood. At the present time it is believed that the adrenocorticotrophic activity of the pituitary gland may be controlled in at least three ways (*a*) by the level of cortical hormones in the blood, (*b*) by the level of adrenaline and noradrenaline in the blood, and (*c*) by some neural or neuro-humoral mechanism.

(*a*) *Control of Pituitary Adrenocorticotrophic Activity by the Level of Cortical Hormones in the Blood.* The results of experiments performed in many laboratories indicate that the adrenocorticotrophic activity of the pituitary gland is controlled by the blood level of adrenocortical hormones. Ingle, Higgins and Kendall[47] and Ingle and Kendall[48] found that atrophy of the adrenal cortex was one of the results of the chronic administration of adrenocortical extracts to rats. Selye[49] showed that desoxycorticosterone acetate caused adrenal cortical atrophy in the rat and the mouse. Ingle[50] demonstrated that the adrenal hypertrophy, which occurred in rats after a 12-hour period of muscular exercise, could be prevented by pre-treating the animals with adrenocortical extracts. Selye and Dosne[51] found that the previous administration of desoxycortone acetate to rats partially prevented the increase in adrenal weight caused by stress. Winter, Silber and Stoerk[52] found, in rats, that the administration of large doses of cortisone caused adrenal atrophy. The histological changes in the atrophied adrenals involved the zona fasciculata and zona reticularis but not the zona glomerulosa. Similar findings have been reported by Stebbins.[53] Since Deane and Greep[54] showed that hypophysectomy resulted in adrenal atrophy, which was confined to the zona fasciculata and zona reticularis, it was probable that this effect of cortisone was due to the inhibition of pituitary adrenocorticotrophic activity. Sayers and Sayers,[55,56] Long[57] and Gershberg, Fry, Brobeck and Long[58] demonstrated, in rats, that the fall in adrenal ascorbic acid concentration, which followed the application of various types of stress stimuli, could be prevented by pre-treatment of the animals with adrenocortical extracts.

Sayers and Sayers[56] put forward the theory that the adrenocorticotrophic activity of the pituitary gland was controlled by the concentration of adrenocortical hormones in the blood. They considered that the anterior pituitary gland elaborated adrenocorticotrophic hormone at a rate inversely proportional to the blood level of adrenocortical hormones. They suggested that stress caused increased adrenocortical activity by increasing the requirement of the organism for cortical hormones. This increased demand for cortical hormones was satisfied by pre-treatment with adrenocortical extracts and in this way the necessity for the adrenal cortices to increase their activity was obviated. Sayers and Sayers[56] found that the mobilisation of endogenous adrenocorticotrophic hormone in response to stress could be completely or partially blocked depending upon the dose of cortical hormones administered. If the intensity of the stress applied was increased, the amount of cortical hormones required to inhibit the adrenocorticotrophic activity of the pituitary gland was increased. Similar findings have been made in this laboratory. Sayers and Sayers[56] attempted to explain the quantitative relationship by assuming that the rate of secretion of adrenocorticotrophic hormone varied according to the

requirement of the organism for cortical hormones. They suggested that the pituitary-adrenocortical system attempted to maintain the tissues in a state of well-being in regard to adrenocortical hormones, to which condition they gave the name "eucortism." They considered that, under optimal environmental conditions, the peripheral tissue cells required small amounts of cortical hormones, and the level in the blood was sufficient to depress the adrenocorticotrophic activity of the pituitary gland. In conditions of stress the demand of the peripheral cells for cortical hormones was increased, with the result that the concentration of cortical hormones in the blood was reduced temporarily. The pituitary gland responded by increasing its output of adrenocorticotrophic hormone which resulted in increased activity of the adrenal cortex. In this way the increased demand of the peripheral cells for cortical hormones might be satisfied. The pituitary-adrenocortical system remained active until the stress was removed or until adaptation occurred when the requirement of the peripheral cells for cortical hormones was diminished.

There exists, therefore, a considerable amount of evidence that the adrenocorticotrophic activity of the pituitary gland is controlled by a "peripheral-humoral" mechanism. It should be emphasised, however, that several workers have been unable to demonstrate the pituitary inhibitory effect of adrenocortical steroids. Moya and Selye,[59] Hall, Finerty, Hall and Hess[60] and Gershberg *et al.*[58] have all been unable to show that desoxycortone acetate could prevent the release of adrenocorticotrophic hormone in response to stress. Fortier, Yrarrazaval and Selye[61] were unable to demonstrate the pituitary inhibitory effect of cortisone. In this laboratory it was confirmed that both desoxycortone acetate and cortisone acetate were effective in preventing the stress-induced secretion of adrenocorticotrophic hormone.

