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STUDIES ON THE METABOLISM OF NUCLEIC ACIDS AND OTHER CONSTITUENTS IN THE ADRENAL GLAND

by

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Thesis presented for the Degree of Doctor of Philosophy of The University of Glasgow

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PART I

INTRODUCTION

1.1 Endocrine glands.

One of the devices by which an animal maintains a proper control over its numerous physiological functions is by the activity of the endocrine glands which secrete their appropriate hormones into the blood stream. These hormones have specific actions on other tissues, sometimes referred to as "target organs." It is now known that some of the endocrine glands, especially those that produce steroid hormones, secrete more than one hormone. Also it is beginning to be evident that hormones may have, in some cases, wider effects than those suggested by the specific relationship of the endocrine gland and its target organ.

In many cases, the secretion of hormones by the endocr[ine] organs is under the control of the pituitary gland situated at the base of the brain and itself an endocrine organ. Thus in the adrenal cortex with which this thesis is concerned, the production of adreno-corticoid hormones appears to be controlled by the adrenocorticotrophic hormone (ACTH) secreted by the adenohypophysis.

1.2 Structure and composition of the Adrenal Gland.

Historical

The first description of the adrenal glands was given [in] 1563 by Bartholomaeus Eustachius in his account of the kidneys and the name 'suprarenal capsules' was introduced

in 1629 by Jean Riolan. Though there was an early belief that these glands produce some substance with a special function, it was only by the middle of the nineteenth century that the idea was generally accepted. The first outstanding contribution to the understanding of these glands in man was made by Addison (1855) who observed the effects of the disease of the adrenal gland showing the characteristics of adrenal insufficiency, now known as Addison's disease. Following this discovery Brown-Séquard (1858) by removing the glands from various animals and finding that this operation proved fatal, established that the adrenals were essential for life.

Gross morphology.

The adrenal gland consists of two distinct parts, the cortex and the medulla which have little resemblance to each other and are embryologically distinct. The central medulla consists of cells derived from the sympathetic nervous system while the peripheral cortex is composed of cells derived from mesoderm. The medulla has been shown not to be essential for life, since its loss is not necessarily attended by fatal consequences. The cortex, on the other hand, cannot be completely

removed without disastrous results. It is therefore the cortex that is more important in regulating physiological function.

It is interesting to note that the adrenal cortex occupies a relatively large portion of the whole gland. Donaldson (1928) found that in female albino rats the cortex and medulla weighed respectively 47.1 mg. and 3.1 mg. while Fiala, Sproul and Fiala (1956) calculated that in rats the volume of the medulla is not more than 12% of that of the whole gland. For man, Quinan and Berger (1933) have reported that the medulla occupies 10% of the whole gland.

The weight of the adrenal glands increases with age and tends to a plateau. In addition to varying with age the weight also varies in accord with such physiological episodes as oestrus and pregnancy. It also varies from strain to strain and from species to species. Generally, too, there is a distinct sex difference. In rats the female has the bigger gland (Donaldson, 1928), while in the guinea-pig, which is remarkable in possessing a very large adrenal for its size, Eaton (1938) found that, for animals of about 700 g. body weight, in the female the glands weighed 117.5 mg. per 100 g. and in the

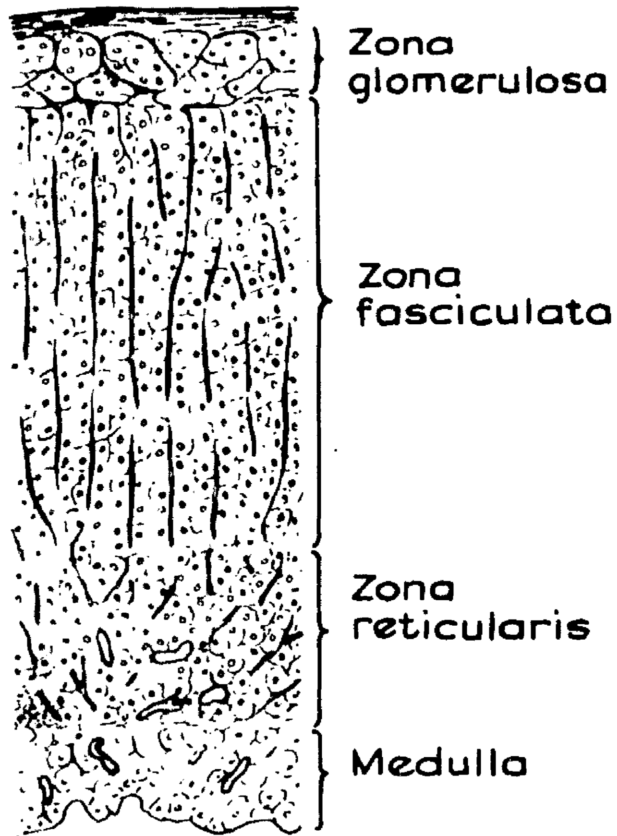
male 126 mg. per 100 g. body weight. These figures are to be compared with 13.3 mg. and 25.6 mg. per 100 g. body weight for male and female mice reported by Jones (1955). An examination of Kojima's (1928) data on adrenal weights in guinea-pigs brings out the further interesting point that the left and the right adrenal glands are not necessarily of the same weight, the left gland being 10-15% greater than the right. No functional significance has been attributed to this difference in the paired organs which occurs in most species and the factor involved may well be their anatomical position.

Histology.

Histologists have reported three main types of cells in the cortical portion of the adrenal gland which are named from their distinctive appearance. The afrangement of these cells is shown diagramatically in Fig. 1.

Immediately below the outer connective tissue capsule is the zona glomerulosa so called since the cells form spherical clusters. This is followed by the zona fasciculata in which the cells are arranged in columns forming radial rows. In man and some other mammals this zone forms by far the most extensive part of the cortex, and between it and the chromaffin tissue consisting of the

Fig. 1.



medulla, lies the comparatively narrow zona reticularis which appears disrupted and broken in its cellular arrangeme The relative size of these zones varies very widely in different species; in man it has been estimated that the outer zona glomerulosa occupies 15%, the zona fasciculata 78.4% and the zona reticularis 6.4% of the total cortical volume (Swinyard, 1940).

Adrenocortical hormones.

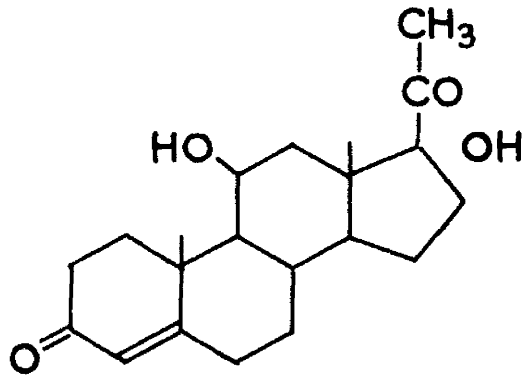
Although interest has been evinced ever since the classical description by Addison of the disease now known by his name it was not until 1930 that very potent extracts were made by Swingle and Pfiffner which were active in adrenal insufficiency. Cortisone was isolated in a pure state in 1934 and since then nearly thirty compounds have been isolated and characterised and this is probably not the final list. Not all the compounds that have been isolated from the gland are biologically active. It is probable that most of them are derivatives of a small number of biologically active hormones. The most important active compounds are cortisol, corticosterone and aldosterone which like the other adrenocorticoids belong to the group of chemical compounds known generally as 'steroids', derived from the parent compound perhydrocyclopentanophenanthrene.

The chemical structures of these compounds are shown in Fig. 2.

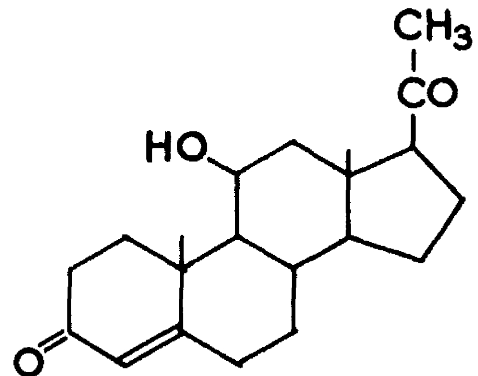
A great deal of attention has been paid in recent years to the elucidation of the chemical nature and biosynthesis of the substances actually secreted by the adrenal cortex. It has been found (Bush, 1953) that in most species, except in the cat, cortisol, corticosterone and aldosterone constitute 85-100% of the αβ-unsaturated 3-keto steroids in the adrenal venous blood. Of the larg number of other compounds produced none exceeds 2.5% of th total. In these investigations use has been made of invitro methods in which the adrenal is arterially perfused or the adrenal vein is cannulated and citrated homologous blood circulated through it (Hechter, 1953; Ma and Hechter, 1954).

The levels of adrenocortical hormones circulating in the blood are not very high. Their rate of synthesis by the cortex is probably determined by the needs of the body and the rate at which they are metabolised. In normal human peripheral blood the amounts of cortisol and corticosterone present are respectively about 4.5 µg. and 7.0 µg. per 100 ml. plasma (Morris and Williams, 1953)

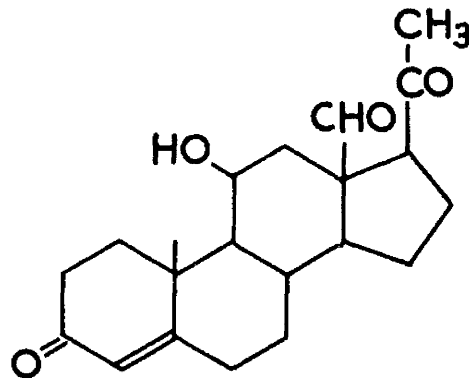
Fig. 2.



Cortisol
Δ^4-pregnene-11β, 17α-
21-triol-3,20-dione



Corticosterone
Δ^4- pregnene-11β, 21-diol-
3,20-dione



Aldosterone
Δ^4- pregnene - 11β, 21-diol-
3,20-dione-18-al.

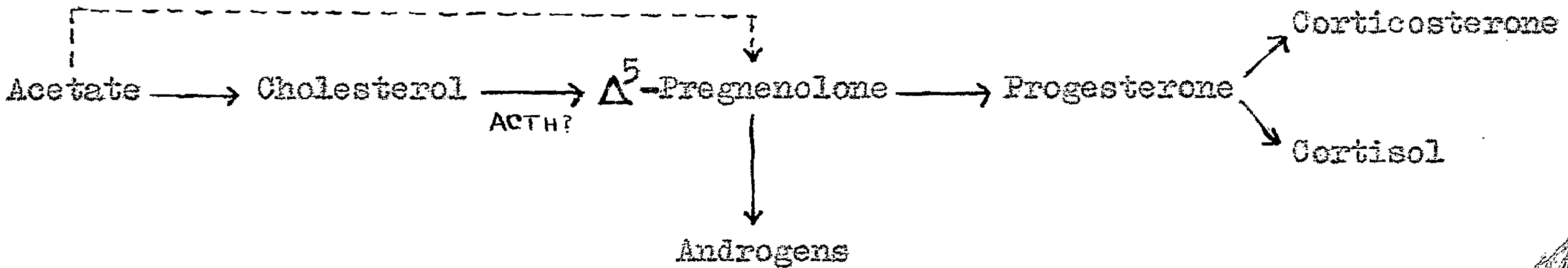
though these levels are subject to diurnal variation (Tyler, Migeon, Florentin, and Samuels, 1954).

The method of formation of cortico-steroids in the cells of the adrenal cortex is not fully known in spite of a vast amount of research in this direction. However, light has been thrown on various possible mechanisms and attempts to isolate the enzyme systems have met with partial success (Hechter and Pincus, 1954; Dorfman, 1955). The possible routes of biosynthesis of corticosteroids from acetate are outlined in Fig. 3.

The synthesis of corticosteroids is usually assumed to occur by a route involving cholesterol, though there is some evidence for a more direct pathway which does not involve this substance. The suggestion has been made (Haynes and Berthet, 1957) that ACTH may act at the step involving the conversion of cholesterol to Δ^5-pregnenolone. If this is so, it is possible that the alternative bisynthetic route which does not involve cholesterol may not be ACTH-dependent. The steps involved in the conversion of cholesterol to cortisol have been described by Hechtor and Pincus (1954) and the enzymes involved by Hayano and Dorfman (1952) and Plager and Samuels (1952).

Methods used to elucidate the details of adreno-corticosteroid biosynthesis include studies with adrenal

Fig. 3.



slices and homogenates as well as cell-free systems (Hayano and Dorfman, 1952; Hofmann and Davison, 1954; Bligh, Heard, O'Donnel, Webb, Saffran and Schönbaum, 1955; Giroud, Stachenko and Venning, 1956). Extensive use has also been made of hyperactive and tumour-bearing human glands (Dorfman, 1958). Unfortunately the latter type of study is limited to humans because adrenocortical tumour in laboratory animals is extremely rare. Experimentally induced tumours in the adrenal cortex of mice have been studied by Woolley (1958).

It will be seen from Fig. 3 that progesterone may give rise to either corticosterone or cortisol. Both of these compounds appear to have similar functions and the proportions of these occurring in the blood of different species varies widely. The ratio of cortisol to corticosterone in man is about 10, in the sheep 15-20, and in the ox 1. The rat and the rabbit excrete mainly corticosterone (Bush, 1953) while the guinea-pig excretes mainly cortisol (Burstein and Dorfman, 1954). The main factor involved in determining the relative amounts of these substances secreted may be the availibility of the various hydroxylating enzymes involved.

Cholesterol and ascorbic acid.

Mention must be made, at this stage, of two chemical substances that are found in the adrenal cortex to a

considerable extent and whose chemical determination has been used to estimate the activity of the gland. These are ascorbic acid and cholesterol both of which decrease in amount with activity of the gland..

Cholesterol has been implicated in the synthesis of corticosteroids and so it is presumably used up when there is a demand for corticoids, and the variations in the total cholesterol content of the adrenal gland estimated chemically has been seen, in the rat, to correspond to functional changes (Sayers and Sayers, 1948).