Although it was a relatively simple matter to demonstrate, in laboratory animals, that an increased concentration of cortical hormones in the blood prevented the increased pituitary adrenocorticotrophic activity which normally followed stress, very little experimental evidence has been produced to support the theory that low blood levels of adrenocortical hormones cause increased pituitary adrenocorticotrophic activity. However, Taylor *et al.*[33] demonstrated the presence of adrenocorticotrophic hormone in the blood of patients with Addison's disease, but were unable to detect the hormone in the blood of normal subjects. Gemzell, Van Dyke, Tobias and Evans[62] showed that removal of the adrenal glands from rats caused a considerable increase in the concentration of adrenocorticotrophic hormone in the blood. These findings added some support to the "peripheral-humoral" theory for the control of pituitary adrenocorticotrophic activity.

There is little doubt that the adrenocorticotrophic activity of the pituitary gland is controlled by a peripheral-humoral mechanism. However, there is still some confusion in the literature concerning the ability of desoxycortone acetate and cortisone acetate to depress the increased secretion of adrenocorticotrophic hormone normally caused by stress.

(*b*) *Control of Pituitary Adrenocorticotrophic Activity by the Level of Adrenaline and Noradrenaline in the Blood.* There exists a considerable amount of evidence that the sympathetic nervous system plays some part in the activation of the pituitary-adrenocortical system. Vogt[63] found that adrenaline stimulates the secretion of the adrenal cortex. She demonstrated the presence of increased amounts of adrenocortical hormones in the blood leaving the adrenal glands of cats and dogs after intravenous infusions of adrenaline. She also found that adrenaline produced these effects in a decapitated dog and she suggested that adrenaline might act directly on the adrenal cortex. Further evidence for a direct action of adrenaline was provided by Hungerford,[64] who showed that adrenaline caused lymphocytopenia in hypophysectomised rats, and by Spiers and Meyer,[65] who found that it produced eosinopenia in hypophysectomised mice.

On the other hand Vogt[66] found that the histological changes and weight increases in the adrenal glands, caused by the chronic administration of adrenaline to rats, were prevented by removal of the pituitary gland. Long and Fry[67] showed that depletion in adrenal cholesterol and ascorbic acid concentration followed the injection of adrenaline into normal rats. These changes did not occur after hypophysectomy.

It is probable, therefore, that the effects of adrenaline on the adrenal cortex are mediated by the pituitary gland. However, recently Pickford and Vogt,[68] working on hypophysectomised dogs, have produced more evidence that adrenaline can exert a direct effect on the adrenal cortex.

Long[57] considered that the release of adrenaline by the sympatho-adrenal system, in conditions of stress, was the most important factor in the production of pituitary adrenocorticotrophic activity. He suggested that all forms of stress caused an increased concentration of adrenaline in the blood which stimulated the pituitary gland to secrete adrenocorticotrophic hormone. Sayers and Sayers[16] considered that the action of adrenaline was an indirect effect on the pituitary gland and was due to its ability to increase the rate of utilisation of cortical hormones, thus producing a decreased concentration of the hormones in the blood.

Gershberg *et al.*[58] showed that small doses of adrenaline, which they considered to be within the physiological range, caused significant falls in the concentration of ascorbic acid in the adrenal glands of rats. They claimed that similar effects caused by cold, trauma and hæmorrhage were due to the release of endogenous adrenaline. These workers confirmed that the effects could be prevented by cortical hormones and were abolished by hypophysectomy. Gellhorn and Frank[69] found that stress caused lymphocytopenia in normal, but not in adrenal-demedullated, rats. McDermott *et al.*[45] compared adrenocortical activity in normal rats with that of those unable to secrete adrenaline as a result of demedullation of the adrenal glands, transection of the spinal cord or electrolytic diencephalic lesions. They found that the eosinopenic responses in these animals when subjected to stress were smaller than in normal animals. Adrenal-demedullated rats and animals, in which the neural pathway responsible for the secretion of adrenaline had been interrupted, no longer responded to mild stress stimuli but showed a response to more severe stress. These

workers showed that, in normal animals, a marked response was obtained within 1 hour of the application of the stress, and that a maximal effect was obtained within 4 hours. In animals in which adrenaline secretion had been prevented, they found that stress caused no response within 1 hour, but some effect within 4 hours of its application. For this reason they suggested that the secretion of adrenocorticotrophic hormone was controlled by a double mechanism: (1) a rapid autonomic mechanism depending upon the secretion of adrenaline, and (2) a slower metabolic mechanism independent of the secretion of adrenaline.