The variation in ascorbic acid content has also been used very frequently as an index of gland actively (Sayers, Sayers, Liang and Long, 1945, 1946). However there is considerable disagreement as to the exact role played by ascorbic acid in the formation of adrenocorticosteroids. Lowenstein and Zwemmer (1946) implicate ascorbic acid directly in steroidogenesis in the cortex and claim to have evidence for a steroid-ascorbic acid complex, while Schmidt and Staudinger (1954) claim increased steroidogenesis *in vitro* in swine adrenal glands when ascorbic acid is present. Dugal and Therien (1949) reported that ascorbic acid administration inhibited the adrenal enlargement following recurrent exposure to cold, and this was confirmed by Nicholls (1956) who observed that the increased incorporation of ^{32}P into inorganic phosphate and acid-soluble phosphate of the adrenal gland following

exposure of rats to cold is abolished by administration of sodium ascorbate.

The continuation of cortical activity in acute conditions of scurvy when there is virtual absence of ascorbic acid indicates that this compound may not be essential for steroidogenesis (Long, 1950) and Jailor and Boas (1950) have observed that in the adrenal of the fowl, which apparently has no ascorbic acid, the synthesis of steroid is quite normal. Prunty, Clayton, McSwiney and Mills (1955), however, point out that methods used are not sensitive enough to detect the presence of traces of ascorbic acid. Skelton and Fortier (1951) while studying the response to ACTH following administration of large doses of ascorbic acid found that there was no change in the adrenal cholesterol response to ACTH. Dugal and Therien (1952) in further experiments of a more chromic nature reached different conclusions. They reported that the effect of ACTH on the adrenal weight and cholesterol content is potentiated by the concurrent administration of large doses of ascorbic acid, when such treatment is continued for a period of ten days after hypophysectomy in the rat. A similar synergistic effect of ascorbic acid and ACTH on the secretory activity of adrenal cortex is reported by Des Morois and Leblanc

(1952). Hayano, Saba, Dorfman and Hechter (1956) have produced evidence that the conversion of cholesterol to progesterone by isolated mitochondrial preparations of adrenal cortex is inhibited up to 40% by addition of ascorbic acid, and put forward the important suggestion that ascorbic acid acts as a restraining factor in the biosynthetic sequence and this suggestion is supported by the recent work of Jones, Peria-Golia and Eik-Nes (1958) on scorbutic guinea-pigs.

Even though the rate of ascorbic acid in corticosteroi biosynthesis is one of the great mysteries of adrenocortica physiology, there is no doubt about the gross effect of acute deficiency of this vitamin on the adrenal gland, which undergoes a significant increase in size (Rodony, 1919), due to enlargement of the cortex (Meyer and McCormi 1928). Interpretation of this enlargement is again a matter of controversy. Meyer and McCormic claim that the enlargement is due to congestion and fatty infiltratio large deposits of which occur in the zonae fasciculata and reticularis, and that this is therefore not true hypertrophy. Gergely (1943) also suggests that the enlargement is caused by mere swelling, perhaps meaning an increase in water content. On the otherhand, Stepto, Pirani, Consolazio and Bell (1951), who made measurements

of the composition of the gland, find a significant increase in dry weight, particularly in protein content, and conclude that there is true hypertrophy. Prunty, et al also made the important observation that the adrenal glands in scorbutic guinea-pigs respond to the administration of ACTH by showing very much larger increases in weight than normal animals.

The Nucleic Acids.

The presence of basophilic granules in the cytoplasm of human adrenal cortical cells was noted by Santee (1936) and following on the development by Brachet (1941) of ribonuclease as a cytochemical tool for the detection of ribonucleic acid (RNA), Rich and Berthong (1949) demonstrated using ribonuclease that these basophilic granules were composed of RNA and that the granules were particularly prominent in the adrenals of patients who had died of severe infections. In 1956 Symington and Davidson reported a cytochemical and biochemical study of the nucleic acids of the adrenal and showed that in stressful conditions and also following stimulation with ACTH there was an increase in the concentration of RNA and in the ratio of RNA to deoxyribonucleic acid (DNA). Since the work to be reported in this thesis is mainly concerned with the nucleic acids of the adrenal gland some

consideration must now be given to the nature and function of these substances in cells and tissues in general.

Occurrence of nucleic acids.

Histological as well as biochemical methods have been used to detect and to estimate the nucleic acids in tissues. The former make use of the strongly acidic nature of nucleic acids and hence their affinity for basic dyes like pyronin and methyl green. Brachet (1941) used these dyes in conjunction with ribonuclease to determine the localization of nucleic acids in tissue sections. The elegant method of ultraviolet spectrophotometry of Caspersson (1941) enabled the detection and quantitative measurement of nucleic acids in the cytoplasm and various other parts of the cell, while Davidson and Waymouth (1943, 1944) carried out chemical estimations of RNA and DNA. From all these early studies it was possible to draw the broad conclusions that nucleic acids are present in all types of cells and that while DNA is mainly confined to the nucleus and probably constitutes the chromosomal material, the RNA is present to a large extent in the cytoplasm.

Ribonucleic Acid.

RNA is a polymeric substance of high molecular weight consisting of repeating nucleotide units joined together through phosphodiester linkages, whose molecular structure has been elucidated (Brown, and Todd, 1952; Cohn and Volkin, 1953; Markham, 1957), except for the sequence of nucleohide units in the polymer. Most of the evidence to date suggests that the molecule of RNA is linear. There is, however, a possibility of it having some branched chains (Cohn, 1958) and sugars other than d-ribose (Smith and Dunn, 1959).

It has been shown by differential centrifugation of cell fractions that RNA is heterogeneous in character and that the tissue or even a single cell might contain different types of RNA. The small amount of RNA associated with the nuclear fraction has been shown to be very much more active than cytoplasmic RNA by isotope incorporation studies (Bergstrand, Eliasson, Hammersten, Norberg, Riechard and von Ubisch, 1948). These earlier results have been confirmed by a large number of other investigators (Barnum and Huseby, 1950; McIndoe and Davidson, 1952; Smellie, McIndoe, Logan, Davidson and Dawson, 1953) and the subject has been reviewed by Brown and Roll (1955). In these studies on RNA, which usually involve the degradation of

the compound to the mono-nucleotides, nuclesides or bases by alkaline or acid hydrolysis, it is emphasized that sufficient care must be taken to eliminate likely contamination (Barnum, Nash, Jennings, Nygaard and Vermund, 1950; Davidson, Frazer and Hutchison, 1951). The ionophoretic technique evolved by Davidson and Smellie (1952) appears to be one of the most satisfactory methods for the separation of the ribomononucleotides in a fairly pure state, free from concomitant impurities.

The main bulk of the cytoplasmic RNA appears to be present in the microsomal fraction while smaller amounts are present in mitochondrial and non-sedimentable supernatant fractions. In general the uptake of radioactive precursors into the microsomal fraction is closely similar to that of mitochondria whereas the activity of RNA of the cell sap tends to be higher than that of the particulate fractions (Smellie, et al., 1953).

The effect of diet on the metabolism of RNA has been investigated by Munro, Naismith and Wikramanayake (1953), Wikramanayake, Heagy and Munro (1953) who have shown that changes in protein intake and in the energy content of the diet have a profound influence on the content and phosphorus turn-over of rat liver RNA. The subject has been

reviewed by Munro (1954).

For some years it has been recognised that RNA is somehow concerned with protein synthesis. Caspersson (1950) and Brachet (1950) showed that cells in growth and secretion are rich in RNA. Gale and Folkes (1953) observed in disrupted staphylococcal cells that the synthesis of proteins or the incorporation of amino acids depends on the nucleic and content and is inhibited by ribonuclease. Biochemical work on embryonic material (Davidson, Leslie and Waymouth, 1949; Leslie and Davidson, 1951) has shown that synthesis of RNA always precedes protein synthesis.

However, recent evidence in a large number of cases has been provided to show that protein synthesis can take place without *de novo* formation of RNA. Inhibition of amino acid incorporation into proteins by ribonuclease observed by Gale and Folkes (1953) and others is not observed always and in all systems. Rat skeletal muscle mitochondria which contain RNA can incorporate labelled leucine, a process which is stimulated by ribonuclease according to Simpson and McLean (1957). Of particular interest are the reports of Landman and Spiegelman (1955) that the enzymes ribonuclease, lipase and trypsin do not inhibit the formation of the adaptive enzyme β-galactosidase

in whole B. megatherium cells but do so in homogenates. Beljanski (1954) found that amino acid incorporation into the protoplasts of M. lysodeikticus cells was inhibited by ribonuclease whereas no inhibition was observed when whole cells were used. Liss (1958) has reported rapidly increased synthesis of proteins in yeast cells, which were incubated in a nutrient medium in the absence of phosphate, without de novo formation of RNA.

Deoxyribonucleic Acid.

DNA, which occurs exclusively in the nucleus of the cell, is now accepted as being the chromosomal material, so extensively studied by the histologists by Feulgen staining (Feulgen and Rossenbeck, 1924). Strong support for the view that DNA carries the hereditary characteristics during cell division was obtained by the discovery of the bacterial transforming principles (Avery, McLeod and McCarty, 1944) and by the recent work on the mechanism of invasion of Esch. coli by the bacteriophage T_2 and other viruses (Cohen, 1951; Kozloff, 1952; Burton, 1957). The work of Chargaff (1955) and others and the X-ray diffraction studies by Watson and Crick (1953) have led to an understanding of the structure of DNA and its possible mode of duplication during cell division (Levinthal and Crane, 1956; Meselson and Stahl, 1958).

Compared to RNA, DNA is a relatively stable molecule. A large number of studies involving the use of radioactive precursors such as inorganic ^{32}P or substances labelled with ^{14}C have confirmed this concept of the stability of DNA. There is very little incorporation into the DNA of tissues in which the mitotic activity is low and in tissues having a large number of mitoses, such as intestinal micosa and bone marrow, the incorporation is greatly enhanced (Smellie, Humphrey, Kay and Davidson, 1955). Earlier work of Hevesy (1951) and of Furst and Brown (1951) had showed very little incorporation of ^{32}P or labelled adenine into the DNA of the nuclei of non-dividing cells. In rapidly growing tissues as in liver which is regenerating after partial hepatectomy (Nygaard and Rusch, 1955) there is a greatly increased incorporation into DNA which is due to the new DNA that is being synthesised. The investigations of Barton (1954) have shown that the isotopes once incorporated into DNA are not exchanged, so that DNA is not in dynamic equilibrium with other constituents of the cell.

Histochemical Studies.

Much of the work that has been done on the adrenal gland is histochemical. In man such studies in normal (Zamchek, 1951; Stoner, Whitley and Emery, 1953) and

stressful (Symington, Currie, Curran and Davidson, 1955) conditions have been made in order to correlate the changes observed with the functional state of the gland. The usual method is to make use of the sudan staining property of the lipids. When the gland is stimulated to secrete more corticoids this is usually accompanied by a decrease in the stainable lipid in the various zones of the cortex and under extreme conditions of stress, it has sometimes been observed that complete loss of this material takes place and the clear demarcation between the zona reticularis and zona fasciculata becomes indistinct. It must, however, be pointed out that at the same time as this decrease in this sudanophilic lipid takes place there is an increase in the phospho-lipid concentration in the gland (Symington and Davidson, 1956) and since the gland weight is increased under conditions of stress, there is presumably, therefore, an increase in the total phospho-lipid content of a gland. It is perhaps possible that the lipid loses its staining property due to this phosphorylation.

The basophilic nature of the nucleic acids has also been used to study the normal and stressed adrenal gland. Rich and Berthong (1949) noticed that in patients who had died of severe infection, the basophilic granules which consisted of RNA were particularly prominent in the adrenal.

Administration of exogenous ACTH stimulates the adrenal cortex in a manner similar to that occurring when a stress is applied. The histological changes which accompany this are, with mild stimulation, increase in cell size in zona fasciculata, i.e. hypertrophy and an increase in cell division with consequent increase in the number of cortical cells, i.e. hyperplasia. Mitotic figures occur principally in the zona fasciculata, but they may also be found in other parts of the cortex including the zona reticularis (Baxter, 1946; Baker, 1952). The lipid droplets in the zona fasciculata become finer and less concentrated, while the zona glomerulosa narrows without much variation in lipid. Stimulation may also bring about hypertrophy of the Golgi apparatus in the cells of the outer zona fasciculata (Reese and Moon, 1938), and an increase in the number of mitochondria especially in this area (Miller, 1950). Intense stimulation brings about an apparently complete disappearance of the zona glomerulosa accompanied by a vast increase in the vascularity of the gland. Indeed massive administration of ACTH can result in a haemorrhagic breakdown of the cortex (Ingle, 1951).

1.3. Functions of the Adrenal Cortex.

Functions of the cortical hormones.

An impressive number of physiological functions

have been listed as coming under adreno-cortical control, though many of these may be only secondary effects. Ingle (1944) has made a survey of these various functions. Only two of them will be considered briefly here, the effect on carbohydrate metabolism and on electrolyte balance. These two are not necessarily divorced from each other.

In carbohydrate metabolism, the cortical hormones known as glucocorticoids, work conjointly with insulin, the hormone of the pancreas, and the growth hormone. Among the main effects of glucocorticoids are the increased production of carbohydrate accompanied by a concomitant increase in urinary nitrogen, suggesting therefore that gluconeogenesis occurs with protein catabolism as the source of the newly formed carbohydrate (Long, Katzin and Fry, 1940). In addition, there is an inhibition of utilization of carbohydrate by the peripheral tissues (Ingle, 1948). Cori and his coleagues (Colowick, Cori and Slein, 1947) have demonstrated that hexokinase activity which is stimulated by insulin is inhibited by adrenocortical and anterior pituitary hormones. This result has been confirmed by Bacila and Barron (1954) who also suggest that these hormones combine with the -SH groups to bring about this inhibition. Clark (1950) using amino acids labelled with ^{15}N has shown that not only do the gluco-

corticoids increase protein catabolism but prevent protein anabolism as well. Recently Goodlad and Munro (1958) have shown that cortisone administration releases amino acids by protein catabolism in the carcass and that these may be resynthesised into proteins in other organs depending on the energy supply in the diet.

The recent discovery of aldosterone has confirmed that this hormone is the most potent in maintaining a proper electrolyte balance in the body and is in this respect many times more active than deoxycorticosterone in comparable amounts (Simpson and Tait, 1955). One of the most striking facts about the adrenalectomised animal is the loss of sodium and retention of potassium. Since the conservation of sodium is one of the functions of the kidney, the lack of the hormone affects the kidney activity. It is supposed that the adrenal hormones influence the enzymes or carrier systems concerned in the active re-absorption mechanisms in the tubule. Similarly it is possible that these hormones facilitate potassium excretion and in their absence their normal secretion of potassium in the distal tubule is prevented.

Aldosterone appears to be produced in the zona glomerulosa of the cortex which, however, is little affected by hypophysectomy (Deane and Greep, 1946). It is therefore possible that the secretion of aldosterone

is independent of ACTH control. This is borne out by the results of Giroud, Stachenko and Venning (1956) obtained with isolated zona glomerulosa.

Among other functions of adrenocortical hormones a brief mention might be made of their effect on circulating lymphocytes. Adrenal insufficiency, either in the Addisonian patient or in the adrenalectomised animal, is associated with the hyperplasia of the lymphoid tissue and some increase in the number of circulating lymphocytes (Yoffey, 1950). Administration of cortical hormones or their increase endogenously by ACTH or stress or disease results frequently in a reduction in the number of circulating lymphocytes - the condition known as lymphopaenia. This has sometimes been used as an indication of increased adrenocortical activity and an empirical biological assay method (Spiers and Meyer, 1949).

Control of adrenocortical activity.

The adrenal cortex is directly under the influence of the pituitary body at the base of the brain. The pituitary is segmented into the anterior, intermediate and posterior lobes. The adenohypophysis, which includes the anterior and part of the intermediate lobes, produces a number of hormones which are adrenocortico-trophic

hormone, thyrotropic hormone, somatotrophic hormone, gonadotrophic hormone and lactogenic hormone. The removal of the pituitary (Smith, 1927), the operation referred to as hypophysectomy, eliminates all these hormones and brings about, among other effects extensive degeneration of the adrenal cortex. The medulla is, however, very little affected by this operation. Deane and Greep (1946) in a detailed investigation of the effect of hypophysectomy on rats, report a rapid post-operative decline in adrenal weight for the first 10 days followed by a more gradual decrease. The degeneration of the cells takes place in the zonae reticularis and fasciculata whereas the zona glomerulosa appears to function normally and indeed enlarges. The atrophy of the inner zones is accompanied by picnosis of the nuclei and shrinkage of cytoplasm, the number of mitochondria diminish and fuse, and the Golgi apparatus becomes compart, losing its digital processes. Similar observations have been made on the guinea-pig by Schweizer and Long (1950), though they point out that there is a loss of sudanophilic material from the zona glomerulosa in the hypophysectomised guinea-pig which does not appear to be the case with the rat. These workers also show that intraocular graft

of anterior pituitary tissue help to maintain the adrenal cortical activity, though below the normal level.

An atrophy of the adrenal cortex similar to that in the hypophysectomised animal takes place when an extract of the cortex or an adrenocortical hormone is injected to the normal animal (Ingle, Higgins and Kendall, 1938). Clearly there is an interrelationship between the amount of ACTH secreted and the amount of circulating corticosteroids. Sayers (1950) suggests that the corticoids act on the adenohypophysis inhibiting the release of ACTH. A direct evidence for this is provided by the work of Tuchmann-Duplessis (1952) who observed an increase in the number and size of basophils in the pituitary and in its ACTH content by 50-60% after administration of corticosteroids and suggests that these hormones interfere with the secretion of ACTH. There is also the possibility that this action may be indirect occurring via the hypothalamus (Vogt, 1955) which in turn may be stimulated by impulses received from the baroreceptors in the cortoids (Mills, 1959).

Abnormalities of Adrenal function.

Deficiency diseases.

Among the deficiency diseases of the adrenal cortex the most extensively investigated is Addison's disease,

a large number of cases arising from destruction of the cortex by tuberculosis or other diseases as syphilis, amyloidosis and scleroderma. Another similar type of chronic adrenal deficiency disease is Simmond's disease, which is caused as a result of failure of secretions of the anterior lobe of the pituitary. A less chronic type is the Waterhouse-Friderischen syndrome found mostly in the new-born baby after a difficult and prolonged labour and in young children. The adrenal shows considerable haemorrhage and the condition is supposed to be caused by excessive demand for corticoids, with the concomitant outpouring of ACTH stimulating the cortex beyond the limits of endurance resulting in a collapse.

Cortical syndromes.

Continued secretion of excessive amounts of ACTH, results in a permanent over-stimulation of the cortex to produce corticoids and is called 'Cushing's syndrome'. The causes are usually pituitary malfunction and may result in a tumour of the cortex. The syndrome produces the characteristic 'moon face', and carbohydrate metabolism and therefore fat metabolism is disturbed resulting in excessive deposits of fat. In some cases it is seen to result in a

deficiency of minerals - corticoids, perhaps due to destruction of the zona glomerulosa. The other familiar type of disease of the adrenal is the adreno-genital syndrome in which the cortex produces large amounts of androgens or oestrogens. In normal cases the adrenal produces small amounts of these hormones, but when the normal mechanism is grossly disturbed by the presence of a tumour, the production of sex hormones will predominate over the corticosteroids. Dorfmann (1955) has proposed an attractive hypothesis in which he suggests that in cases of adreno-genital syndrome, the enzymes of the C_{21}- hydroxylating system are defective and therefore abnormal amounts of sex hormones are produced. The tumours usually result in virilism, but feminising tumours also occur. Sydnor, Kelly, Raile, Ely and Sayers (1953) have demonstrated increased ACTH in the blood of patients with virilising adrenal hyperplasia so the disease may well be due to hyperpituitary activity.

1.4. Adrenocorticotrophic hormone.

The atrophy of the adrenal cortex brought about by hypophysectomy or by other means can be reversed by the administration of pituitary extracts (Smith, 1930). Numerous attempts were made to isolate and purify the hormone without success until 1942-43 when two independent

announcements were made (Li, Simpson and Evans, 1942; Sayers, White and Long, 1943) of preparations from sheep and pig pituitary glands of highly purified proteins which were free from other pituitary contaminants. These preparations were found to produce selective stimulation of the adrenal cortex of hypophysectomised rats, as evidenced both by increase in gland weight and by repair of adrenal histology. Since that time the use of various procedures involving for example ultra-filtration (Tyslowitz, 1943), oxycellulose adsorption (Astwood, Raben, Payne and Grady, 1951) and ion-exchange resins (Dixon, Moore, Stacke-Dunne and Young, 1951) have resulted in preparations of very high potency. There is, however, no general agreement about the potency of the various preparations, as the methods of bio-assay are themselves the subject of considerable dispute. The methods generally employed are (i) Sayer's ascorbic acid depletion test (Sayers, Sayers, Liang and Long, 1945) and (ii) Saffran's assay of corticoids produced by adrenal slices _in vitro_ (Saffran, Grad and Bayliss, 1952; Saffran and Bayliss, 1953; Saffran and Schally, 1955).

Dixon _et al_. (1951) have claimed that there are two different fractions of ACTH, an ascorbic acid depleting factor and an adrenal weight maintaining factor. These claims, however, have not been substantiated and may well

be due to breakdown of the molecule of ACTH during isolation and purification procedures.

ACTH appears to be very susceptible to inactivation especially when given intramuscularly or subcutaneously. However the action of ACTH is considerably prolonged by making it into a gel with gelatin or a suspension with zinc hydroxide.

Even though the normal functioning of the adrenal gland requires ACTH, over-activity of the pituitary resulting in an excess elaboration of the hormone will stimulate the adrenal cortex to produce very large quantities of cortical steroids. Certain stimuli such as administration of histamine and stressful condition such as shock and infection also bring about such increases in ACTH production. It is, however, doubtful whether all these conditions act directly on the pituitary. There is evidence that histamine acts on the adrenal medulla releasing adrenaline which then acts through the vasomotor control and hypothalamus on the adenohypophysis (Vogt, 1955). Administration of exogenous ACTH stimulates the gland in a manner similar to that occurring when a stress is applied. The changes that accompany such stimulation have already been discussed in the sections describing the histochemistry of the gland and abnormalities of its function.

1.5. Hormones and Nucleic acids

Investigation of nucleic acid composition of tissues following administration of hormones or removal of endocrine glands have not been extensive and the earlier work has been reviewed by Leslie (1955).

The effect of cortisone on nucleic acid composition in liver has been studied by Lowe, Williams and Thomas (1951) who report a decrease in RNA concentration per unit weight of tissue, but it does not follow that RNA synthesis is reduced by cortisone, as the authors suggest. Leslie (1952) added cortisone to the growth promoting medium of cultures of embyronic chick heart explants and found slight increases in total DNA synthesis and RNA/DNA ratio. When cortisone and growth hormone were combined, the total synthesis of RNA and DNA rose as much as 75% above normal over 6 days. Cortisone administration has been found to bring about a general cortabolism of muscle protein, and resynthesis of protein in the liver depends on the intake of energy in the diet (Goodlad and Munro, 1959).

Injection of thyroxine into the rat produces increases in RNA, DNA and the protein content of the kidney and spleen (Mandel, Jacob and Mandel, 1952). Campbell, Innes and Kosterlitz (1953) found by administering oestradiol to intact and hypophysectomised rats that

the hormone produces increases in DNA and in the RNA/DNA ratio in the intact animal but not after hypophysectomy so that the effect of oestradiol appears to be mediated through the pituitary. Di Stepano, Diermier and Tepperman (1952) have also provided evidence for the pituitary control of liver nucleic acids by showing that hypophysectomy decreases the RNA content without change in DNA and that injection of growth hormone restores the RNA and protein to the normal level. Campbell et al. (1953) in their extensive study of the hormonal control of liver nucleic acid in pregnancy conclude that there are two independent RNA fractions in the liver of the pregnant rat , one varies linearly with protein content as in non-pregnant rats; and the other 'excess RNA' is quite independent of either protein content or the amount of protein eaten, but varies directly with the amount of energy consumed with the food and with the weight of the placenta.

Marked stimulation of the pigeon crop gland by lactogenic hormone has been shown to take place (McShan, Davis, Soukup and Meyer, 1950). These authors noted that an increase in DNA takes place on the second and third days of hormone administration, when the histological picture shows maximal mitotic activity.

Fiala, Sproul and Fiala (1957) have noted a marked stimulatory effect of thyrotrophin on the rat thyroid gland. Matovinovic and Vickery (1959) have carried out similar studies on the guinea-pig thyroid gland and note that thyrotrophin produces hypertrophy but no hyperplasia.

Fiala, Sproul and Fiala (1956) have studied the effect of the administration of ACTH on rat adrenal glands and report large increases in DNA, RNA and the protein content of the gland.

Studies on the incorporation of ^{32}P into the adrenal gland of normal and hypophysectomised rats was first made by Gemzell and Samuels (1950) who showed that the incorporation into the inorganic phosphate of the gland in the hypophysectomised rat was reduced but could be restored to normal by a single injection of ACTH. Similar decrease in ^{32}P incorporation due to hypophysectomy was observed in the acid soluble fraction of the gland (Reiss and Halkerston, 1950). Riedel and Russiter (1954) have confirmed these results and further shown that incorporation into lipid phosphorus (LP) and RNA is similarly lowered in hypophysectomised rats and restored to normal by ACTH injections (Riedel, Logan and Rossiter, 1954; Logan, Heagy and Rossiter, 1955). Rossiter and his colleagues in their extensive investigations on the phosphorus metabolism of the

adrenal gland of rats exposed to a cold environment have found that ^{32}P incorporation into tissue inorganic phosphate increases to a maximum during the first 2 hours of cold stress and then gradually falls to a value slightly above normal during 24 hours (Nicholls and Rossiter, 1955). After 8 days in the cold, there is a marked increase in the adrenal weight, LP, RNA and DNA suggesting hyperplasia of the gland (Nicholls, Heagy and Rossiter, 1956 a) and a significant increase of ^{32}P incorporation into LP and into RNA nucleotides after 16 hours in the cold (Nicholls, Heagy and Rossiter, 1956 b). Both this group of workers and Symington and Davidson (1956) emphasise that phospholipids may play an important role in the energy transformation reactions in the active synthesis of corticosteroids. Recently Hutchison, Burns and Hale (1958) have reported that in the guinea-pig adrenal gland the nuclei are entirely diploid and remain so even when ACTH administration is continued for periods up to three weeks. Any increases found in the DNA content of the gland after stimulation therefore represent true hyperplasia.

1.6. Objects of the present investigation.

This brief survey of the literature indicates

the extremely important role that the adrenal cortex plays in the physiology of animals and man. The work of Rossiter and his colleagues indicates the importance of the phosphorus metabolism of the gland and Symington and Davidson have shown that ACTH causes big changes in the RNA of the gland in man and in the calf. In view of the latter finding it seemed desirable to study the effect of ACTH on the adrenal glands of smaller experimental animals, which also offer the possibility of studying the incorporation of isotopically labelled precursors. Measurement of the DNA con-tent of the whole gland would provide direct evidence of the number of cells in the gland under various conditions, and also from what is known of the metabolic stability of DNA, an increased incorporation of labelled precursors would act as a very sensitive indicator of new DNA synthesis. A study of the total phospholipid would be of interest in view of its probable role in supplying energy for other metabolic reactions.

In addition, the use of animals such as the guinea-pig which are easily made scorbutic, gives the opportunity of studying the effect of withdrawal of ascorbic acid on the metabolism of adrenal nucleic acids, which might shed some light on the function of ascorbic acid in the gland.