In another publication[70] the same authors reported that both adrenaline and hypertonic saline solution, when injected into hypophysectomised rats with pituitary transplants in the anterior chamber of the eye, caused marked falls in the eosinophil count. An eosinopenic response was produced when very small doses of adrenaline were instilled into the eyes with the pituitary transpants, but not when injected into the other eyes. They concluded that adrenaline caused increased adrenocorticotrophic activity by direct stimulation of the pituitary gland.

The work which has been described so far indicates that endogenously secreted adrenaline has some function in stimulating an increased secretion of adrenocorticotrophic hormone when an animal is subjected to stress stimuli. However, there exists a considerable amount of evidence that adrenaline does not play such an important part in the production of increased pituitary adrenocorticotrophic activity in response to stress as Long and his colleagues believe. Sayers[17] pointed out that the completely sympathectomised animal may resist stress as well as a normal animal. Colfer *et al.*[46] found that the lymphocytopenic response which followed immobilisation or mild electric shock stimuli, in rabbits, was the same whether the adrenal glands were denervated or not. Tepperman and Bogardus[71] demonstrated that neither dibenamine nor tetraethylammonium bromide prevented the fall in adrenal ascorbic acid in rats subjected to stress. Recant, Hume, Forsham and Thorn[72] found that formaldehyde injections caused eosinopenia in completely sympathectomised dogs. Vogt[73] showed that the depletion of lipides in the adrenals of rats or cats subjected to various types of stress was not prevented by denervation of the glands. Gordon,[74,75] Nasmyth,[76] Fortier[77] and Vogt[78] have all shown that the adrenocorticotrophic response to stress is not prevented by adrenal demedullation. Experiments carried out in this laboratory have produced similar results.

It seems probable that the secretion of the adrenal medulla is not of fundamental importance in the production of pituitary adrenocorticotrophic activity, and that other mechanisms are capable of stimulating the adrenocorticotrophic response in animals in which the secretion of adrenaline is prevented.

(*c*) *Control of Pituitary Adrenocorticotrophic Activity by a Neural or Neuro-Humoral Mechanism.* The adrenocorticotrophic hormone is liberated rapidly from the pituitary glands of animals subjected to stress. It was shown, in this laboratory, that changes in the ascorbic acid concentration in the adrenal glands of rats could be detected within a few

minutes of the application of stress. The rapidity of the pituitary adrenocorticotrophic response suggested to many workers that a neural or neurohumoral mechanism might be involved in the process controlling the secretion of adrenocorticotrophic hormone.

Colfer *et al.*[46] showed that emotional stress stimuli caused lymphocytopenia in rabbits. The response was abolished by hypophysectomy but still occurred in hypophysectomised rabbits which received injections of adrenocorticotrophic hormone. De Groot and Harris[79] showed that lymphocytopenia could be produced in rabbits by electrical stimulation of parts of the tuber cinereum or mamillary body. Stimulation of other regions of the hypothalamus or pituitary gland had no effect on the lymphocyte count. They found that, in rabbits with electrolytic lesions in the hypothalamus and pituitary gland, the lymphocytopenic response to stress was abolished in animals in which the zona tuberalis, the posterior part of the tuber cinereum or the mamillary body were destroyed or damaged. They concluded that, in rabbits, the secretion of adrenocorticotrophic hormone in response to some stress stimuli is regulated by the hypothalamus. A similar conclusion was reached by Hume[80] who showed, in dogs, that the eosinopenic response of the pituitary-adrenal system to stress was abolished by certain lesions of the hypothalamus.