PART II

GENERAL METHODS.

The animals used were guinea-pigs and albino rats from the departmental colonies, except in one experiment in Part III, section 2. All animals were females. In all cases, unless otherwise stated, a control group of animals was included and the body weights were correctly matched between the control and the test groups.

In most of the experiments the long-acting form of ACTH used was either "Acthar-gel" (Armour Laboratories) or "Cortrophin-ZN" (Organon Laboratories). Where rapid response was required lyophilised ACTH (Crookes Laboratories was employed.

Cortisone was administered as cortisone acetate (Merck, Sharp and Dohme) and cortisol as "Hydrocortisyl" (Roussel) or "Hydro-Adresan" (Organon Laboratories), administration in all cases being intramuscular, unless otherwise stated.

Radioactive phosphorus ^{32}P was supplied as inorganic orthophosphate by The Radiochemical Centre, Amersham, England, either as carrier-free solution in dilute hydrochloric acid (PBS 1) or containing 1 mg. carrier-phosphate per ml. in isotonic saline (PBS 2). The required quantity of ^{32}P was injected intramuscularly 2 hours before the animals were killed.

The animals were killed by sudden stunning and were bled from the neck vessels. The adrenal glands were rapidly removed, freed from extraneous tissue, weighed either on a sensitive beam balance or on a torsion balance. In some cases the glands were stored in the deep-freeze cabinet (-15°) until required for analysis.

The glands were homogenised in distilled water in a Potter-Elvehjem homogeniser to give a total of 10 ml. homogenate. Exactly 3 ml. of the homogenate were pipetted into a 10 ml. centrifuge tube (Fraction A) for quantitative estimation (Fig. 4), the remainder being transferred to another 10 ml. centrifuge tube (Fraction B) for radioactivity determinations (Fig. 5). Both fractions were treated with 0.5 vol. 30% ($^{w}/v$) ice-cold trichloracetic acid (TCA) and kept in ice for half an hour before centrifuging. The precipitate was spun down at 2500 r.p.m. for 10 min. at 0° and the supernatant fluid was collected for the determination of the radioactivity of the acid soluble fraction (A_1S) and of tissue inorganic phosphate (Pi). The residue was washed with approximately 5 ml. ice-cold 10% ($^{w}/v$) TCA and the washings added to the original extract. The fluid from a second similar washing was rejected.

The precipitate was then extracted successively with approximately 5 ml. acetone, ethanol, ethanol-chloroform (3:1) (twice), ethanol-ether (3:1) and finally with ether. The lipid extracts from Fraction A were collected for quantitative estimation of lipid phosphorus (LP) and radioactivity. The lipid extracts from Fraction B were rejected. The residues were dried at room temperature.

Fraction A was subjected to a modified Schneider (1945) procedure for extraction of nucleic acids. The residue was treated with 2-3 ml. 0.5 N- perchloric acid (PCA) for 20 min. at 70°. The extraction was repeated with an equal volume of 0.5 N - PCA and the residue washed with cold 0.5 N - PCA. After each extraction the tubes were centrifuged and as much of the supernatant as possible transferred to a stoppered measuring cylinder. The extract was made up to a total volume of 10 ml. with 0.5 N - PCA. Portions of this extract were used for quantitative estimation of DNAP, RNAP and total nucleic acid phosphorus (TNAP).

Estimation of DNAP. (Ceriotti, 1952).

The DNA standard was a purified sample of the sodium salt of calf thymus DNA from the departmental stocks

prepared by the method of Kay, Simmons and Dounce (1952). A stock solution of DNA was prepared by dissolving an accurately weighed amount (about 20 mg.) of pure, dry DNA in distilled water, with a drop of alkali to help solution, and making up to 50 ml. 1 ml. of this solution was diluted with 0.5 N - PCA, heated to 70° for 20 min. to redissolve any precipitated DNA and then made up to 50 ml. Both the stock solution and the standard solution were kept in a refrigerator. The amount of DNAP in the standard was estimated on 1 ml. portions of the solution by the method of Griswold, Humöller and McIntyre (1951) as described later.

Duplicate DNA standards and reagent blanks were carried out with each group of estimations. For the standard, 2 ml. of the DNA solution were pipetted into a 15 ml. glass-stoppered pyrex tube and for the blank 2 ml. distilled water were taken instead. 1 ml. 0.05% ($^{w}/v$) indole in distilled water (stored in the cold) and 1 ml. conc. HCl (AnalaR) were added giving a total volume of 4 ml.

2 ml. of the PCA extract (or 1 ml. extract plus 1 ml. water in cases where the concentration of DNAP in solution was greater than 5 µg./ml.) were taken for estimation along with 1 ml. indole and 1 ml. conc. HCl

as above. The tubes were immersed in a boiling water bath for 10 min., cooled, and the solutions then extracted with 3 successive portions of about 4 ml. chloroform (B.P. grade*). The chloroform layer after the first two extractions was carefully sucked off by means of a Pasteur pipette and after the last extraction the tubes were centrifuged at low speed (500 r.p.m.) for 5 min. to separate the liquid layers. The chloroform extracts the colour due to substances other than DNA, the yellow colour due to DNA itself remaining in the aqueous layer. The colour intensity was measured against the reagent blank in the Unicam SP 600 spectrophotometer at 490 mμ.

*In this laboratory it has been found that purification of chloroform as described by Ceriotti (1952) is neither necessary nor desirable. This has been confirmed in a private communication from Dr. Ceriotti.

Estimation of RNAP.

[modification of the method of Mejbaum (1939)].

Orcinol reagent: 60 mg. crystalline orcinol (BDH) per 10 ml. of 0.02% (w/v) $FeCl_3.6H_2O$ (AnalaR, BDH) in conc. HCl (AnalaR).

Standard: D- ribose solution containing 10μg/ml. (equivalent to 4.13 μg. RNAP)

2 ml. PCA extract and 1 ml. distilled water (or 1 ml. extract plus 2 ml. water if the extract contained more than 10 µg. RNAP/ml.) were pipetted into a well-cleaned* pyrex test tube. The standards contained 1 ml. solution with 2 ml. water and the blanks 3 ml. distilled water. 3 ml. orcinol reagent were added to the tubes and the tubes were placed in a vigorously boiling water bath for exactly 30 min. and then cooled rapidly under the tap. The colour intensity was measured against the reagent blank in the Unicam SP 600 spectrophotometer at 665 mµ.

* Note: Thorough cleaning of glassware was essential to achieve reproducible results. The test tubes after being cleaned with a detergent were left in chromic acid overnight. They were then thoroughly washed with tap water and finally immersed in glass-distilled water for several hours before being dried.

Estimation of TNAP.

(Logan, Mannel and Rossiter, 1952).

The spectrophotometric analysis was carried out on the PCA extract of the nucleic acids by measuring the

total absorption at 265 mμ due to the purine and pyrimidine nucleosides and nucleotides present in the extract. The extraction procedure causes considerable degradation of the nucleic acids which, however, does not result in any net loss of absorbing material (Beaven, Holiday and Johnson, 1955). On the other hand, it is an essential feature of this method that the nucleic acids should be degraded so that a direct correlation between absorptivity and concentration be made.

These measurements were made, however, purely as a check on the values obtained for DNAP and RNAP, and in all cases the total values obtained for the latter by Ceriotti and orcinol methods was equal to that obtained for TNAP by ultraviolet absorption within limits of experimental errors involved in these methods.

Estimation of Protein nitrogen (PrN). (Paul, 1958).

Selenium dioxide: 1%(^{W}/v) SeO_2 (M & B) in 50%(^{V}/v) H_2SO_4 (AnalaR).

Nessler's reagent: A solution of 3.5 g. gum acacia in 350 ml. distilled water added to a solution of 4g. KI (AnalaR) and 4g. HgI_2 (B.P. grade) in 25 ml. distilled water and then made up to 1 l. .

Standard: Solution of ammonium sulphate (AnalaR) to contain 10μg.N/ml.

PrN was estimated in the residue after extraction of nucleic acids with PCA. The résidue was washed with 5% ($^{w}/v$) TCA and dried with ethanol and ether. It was then digested with 0.5 ml. SeO_2 reagent, until the contents were clear. The digest was washed into a measuring cylinder by rinsing with distilled water and made up to a known volume so as to give a concentration of about 10-20 µg. N/ml.

2 ml. of the solution were treated with 2 ml Nessler's reagent and 3 ml 2 N-NaOH (AnalaR). 2 ml. ammonium sulphate standard and 2 ml. water as a blank were similarly treated. The colour was allowed to develop for 15 min. and the intensity measured in the Unicam SP 600 spectrophotometer at 490 mµ against that of the blank.

Estimation of the amount and radioactivity of LP.

The combined lipid extracts from fraction A were made up to a known volume. 10 ml. extract were pipetted into a microkjeldahl flask, 1.2 ml. 10 $N.H_2SO_4$ added, the organic solvents evaporated in a waterbath and the contents then digested over a flame. In the earlier experiments the phosphorus in the digest was

assayed by the method of Allen (1940), and the radioactivity measured in 5 ml. of the blue solution using a liquid counter (Type M6, 20th Century Electronics). coupled to a scaler (Type 100 C, Panax Equipment).

In some experiments the clear digest was made up to 10 ml. with distilled water and radioactivity measured in 5 ml. of this solution. In these cases the phosphorus was determined by a slight modification of the more sensitive method of Griswold _et al._ (1951). Details of the modified method are given below.

Reagents: reducing agent: 54.4 g. of sodium meta bisulphite (M & B, reagent grade), 4 g. $Na_2SO_3 7H_2O$ (AnalaR, BDH) and 1 g. 1-naphthol-2-amino-4-sulphonic acid (BDH, extra pure) dissolved in 300 ml. water, made up to 1 l., filtered, if necessary, and kept in a stoppered brown bottle. (The solution keeps well for several months. A small amount of crystallisation sometimes occurred, but this did not appear to affect the results in any way).

Ammonium molybdate: 2.5% ($^w/v$) (AnalaR) solution in distilled water.

standard: 2.193 g. KH_2PO_4 (AnalaR) in 500 ml. distilled water. 1 ml. of this stock solution was diluted to 500 ml. to give a standard of 2 µg. P/ml.

Method: 1 ml. of the lipid extract (or 2 ml. if the amount of LP was less than 1 µg/ml.) was pipetted into a graduated pyrex test tube and 0.5 ml. each of 10 N-H_2SO_4 and 4N-PCA were pipetted. When the organic solvents had been removed by evaporation in a waterbath the contents were digested. The contents were cooled, diluted to about 3 ml., and 0.5 ml. reducing agent followed by 0.5 ml. ammonium molybdate added, the contents being well mixed after each addition. Standards consisting of 1 ml. of the standard solution and 0.5 ml. 10N-H_2SO_4, and a blank with 0.5 ml. 10 N-H_2SO_4 diluted with water were treated in the same way. The tubes were then immersed in the boiling water bath for 10 min. The solutions were cooled, made up exactly to the 5 ml. mark and the colour intensity measured against the reagent blank in the Unicam SP600 spectrophotometer at 820 mµ.

Estimation of the specific activities of the acid soluble fraction (A_1s) and of tissue inorganic phosphate (P_1).

The TCA extract of the homogenate and the first TCA

washing were pooled and made up to 20 ml., the solution being kept in ice. 1 ml. of this solution was pipetted into a graduated tube, for phosphorus estimation by the method of Griswold et. al. (1951) the radioactivity being assayed on the blue solution as described for ALP.

The remaining portion was neutralised with conc. ammonia (AnalaR) using phenolphthalein as indicator. 1 ml. Mathison's (1909) reagent was added to each tube together with a drop of a fine suspension of powdered glass (which considerably helps the crystallisation of $Mg(NH_4)PO_4$, in cases where the amount of inorganic phosphate present is very small). The tubes were kept overnight at 4° and the resulting precipitate (sometimes very small in amount) was filtered, washed 2 or 3 times with 10% ($^V/v$) ammonium hydroxide, dissolved in 1-2 ml. 2N-HCl and the solution made up to a known volume. 5 ml. of this solution were taken for radioactive assay and 1 ml. transferred to a graduated tube for estimation of phosphorus by the method of Griswold et al. (1951).

Measurement of the specific activity of RNAP and DNAP.

The acid in-soluble non-lipid phosphorus residue (AINLP) from fraction B was subjected to a modification of the separation method of Schmidt and Thannhouser (1945) as described by Davidson and Smellie (1952 a).

The AINLP residue was hydrolysed with 0.3 N-KOH (about 1 ml./5 mg.) for 18 hours at 37°, the mild hydrolysis breaking down the RNA to acid soluble mononucleotides. The DNA and protein, which are considerably more resistant to this alkaline hydrolysis, were precipitated at 0° by acidifying the hydrolysate to pH 1 with 60% (w/w) PCA. The precipitate was centrifuged in the cold, the supernatant being removed by means of a Pasteur pipette. The residue was washed twice with about 5 ml. 0.5N-PCA and the washings rejected.

Purification of RNA mononucleotides by ionophoresis.

The supernatant fluid (A_2s) containing the ribonucleotides and other concomitant phosphorus compounds (Davidson and Smellie, 1952b; Hutchison, Crosbie, Mendes, McIndoe, Childs and Davidson, 1956) was purified by ionophoresis on paper by the method of Davidson and Smellie (1952 a). The supernatant was brought to pH 3.5 with N-KOH in the cold and the precipitated $KClO_4$ centrifuged down. A portion of the supernatant containing about 100 µg. P was applied along a line 7cm. from one end of a strip of Whatman 3 mm paper (72 x 7 cm. in size). The paper was moistened with 0.02 M - citrate

buffer of pH3.5 allowing the excess liquid to drain off, and suspended over a glass rod, the two ends of the paper being immersed in the buffer contained in two glass or perspex dishes. A smoothed D.C. voltage was applied by means of carbon electrodes placed in each dish so that the anode was in the dish further away from the spot. The separation was carried out with a potential gradient of 11 v./cm. for a period of 18 hours. This resulted in the separation of the four mononucleotides. The paper was removed, dried by means of an infrared lamp and the nucleotide bands located visually in ultraviolet light at 260 mμ by the method of Holiday and Johnson (1949). The bands were cut out and eluted by the method of Consden, Gordon and Martin (1947). The phosphorus in the nucleotide fractions was measured by the method of Allen (1940) and the radioactivity assayed on the blue solution as already described. The specific activity of RNAP was taken as the mean of the values obtained for the four individual nucleotides.

Purification of DNA.

The precipitated mixture of DNA and protein was suspended in 0.9% ($^{w}/v$) NaCl, adjusted to pH7 with NaOH, and treated with a solution of duponal (5% $^{w}/v$)

sodium dodecyl sulphate in 45% ($^{v}/v$) ethanol], 1 ml. being added to every 10 ml. mixture. The solution was kept at room temperature for 1 hour with occasional stirring after which enough solid NaCl was added to raise the concentration to 1M. This precipitated the proteins and in order to complete the precipitation the tubes were left in the deep freeze cabinet (-15°) for 1 hour and then allowed to thaw. The precipitate was centrifuged, the supernatant was treated with 2 volumes of ethanol and the tubes were kept at 4° overnight to coagulate the DNA, which was then centrifuged down, washed twice with 70% ethanol and dried with ethanol and ether.

The DNA was dissolved in distilled water with a drop or two of NaOH and a convenient portion was transferred to a microkjeldahl flask for phosphorus estimation by the method of Allen (1940). The radioactivity was measured on the blue solution.

Modified procedure for the isolation of nucleic acids in small amounts of tissue.

A slight modification of the method of Davidson and Smellie (1952 a) was used to isolate the nucleic acids in which the residue was extracted with 2-3 ml. M-NaCl in

a boiling water bath for 1 hour with the addition of 0.2-0.3 ml. duponal (cf. above). After centrifugation, the supernatant was removed and the residue re-extracted as before for another 30 min. The combined supernatants were treated with 2 volumes ethanol and the nucleic acids allowed to coagulate overnight at 4°, centrifuged, washed twice with 70% ethanol and dried with ethanol and ether.

The dry nucleic acid powder which is fairly free from protein was treated as outlined above for separation of the nucleic acids.

In this case the ionephoresis for separation of RNA nucleotides was carried out on washed Whatman 3 MM paper strips, the washing being carried out as follows. A few 73cm. lengths of paper were immersed in 0.01 N-HCl (AnalaR) for several hours, the liquid being kept stirred. The acid was then replaced by distilled water and changed every few hours until all the acid was washed out, after which the papers were taken out and dried.

A very good separation of the nucleotides from the A_2s fraction was obtained, four bands being clearly visible in u.v. light even when as little as 10-15µg. total RNAP was applied to the papers.

The spots were cut out, eluted and the eluates of

the four nucleotides pooled. 5 ml. of this solution were used for radioactive assay and 1 ml. transferred to a graduated tube for phosphorus estimation by the method of Griswold et. al. (1951).

The precipitate of DNA, after being washed twice with 0.5 N. PCA, was dissolved in distilled water with a drop of NaOH added. 5 ml. of the solution was used for determining the radioactivity and 1 ml. for estimation of phosphorus by the method of Griswold et al. (1951).

In some experiments, as indicated in Part III, a further purification of DNA by electrophoresis on paper was carried out. The precipitate of DNA was dissolved in 0.2 ml. distilled water and the pH adjusted to 8 with 0.1 N-KOH. This solution was applied to washed 3 MM paper as in the case of the RNA nucleotides and run at pH 7.5 in 0.05 M-tris (2-amino-2-hydroxymethyl propane-1:3-diol) buffer for 4 hours at 11 v./cm. The DNA spot which remains at the origin was cut out, eluted from the paper with 0.1 N-KOH and the eluate made up to approximately 6 ml. with water. 5 ml. of this DNA solution were used for measurement of radioactivity and 1 ml. for phosphorus estimation as above.

Tests of statistical significance.

Statistical analyses of the results in the present series of experiments will be found in Part III. The

results in the tables are expressed as mean values $\pm$ the standard error of the mean (S.E.) where

$$S.E. = \sqrt{\frac{\Sigma(x-\bar{x})^2}{N(N-1)}}$$

Student's 't' test was used to assess the significance of the difference between the mean values for two groups of data. Where more than two groups of data were compared analysis of variance (Snedecor, 1946) was used. The expressions $P < 0.05$, $P < 0.02$, and $P < 0.01$ are used in the conventional sense to indicate significance at the 5%, 2% and 1% levels respectively and $P << 0.01$ to indicate significance at very much below the 1% level.

Fig. 4.

Outline of the procedure for the quantitative estimation of constituents of the adrenal gland.

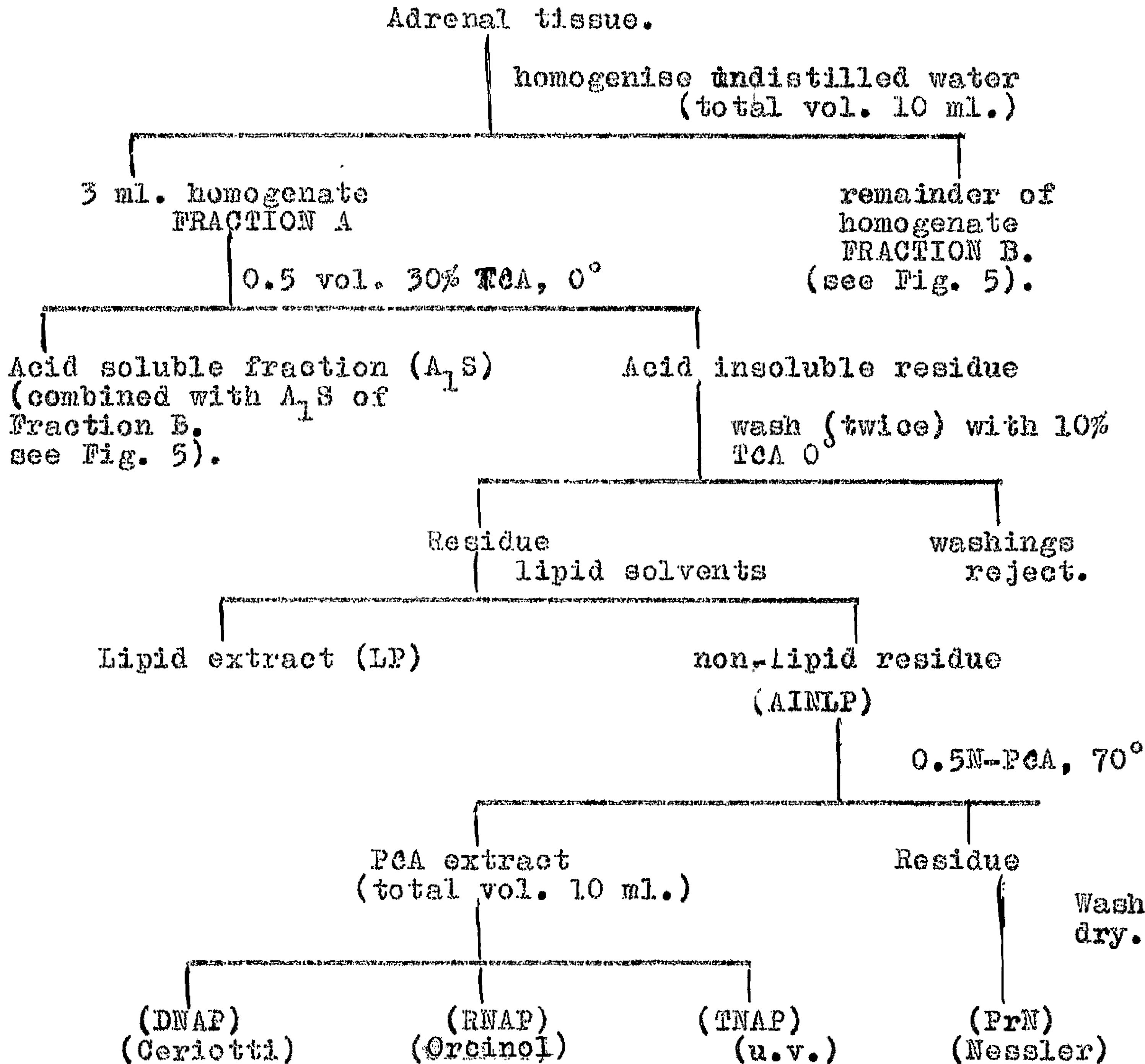


Fig. 5

Outline of the procedure for the determination of phosphorus and radioactivity of various components of adrenal tissue.