On the other hand Uotila,[81] Recant *et al.*[72] Tang and Patton,[82] Cheng, Sayers, Goodman and Swinyard,[83] and Fortier and Selye[84] have performed experiments, on animals with transected pituitary stalks and hypophysectomised animals with pituitary transplants, the results of which indicated that neural or vascular connections between the pituitary gland and the hypothalamus were not essential for the immediate secretion of adrenocorticotrophic hormone in acute stress.

Fortier[85] recently demonstrated that the adrenocorticotrophic response to emotional stress (e.g. sound, immobilisation) was dependent upon the existence of neural and vascular connections between the pituitary gland and the hypothalamus. However, he found that the response to systemic stress stimuli (e.g. trauma, drugs) was independent of the existence of an intact pituitary stalk.

No definite conclusion can yet be reached with regard to the exact mechanism which controls the output of adrenocorticotrophic hormone by the pituitary gland. It is probable that the mechanism controlling a steady secretion of adrenocorticotrophic hormone in optimal environmental conditions may differ from the mechanism governing increased adrenocorticotrophic activity in conditions of stress, Again, the manner in which increased blood levels of adrenocorticotrophic hormone are reached is probably influenced by the nature of the stress.

The Biological Standadrisation of Adrenocorticotrophic Hormone

The discovery of the existence of this hormone naturally led to attempts to devise methods for the determination of adrenocorticotrophic activity in anterior pituitary extracts. Almost all the methods developed so far for the biological standardisation of this hormone have been inaccurate and

difficult to perform and no really satisfactory technique has yet been described. Most of the bioassay methods employed have depended upon the changes which it produces in the adrenal glands of normal and hypophysectomised animals.

The earliest methods of Moon[86,87] and Bates, Riddle and Miller[88] depended upon the increases in adrenal weight which a series of injections caused in rats and chicks. The results of such methods, obtained using normal instead of hypophysectomised animals, were practically valueless since the subjection of intact animals to any environmental change causes increased pituitary adrenocorticotrophic activity with subsequent adrenocortical changes. In hypophysectomised animals the mobilisation of endogenous adrenocorticotrophic hormone is prevented and the effects produced are due entirely to the activity of the samples under test. Bioassay methods using hypophysectomised rats are more sensitive and more accurate than methods employing normal animals. Most of the methods using hypophysectomised animals are performed on rats.

Methods based upon the changes produced in the weight and histology of the adrenal glands of hypophysectomised animals fall into two main groups: (1) adrenal "repair" tests,[3,5,19,89] (2) adrenal "maintenance" tests.[5,19,89,90] In the repair tests, rats were hypophysectomised and their adrenal glands were allowed to regress. Several days later the animals were given frequent injections of adrenocorticotrophic hormone. The dose necessary to restore the weight and histology of the adrenals to normal was determined. In the maintenance tests hypophysectomised rats were given frequent injections commencing immediately after hypophysectomy. The dose necessary to maintain the weight of the adrenal glands at the normal level was determined. Repair methods generally utilised histological changes in the adrenal cortex and maintenance tests were usually based upon changes in adrenal weight.

In 1948, interest in adrenal repair and maintenance methods waned as a result of the development of the adrenal ascorbic acid depletion technique by Sayers *et al.*[20] These workers showed, in hypophysectomised rats, that the adrenal ascorbic acid depletion produced by the intravenous injection of adrenocorticotrophic hormone was proportional to the dose administered. They developed this finding into an extremely sensitive and accurate method of bioassay. Hypophysectomised rats were used 24 hours after the removal of their pituitary glands. The left adrenal glands of the animals were excised and injections of the hormone were administered. Later the animals were killed and their right adrenal glands were removed. The ascorbic acid concentrations in the adrenals were determined. The response was expressed as the difference between the concentration of ascorbic acid in the left adrenal, removed immediately before the injection, and the concentration of the acid in the right adrenal, removed 1 hour after the intravenous administration of the hormone. Sayers *et al.*[20] found that a rectilinear relationship existed between the adrenal ascorbic acid depletion and the logarithm of the dose. The authors claimed that the adrenal ascorbic acid depletion method possessed several advantages over methods employing changes in the weight and histology of the adrenal

glands. It was rapid, more accurate and possessed a greater degree of sensitivity. They suggested that the method was specific for adrenocorticotrophic hormone since they found that extracts of animal tissues, except pituitary glands, caused no adrenal ascorbic acid depletion.