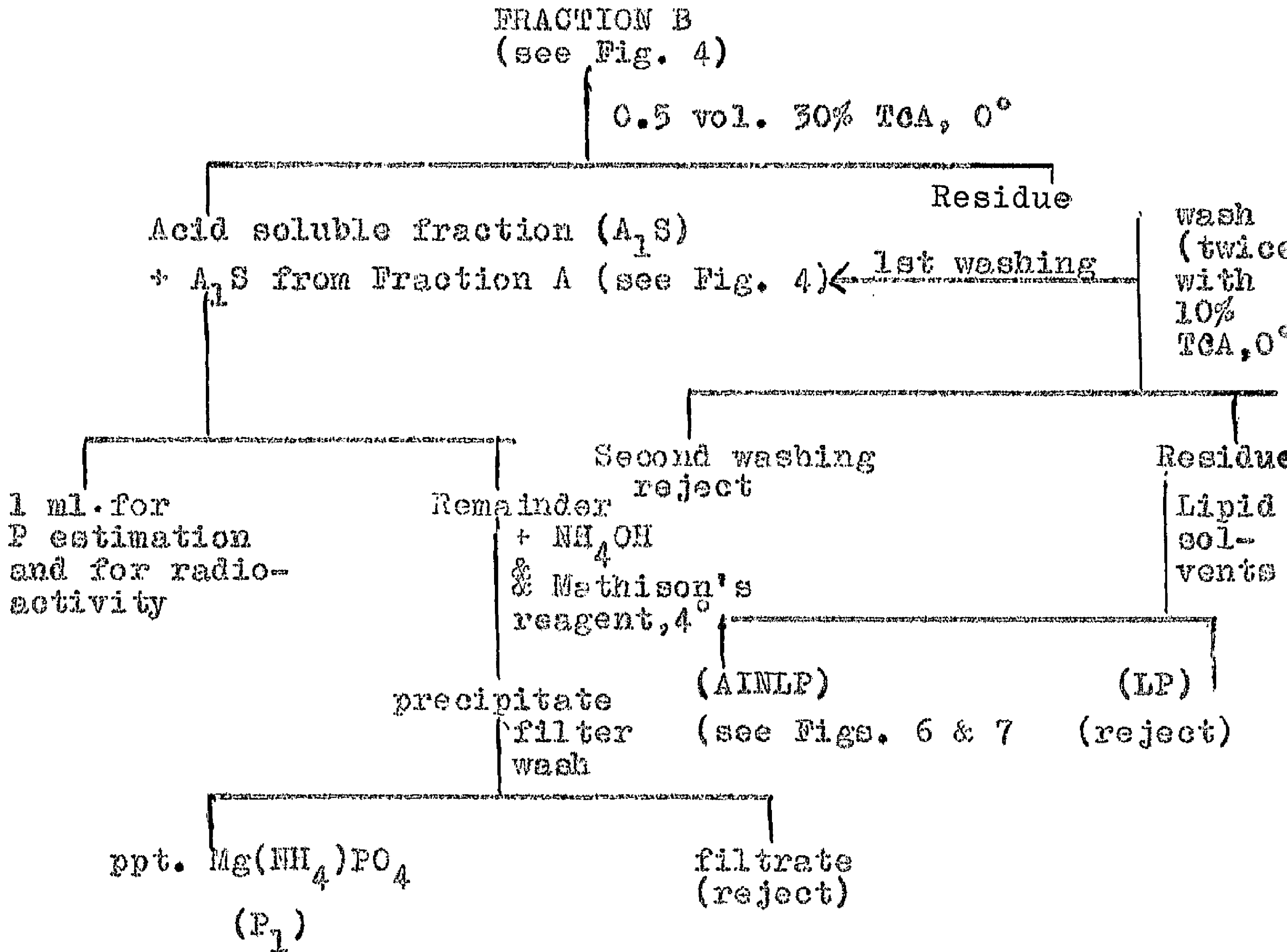


Fig. 6

Separation of nucleic acids for determination of specific activities

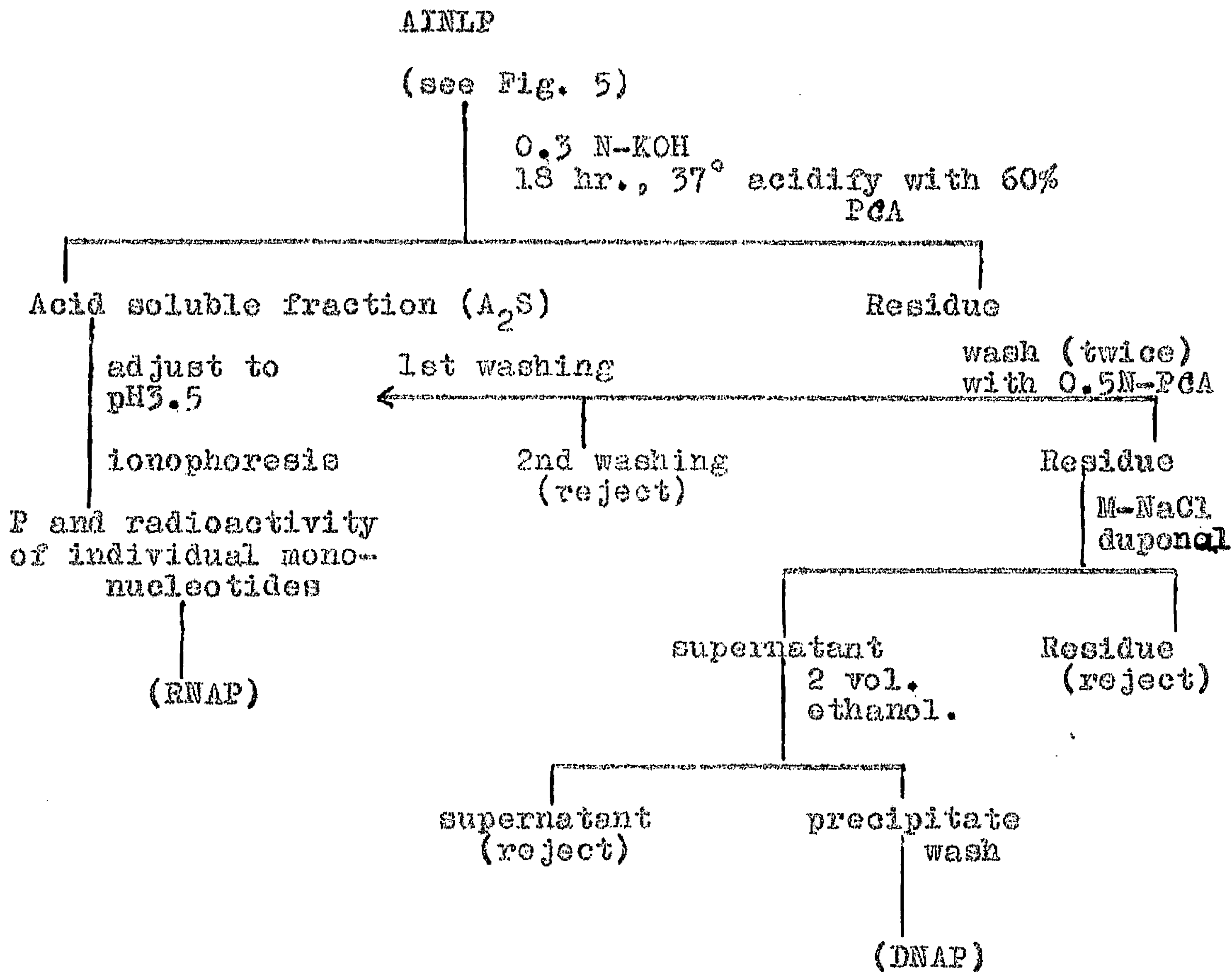
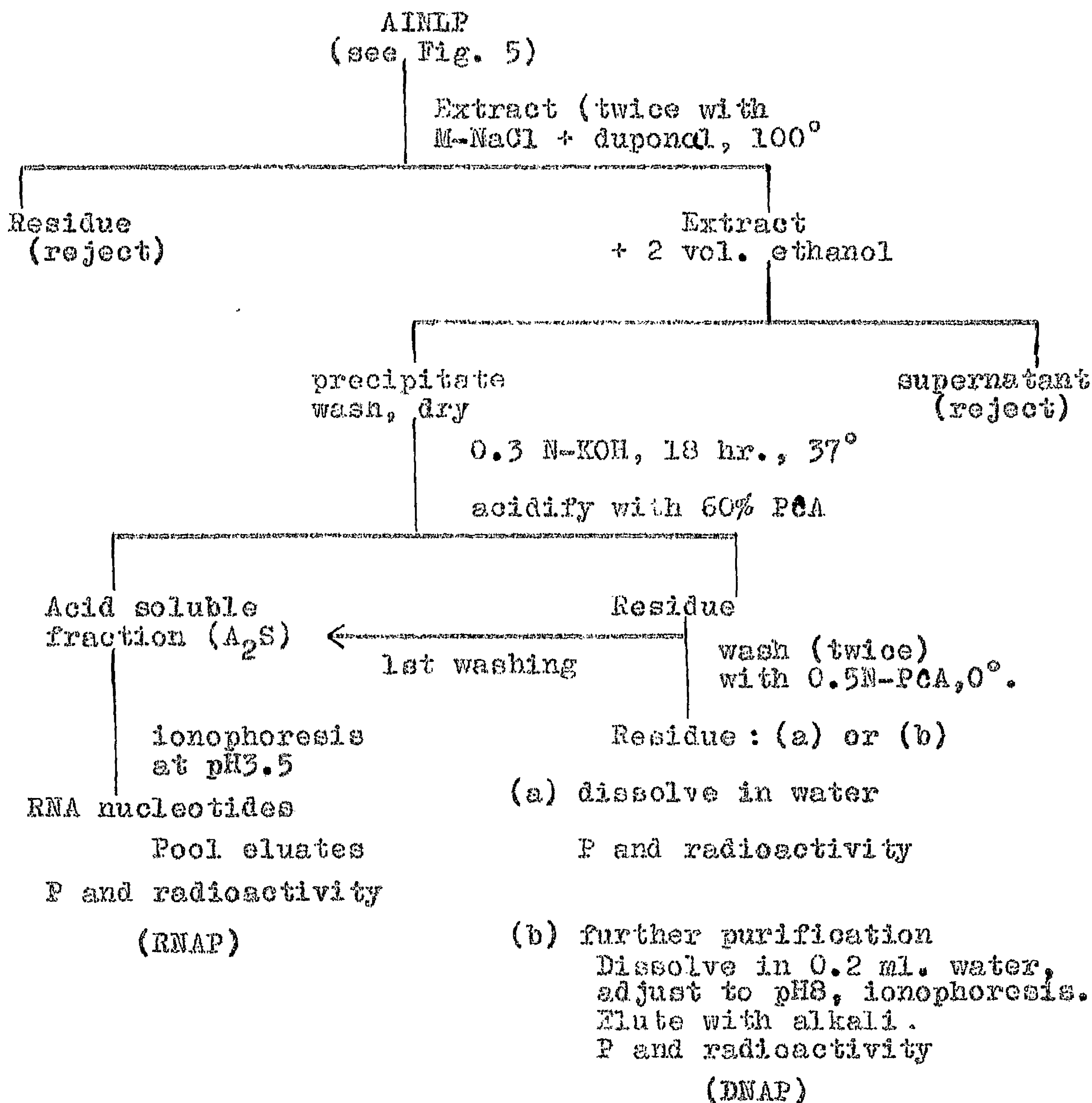


Fig. 7

Modified procedure for isolation of pure nucleic acids from small amounts of tissue.



PART III

RESULTS

Section 1.

The Action of ACTH on the Adrenal Glands of Guinea-pigs and Rats for Varying Periods.

The initial experiments were designed primarily to study the effect of ACTH on the adrenal nucleic acids of guinea-pigs. 24 animals of body weight 200-250 g. were arranged in 4 groups of 6 animals each. One of the groups was taken as the control and was untreated. The other groups received intramuscular injections of Acthar-gel, each animal receiving a total of 10 mg. per day given in two doses. The 3 groups were treated for 1, 3, and 5 days respectively and the animals were killed on the morning following the last injection.

Analyses were carried out on 6 pooled glands from 3 animals of each group and the results, which are shown in Table k, represent the mean values of the constituents per pair of glands and also the concentrations per 100 mg. wet weight of adrenal tissue.

From the results of the experiment it is evident that the glands undergo considerable enlargement between the first and third days of treatment. It was therefore decided in a further experiment to include a 2-day treatment, and also to study the incorporation of radioactive phosphorus. The experiment was slightly modified to include only two animals (body wt. about 320 g.) in each group. The animals received ACTH for 1, 2, 3, and 5 days respectively exactly as before, but the control group, in this case, received

Table 1

Effect of ACTH administration for varying periods on guinea-pig adrenals

		Days of ACTH Administration			
		0 (control)	1	3	5
wt. per 2 glands (mg.)		131 ± 3.8	154 ± 10.7	220 ± 3.5	267 ± 8.[illegible]
LP	µg/2 glands	218 ± 6.1	241 ± 22.0	311 ± 8.0	371 ± 3.[illegible]
	µg/100 mg.	167 ± 0.2	157 ± 4.0	141 ± 1.2	139 ± 5.[illegible]
RNAP	µg/2 glands	69.9 ± 0.6	84.5 ± 6.5	129.5 ± 0.5	149.5 ± 1.[illegible]
	µg/200 mg.	53.8 ± 1.0	54.9 ± 0.3	58.9 ± 1.0	56.1 ± 1.[illegible]
DNAP	µg/2 glands	26.8 ± 0.1	26.3 ± 3.2	35.1 ± 1.5	44.8 ± 1.[illegible]
	µg/100 mg.	20.5 ± 0.7	17.0 ± 0.9	13.7 ± 1.4	16.8 ± 0.[illegible]
RNAP/DNAP		2.61	3.25	3.69	3.39
LP/DNAP		8.18	9.20	8.87	8.28

Table 2

Analytical values (µg/2 glands) for the adrenals of guinea-pigs treated with ACTH for varying periods expressed as percentage increases over the control group (Data from Table 1)

Days of ACTH	1	3	5
Gland wt.	18	68	104
LP	11	43	70
RNAP	21	85	114
DNAP	0	31	67

0.9% saline injections to compensate for any stress due to handling and injecting. In addition all animals received 300 µc ^{32}P 2 hours prior to killing.

The results are given in Tables 3 and 4. The analytical values (µg./2glands) of the two experiments are also expressed as percentage increases of the test groups over the control groups, and are shown in Tables 2 and 5. Table 6 shows the statistical analysis of data presented in Table 4 for the uptake of ^{32}P into RNAP and DNAP.

The glands show a response to the administration of ACTH by the first day as indicated by the increase in gland weight as well as in RNAP and LP. The DNAP, however, does not show any measurable increase over 1 day. The increases become more marked with continued administration of ACTH, and after 3 days there is a 60-70% increase in gland weight and an even greater increase in RNAP. Expressed as percentage increases over the controls, the LP increases much less than the RNAP and the gland weight. Over the 5 day period there is no consistent picture because the continued increase shown in one experiment is not shown in the other. Measurable increases in DNAP are seen after the second day, and amount to 25-30% over the control values after 3 days of treatment.

Table 3

Effect of ACTH administration for varying periods

on guinea-pig adrenals

		Days of ACTH Administration				
		0 (control)	1	2	3	5
wt./2 glands (mg.)		197±0.3	227±4.5	254±15.2	319±16.1	296±34.8
LP	µg/2 glands	296±7.6	321±8.0	345±21.0	433±15.5	367±12.5
	µg/100 mg.	150±3.4	142±0.5	136± 0.1	135± 1.2	141± 4.0
RNAP	µg/2 glands	68.7±0.2	84.2±3.3	99.3±7.7	128.2±8.8	124±16.5
	µg/100 mg.	34.9±0.2	37.2±0.7	39.1±0.8	40.2±0.7	42± 0.7
DNAP	µg/2 glands	44.4±0.8	38.2±6.2	48.9±0.3	55.3±0.4	57± 8.8
	µg/100 mg.	23.1±0.2	17.2±2.6	19.8±0.6	18.9±0.5	19.3±0.5
RNAP/DNAP		1.55	2.25	2.02	2.32	2.19
LP/DNAP		6.69	8.57	8.05	8.33	6.54

Table 4

Specific activities (relative to tissue inorganic phosphate x 10^3) of RNAP and DNAP from the adrenals of guinea-pigs treated with ACTH for varying periods

Days of ACTH	0	1	2	3	5
RNAP	33.8 ± 0.2	32.2±3.2	40.3±0.3	47.4±0.1	49.5±1.5
DNAP	3.4 ± 0.5	4.9±0.3	10.8±0.8	12.6±1.0	12.9±1.7

Table 5

Analytical values (µg/2 glands) for the adrenals of guinea-pigs treated with ACTH for varying periods expressed as percentage increases over the control group

(Data from Table 3)

Days of ACTH	1	2	3	5
Glands wt.	15	29	62	50
LP	9	17	46	24
RNAP	23	44	87	81
DNAP	0	10	25	28

Table 6

Statistical analysis of data presented in Table 4.

Analysis of variance to test the significance of differences between the mean values of the relative specific activities of RNAP and DNAP from control and ACTH-treated groups.

	source of variation	degrees of freedom	sum of squares	mean squares	variance ratio, F
	Total	9	509.8		
RNAP	Treatment	4	485.1	121.3	$\frac{121.3}{4.9} = 24.8$
	Residual	5	24.7	4.9	
	Total	9	168.8		
DNAP	Treatment	4	159.1	39.8	$\frac{39.8}{1.94} = 20.5$
	Residual	5	9.7	1.94	

For $n_1 = 4$ and $n_2 = 5$, $F = 11.39$ at 1% significant level. There is therefore a very highly significant difference between the mean values for control and ACTH-treated groups for both RNAP and DNAP.

Significant increases of ^{32}P uptake into RNAP and DNAP are shown after 2 days and these increases continue with further administration of ACTH.

The preceding experiments showed a lag period of up to 1 day in the action of Acthar-gel in inducing a significant change in the amount of and the ^{32}P incorporation into the nucleic acids and LP. It was therefore decided to study whether this delay was due mainly to the slow action of Acthar-gel used and an experiment was carried out with guinea-pigs combining the Acthar-gel with lyophilised ACTH.

Three groups of 4 guinea-pigs (body wt. 430-470 g.) were taken. The control group received 0.9% saline injections. Each animal in the other groups received 2 mg. lyophilised ACTH every 4 hours during the day and one injection of 5 mg. Acthar-gel during the night. The two groups were treated for 1 and 2 days respectively. The animals were killed on the morning following the last injection. 500 μc ^{32}P was given to each animal 2 hours before sacrifice.

The DNA was purified in this and the following experiment by ionophoresis on paper as described in the section on general methods.

The results are shown in Tables 7 and 8.

Table 7

Stimulation of guinea-pig adrenal glands by a combination of Acthar-gel and lyophilised ACTH

		Days ACTH administration		
		0 (control)	1	2
wt./2 glands (mg.)		253 ± 14.6	287 ± 18.7	319 ± 15.6
LP	μg/2 glands	444 ± 32	575 ± 81	619 ± 40
	μg/100 mg.	177 ± 3	183 ± 4	183 ± 14
RNAP	μg/2 glands	114 ± 10.7	162 ± 21.8	188 ± 7.8
	μg/100 mg.	45.5 ± 1.8	60 ± 4	59 ± 1.4
DNAP	μg/2 glands	39.3 ± 3.7	49.3 ± 4.0	48.4 ± 0.8
	μg/100 mg.	15.6 ± 0.6	17.2 ± 0.7	15.2 ± 0.8
PrN	μg/2 glands	3690 ± 325	5080 ± 830	5520 ± 160
	μg/100 mg.	1460	1740	1750
RNAP/DNAP		2.91	3.29	3.88
LP/DNAP		11.3	11.7	12.8

Table 7(a)

Mean body weights of animals used in the experiment reported in Table 7.

Days of ACTH	0 (control)	1	2
mean body wt. (g.)	452 ± 15	442 ± 12	437 ± 8

In order to find the effect of ACTH on guinea-pig adrenals over short periods of a few hours, an experiment was carried out with 3 groups of 4 animals (body wt. 430-470 g.). In this experiment, only lyophilised ACTH was used. One of the test groups received 10 mg. lyophilised ACTH and was killed 4 hours later. The two other groups were given 5 mg. lyophilised ACTH per animal every 4 hours and killed 8 and 16 hours after the initial injection. Each animal received 500 μc ^{32}P 2 hours before sacrifice.

The analyses were carried out with the pair of glands from each animal and the results are given in Tables 9 and 10. The mean body weights of the animals used in these two experiments are given in Tables 7(a) and 9(a).
(Note: The control values in Tables 9 and 10 are the same as those given in Tables 7 and 8 since the two experiment were carried out simultaneously with animals of similar body weights, and the same control group served for both).

In these experiments it is seen that the administration of lyophilised ACTH plus Acthar-gel gives a rapid response which appears to be maximal after 1 day, any further increases observed with continued treatment being without statistical significance. Although this applied to the

Table 8

Specific Activities (relative to tissue inorganic phosphate x 10^3) of LP, RNAP and DNAP from adrenals of guinea-pigs receiving both Acthar-gel and lyophilised ACTH.

Days of ACTH	0	1	2
LP	16.5 ± 1.4	12.7 ± 1.3	22.0 ± 2.4
RNAP	12.6 ± 1.6	22.8 ± 1.7	22.6 ± 1.6
DNAP	4.6 ± 0.4	18.1 ± 2.9	17.8 ± 4.8

Table 9

Effect of lyophilised ACTH on guinea-pig adrenals over short intervals of time

		Hours of ACTH administration			
		0 (control)	4	8	16
wt./2 glands (mg.)		257 ± 14.6	229± 9.6	244±11.2	260 ± 10.5
LP	µg/2 glands	444 ± 32	404± 13	425±20	470 ± 37
	µg/100 mg.	177 ± 3	177± 3	177± 6	180 ± 8
RNAP	µg/2 glands	114 ± 10.7	105± 4.2	128± 12	125 ± 4.4
	µg/100 mg.	45.5± 1.8	45.9±1.2	50.6±1.8	48.0± 1.1
DNAP	µg/2 glands	39.3± 3.7	41.2±2.2	41.7±2.2	45.1± 1.3
	µg/100 mg.	15.6± 0.6	18.1±1.2	16.5±0.6	17.4± 0.8
PrN	µg/2 glands	3690± 325	3480±200	3710±171	4040± 260
	µg/100 mg.	1460	1520	1480	1560
RNAP/DNAP		2.91	2.55	3.07	2.80
LP/DNAP		11.4	9.9	11.1	10.7

Table 9(a)

Mean body weights of animals used in the experiment reported in Table 9.

Hours of ACTH	0	4	8	16
Body wt. (g.)	452 ± 15	449 ± 16	445 ± 23	450 ± 20

Table 10

Specific Activities (relative to tissue inorganic phosphate x 10^3) of LP, RNAP, DNAP from adrenals of guinea-pigs receiving lyophilised ACTH for short periods.

Hours of ACTH	0	4	8	16
LP	16.5 ± 1.4	18.9 ± 0.6	16.1 ± 0.7	16.3 ± 1.2
RNAP	12.6 ± 1.6	15.6 ± 0.6	21.4 ± 3.1	15.9 ± 0.6
DNAP	4.6 ± 0.4	5.0 ± 0.8	5.8 ± 1.2	5.3 ± 1.0

uptake of ^{32}P into RNA and DNA, the relative specific activity of LP does not conform to this pattern. A possible interpretation of this is discussed later.

The effect of Acthar-gel alone is never so great and develops more gradually, treatment for 1 day inducing a slight hypertrophy in the gland with no measurable increase in DNAP. Stimulation for 2 days or more causes an increase in the amount of DNAP, and this is also shown by a very significant increase in the uptake of ^{32}P into the DNA (Fig. 8).

On the other hand administration of ACTH to guinea-pigs for shorter periods of time up to 16 hours does not produce any such stimulation. The group treated for 4 hours gives values for gland weight and amounts of LP and RNAP actually below the control values though these differences are not statistically significant. The relative specific activities of LP, RNAP and DNAP also do not show significant differences between the test and control groups except for RNAP in the case of the group receiving ACTH for 8 hrs.

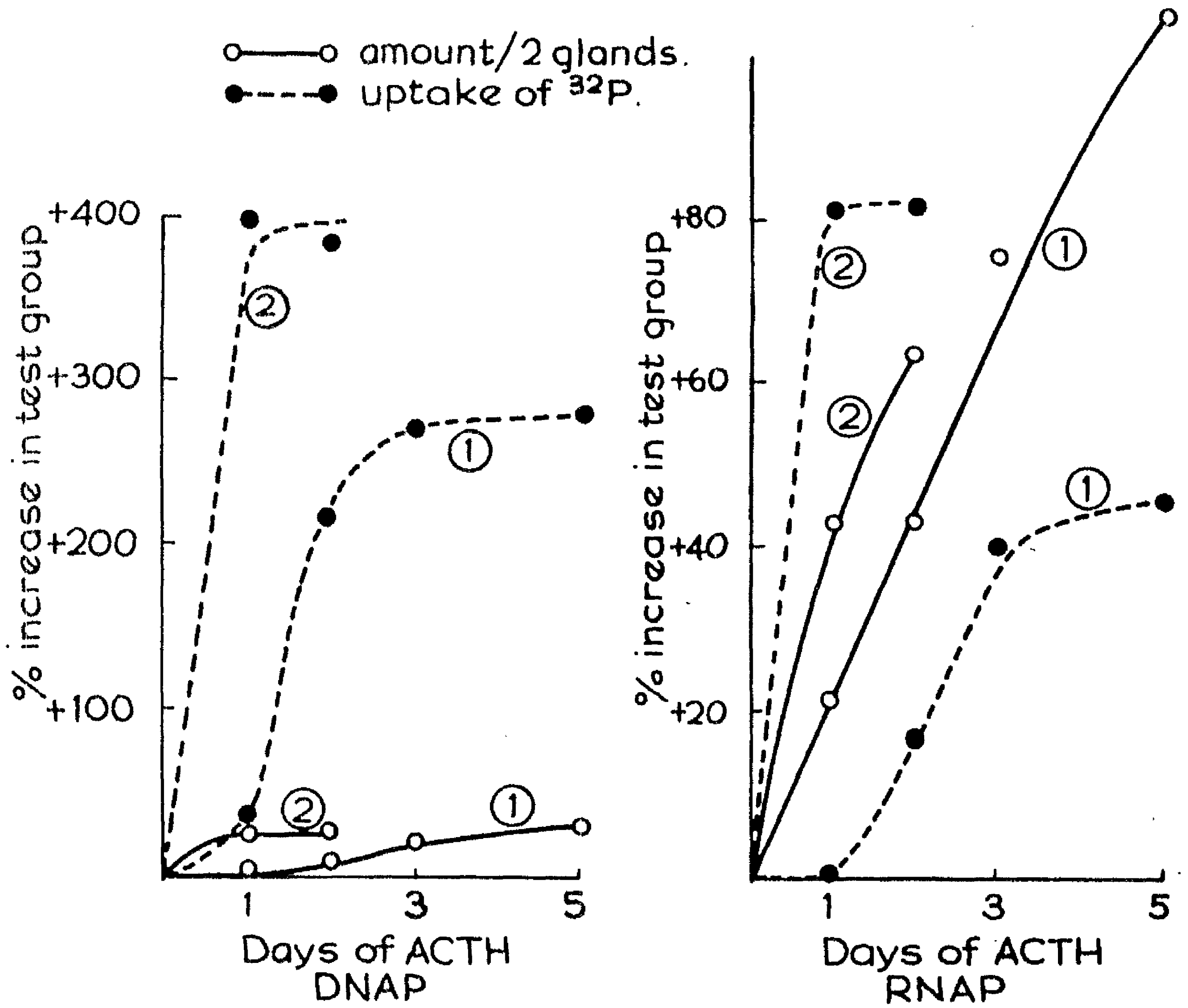
Effect of ACTH for varying periods on rat adrenal glands.

The experiments with guinea-pigs showed that the degree of stimulation of the adrenal gland depends on the duration

Fig. 8.

Comparison of the effect of Acthar-gel alone or Acthar-gel in combination with lyophilised ACTH on adrenal nucleic acids in guinea-pigs. Observed increases in the test groups represented as percentage over control group. (Note the difference in scales for DNAP and RNAP).

Fig. 8



① - *Stimulation with Acthar-gel.*
② - *Stimulation with Acthar-gel + lyophilised ACTH.*

of ACTH administration. It was of interest, therefore to carry out a similar experiment with rats.

Four groups of 6 female albino rats (body wt. 160-180 g.) were treated with 10 mg. Acthar-gel daily for 1, 2, 3 and 5 days respectively. A control group of rats received 0.9% saline injections. 500 μc of ^{32}P was given to each rat 2 hours before killing on the day following the last injection. Analyses were carried out on 6 pooled glands from 3 animals of each group, and the results are shown in Tables 11 and 12.

The analytical values (μg./2 gland) of Table 11 are also expressed in Table 13 as percentage increases for the ACTH-treated groups over the control group.

The statistical analyses of data in Table 12 are given in Table 14 and show the significance of the differences in the mean values for the uptake of ^{32}P into RNA and DNA.

Treatment for 1 day produces no noticeable increase either in the gland weight or in any of the constituents measured. A marked increase, however, appears to occur between 1 and 2 days and no further increases are observable with continued treatment. Although there is no measurable increase in DNAP over the 5 day period tested, an increased uptake of ^{32}P is observed. The relative specific

activities of LP and RNAP show a significant decrease compared with the control values. The total radioactivity of RNAP (Table 12) remains fairly constant.

The changes observed in nucleic acids of the adrenal glands of both guinea-pigs and rats upon ACTH administration are compared in Fig. 9.

Table 11

Effect of ACTH on rat adrenals for varying periods

		Days of ACTH				
		0 (control)	1	2	3	5
wt/2 glands (mg.)		55.2±3.7	55.9±3.2	70.1±2.5	66.8±0.8	69.6±4.6
LP	µg/2 glands	98.3±5.5	89.6±9.6	108 ±2.1	102.6±3.3	107 ±5.5
	µg/100 mg.	168±6.9	160±7.0	155 ±4.6	154 ±6.6	153.5±2.0
RNAP	µg/2 glands	29.9±1.0	30.7±2.6	39.1±4.8	37.7±1.4	39.0±5.0
	µg/100 mg.	54.7±1.2	54.8±1.3	55.6±4.9	55.6±2.9	55.9±3.5
DNAP	µg/2 glands	13.8±0.4	14.3±0.7	14.4±1.1	13.6±0.4	13.8±1.4
	µg/100 mg.	25.1±1.6	25.8±0.1	20.4±0.8	20.4±0.9	19.7±0.4
RNAP/DNAP		2.17	2.14	2.71	2.77	2.99
LP/DNAP		7.13	6.27	7.55	7.55	7.75

Table 12

Specific activities (relative to Tissue inorganic phosphate x 10^3) of LP, RNAP and DNAP from the adrenals of rats given ACTH for periods up to 5 days.

Days of ACTH	0	1	2	3	5
LP	29.3 ± 0.1	24.8±1.7	24.3±3.5	19.8±1.0	21.2±2.0
RNAP	64.3 ± 0.2	65.4±2.9	60 ±4.6	50 ±1.4	50.6±0.2
DNAP	4.2 ± 0.5	6.0±0.1	7.3±0.4	8.1±1.0	8.8±0.2
*Total radio-activity of RNAP	1920	1940	2340	1880	1920

* Total radioactivity of RNAP = µg/2 glands x relative specific activity.

Table 13

Analytical values (µg/2 glands) from Table 11 for the ACTH treated groups expressed as percentage increases over the control group.

Days of ACTH	1	2	3	5
Gland wt.	1	27	20	25
LP	0	11	5	9
RNAP	3	31	26	31
DNAP	0	0	0	0

Table 14

Statistical analysis of results given in Table 12. Analysis of variance to test the significance of differences between the mean values of relative specific activities of RNAP and DNAP for the ACTH-treated and control groups of rats.

	source of variation	degrees of freedom	sum of squares	mean squares	variance ratio F
	Total	9	477.3		
RNAP	Treatment	4	410.9	102.7	7.72
	Residual	5	66.4	13.3	
	Total	9	29.5		
DNAP	Treatment	4	26.6	6.65	11.48
	Residual	5	2.9	0.58	

For $n_1 = 4$, $n_2 = 5$ the value of $F = 11.39$ at the 1% level of significance and 5.19 at the 5% level of significance.

It is seen that the differences between the mean values of the relative specific activities for RNAP are significant at less than the 5% level and for DNAP at less than the 1% level.

Fig. 9.