Munson, Barry and Koch[91] modified the Sayers technique. They carried out no unilateral adrenalectomy before the injection but compared the mean total adrenal ascorbic acid concentration in an injected group with the mean of a control group. Sayers *et al.*[20] reported that, using this modification, it was necessary to use twice the number of animals to obtain the same accuracy as their method provided.

Morris,[92] using certain modifications of the adrenal ascorbic acid depletion technique for the biological standardisation, obtained results as accurate as those of Sayers *et al.*[20] Similar results have been obtained by Clayton and Prunty.[93] On the other hand Reiss *et al.*[89] found that, in their laboratory, the method did not always give satisfactory results. Greenspan, Li, Simpson and Evans[94] also found that the adrenal ascorbic acid depletion method provided them with results the accuracy of which was not as great as that achieved by Sayers *et al.*,[20] although they were able to confirm the existence of a linear relationship between the logarithm of the dose of adrenocorticotrophic hormone injected and the adrenal ascorbic acid depletion.

Their disappointment with the accuracy they obtained using the adrenal ascorbic acid depletion technique stimulated Reiss *et al.*[89] to investigate other methods of biological standardisation. They found that the adrenal glands showed an increased uptake of phosphorus-32 in conditions of increased activity. They demonstrated, in hypophysectomised rats, that if adrenocorticotrophic hormone and phosphorous-32 were injected at the same time there existed a dose-response relationship between the amount of hormone injected and the percentage of phosphorus-32 taken up by the adrenals.

Rheinhardt and Li[95] found that the values obtained in the assessment of adrenocorticotrophic hormone activity depended upon the method of bioassay employed. They showed that the results produced using adrenal maintenance tests differed widely from those obtained employing the adrenal ascorbic acid depletion technique. Their findings indicated that more than one factor existed in some preparations of the hormone. Stack-Dunne and Young[12] have actually separated two factors. One is the "adrenal weight factor" which, they suggested, is concerned with the synthesis and deposition of adrenal cholesterol, and the other is the "ascorbic acid factor" which is probably associated with the release of cortical hormones. The existence of at least two factors in adrenocorticotrophic hormone makes it advisable to utilise at least two bioassay methods in assessing the adrenocorticotrophic activity of commercial preparations of the hormone. An adrenal repair or maintenance method should be used in addition to the ascorbic acid depletion method. However, it should be mentioned that the clinical effectiveness of the hormone is apparently most closely related to its potency in terms of ascorbic acid factor.

The methods of biological standardisation, which have been considered so far, all depend upon the direct effects which the hormone produces on the adrenal cortex. Other methods have been developed which are based upon the effects of the increased secretion of adrenocortical hormones which adrenocorticotrophic hormone causes. Clayton and Prunty[93] described a method which depends upon the antagonistic effect of adrenocorticotrophic hormone to wound healing in mice. Recently, Bruce, Parkes and Perry[96] have developed a bioassay method which depends upon the fact that the hormone causes involution of the thymus gland. Nestling rats are used in this method, which is considered to be specific, since they are incapable of the mobilisation of endogenous adrenocorticotrophic hormone in response to stress.

Hypophysectomy renders animals very sensitive to stress. Tyslowitz and Astwood[21,97] found that adrenocorticotrophic hormone increased the resistance of hypophysectomised rats to cold. Li, Simpson and Evans[98] showed that the hormone increased the resistance of hypophysectomised and normal rats to cold, starvation and anoxia. Reiss *et al.*[89] found that it increased the resistance of normal mice to insulin. It is probable that these and many similar actions could be developed into quantitative methods for the bioassay. However, the accuracy and specificity of such methods are not likely to be great unless hypophysectomised animals are used. In this event, no advantage is gained, and it is probably more reliable to measure the direct effects on the adrenal cortex.

The technique of hypophysectomy in the rat is not easy, and complete success is difficult to attain except after considerable practice on the part of the operator. For this reason, attempts are being made in this laboratory to modify the Sayers' technique by substituting, for hypophysectomised animals, rats in which pituitary adrenocorticotrophic activity has been "blocked" with cortical hormones. The investigations are still in progress but, so far, a method has been devised which is at least as sensitive as the Sayers' method although its accuracy has yet to be ascertained. Such a method has the advantage that it is less time consuming and readily performed by comparatively unskilled workers.

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