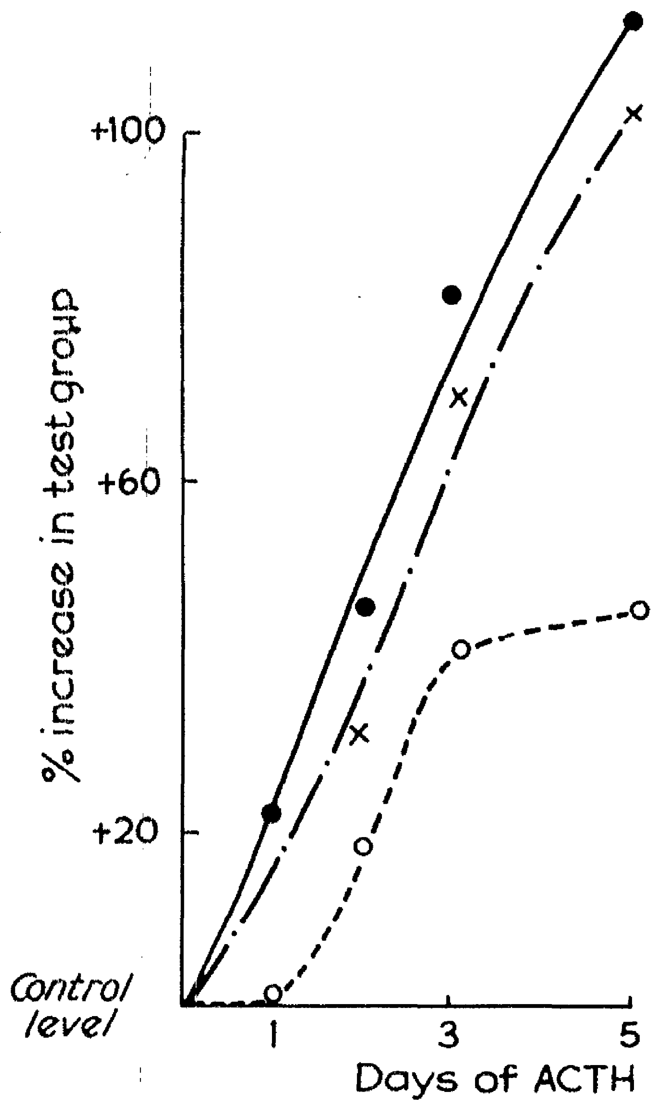
Comparison of the action of Acthar-gel on guinea-pig and rat adrenal glands.

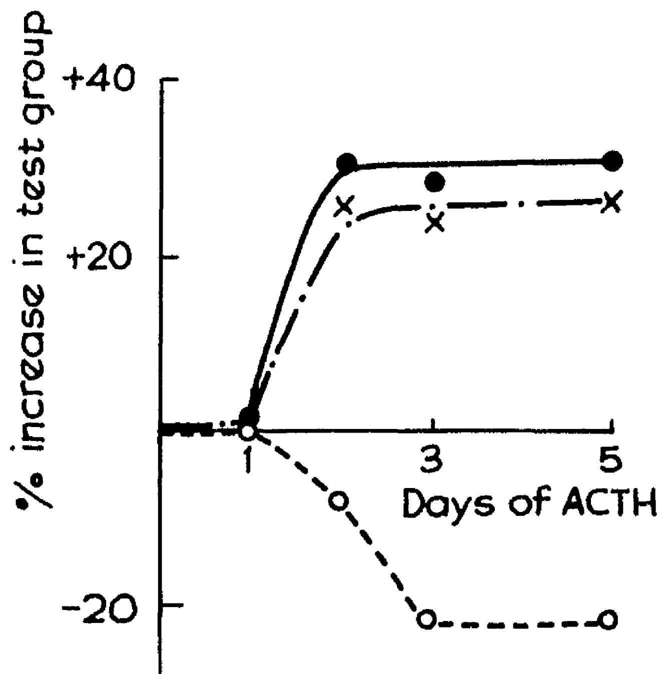
Top: increases in gland weight, amount and relative specific activity of RNAP.

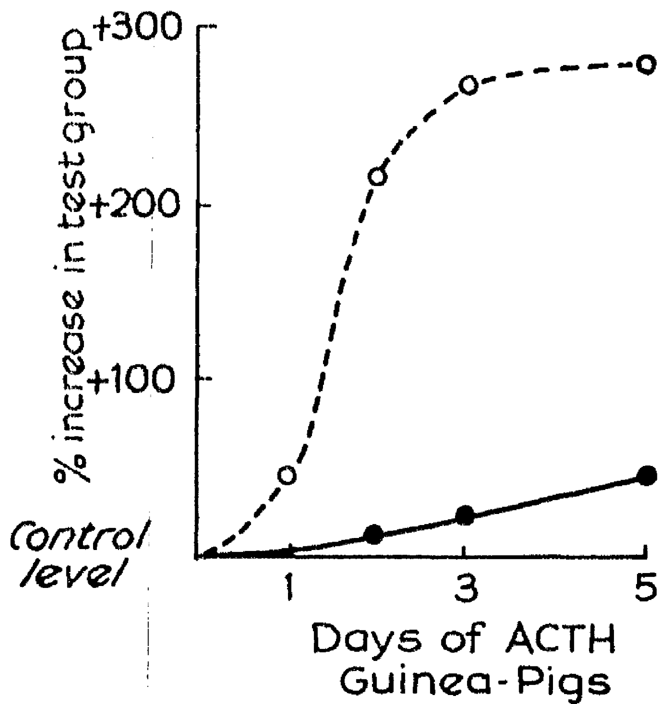
Bottom: increases in amount and relative specific activity of DNAP

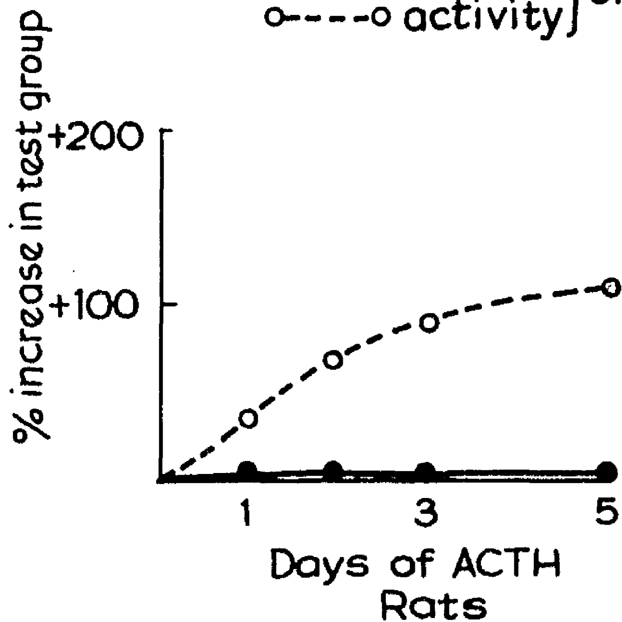
(Note the difference in scales for top and bottom figures).

Fig. 9









Section 2.

Effect of Exogenous ACTH in Rats whose Endogenous ACTH is suppressed.

The results reported in Section 1 indicate that the rats from the departmental colony respond to ACTH to a lesser degree than do the guinea-pigs. This could be because the endogenously secreted ACTH in the rats is obscuring the effect of the ACTH administered. Experiments were therefore designed, and are described in this section, to suppress the endogenous ACTH before carrying out injections of ACTH.

The most obvious way to do this is to use hypophysectomised animals but this method involves the withdrawal of other pituitary hormones besides ACTH and is also limited by the supply of an adequate number of animals which have been successfully operated upon. It is also worth noting that Vogt (1953) has claimed that, in the dog, only a minute part of the anterior lobe or of the pars tuberalis will maintain the adrenals in a normal condition and that Zuckerman (1953) has stated that, in the rat, the removal of pars tuberalis for complete hypophysectomy is quite impossible.

There is evidence, on the other hand, that administration of adreno-corticoids such as cortisone or cortisol brings about changes in the adrenal cortex similar to those obtaining in hypophysectomised rats (Sayers, 1950; Tuthmann-Duplessis, 1952; Krohn, 1955). It was therefore decided to investigate the administration of such corticoids

as an alternative to surgical removal of the pituitary, though it was felt essential to carry out some preliminary studies with hypophysectomised rats to serve as a basis for comparison.

Hypophysectomy was carried out under tribromethanol anaesthesia, using the parapharyangeal approach (Smith, 1927). Either Bromethol (Boots) or Avertin (Winthrop Products) was used and each rat was given intraperitoneally 0.8 ml./100 g. body weight of a 2.5% ($^{V}/v$) solution in distilled water. After operation the rats were allowed to recover for 10 days during which time they were maintained on 5% ($^{W}/v$) glucose solution and 0.9% saline in addition to their normal food. After killing every rat was examined for any possible remnant of the pituitary.

<u>Effect of ACTH on hypophysectomised rats.</u>

Twelve August rats (body wt. 150-180 g) were hypophysectomised and allowed to recover for 10 days, six animals receiving 10 mg. Acthar-gel per day from the 8th till the 10th day. On the 11th day both groups were killed 2 hours after administration of 250 μc ^{32}P. Four glands from two animals of each group were pooled and analysed.

In a subsequent experiment 24 albino rats (body wt.

150-180 g.) were hypophysectomised and divided into two groups of 12 animals, one of which received ACTH as above. All the animals were killed on the 11th day 2 hours after receiving 250 µc ^{32}P six glands from 3 rats of each group were pooled in this case for analysis.

The results are shown in Tables 15 and 16 as well as in Fig. 10 and Fig. 11.

It is to be observed that hypophysectomised rats respond to the administration of ACTH, but the effect appears to be essentially hypertrophy. Gland weight increases by about 100% and similar increases are seen in the amount of RNAP and LP. This response, however, is not reflected by any significant increase in the DNAP content of the gland either in August or albino rats. The large increase in LP and RNAP due to hypertrophy is likewise reflected in the concentrations of these two constituents which show a marked increase after ACTH administration.

The relative specific activity of RNAP shows a significant drop in the ACTH-treated group whereas the differences between the groups in the uptake of ^{32}P into LP and DNAP are perhaps not significant.

It is interesting, however, to note the differences

Table 15

Effect of ACTH on hypophysectomised rats killed 10 days after operation. The test group was given 10 mg. ACTH daily for 3 days prior to killing.

		August rats		Albino rats	
		no ACTH	ACTH	no ACTH	ACTH
wt/2 glands (mg.)		22.0±3.1	36.3±0.5	20.2±2.5	40.8±1.0
LP	µg/2 glands	22.7±1.7	46.9±0.2	29.6±4.9	67.4±2.1
	µg/100 mg.	100 ±6.5	129±2.0	146±6.2	165±0.9
RNAP	µg/2 glands	13.9±0.8	25.8±0.3	10.6±0.8	32.7±4.2
	µg/100 mg.	61±6.0	71±0.2	52.8±2.7	68.5±0.5
DNAP	µg/2 glands	8.7±1.0	9.6±0.3	11.6±0.6	13.7±0.6
	µg/100 mg.	40 ±7.0	26.5±0.6	57.5±4.3	33.6±0.6
RNAP/DNAP		1.60	2.69	0.91	2.37
LP/DNAP		2.61	4.88	2.55	4.92

Table 16

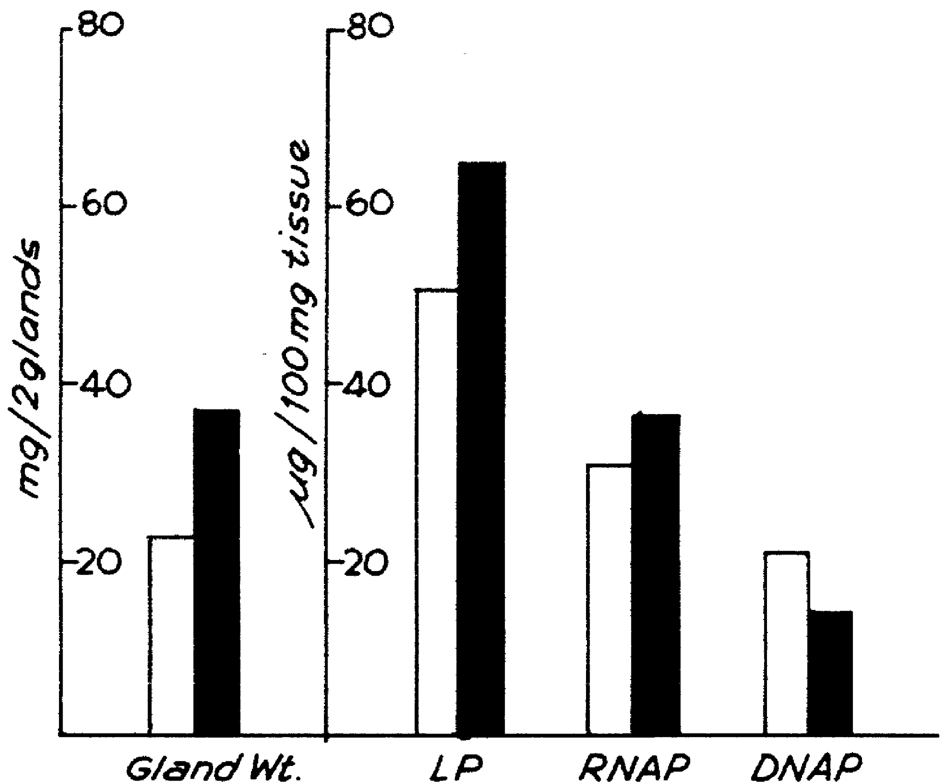
Specific activities (relative to tissue inorganic phosphate x 10^3) of LP, RNAP and DNAP from the adrenals of hypophysectomised August rats

	August rats	
	no ACTH	ACTH
LP	2.3	1.8
RNAP	5.5	1.8
DNAP	0.28	0.33

Fig. 10

The effect of hypophysectomy on the adrenal glands of female August rats killed 10 days after operation.

Fig. 10

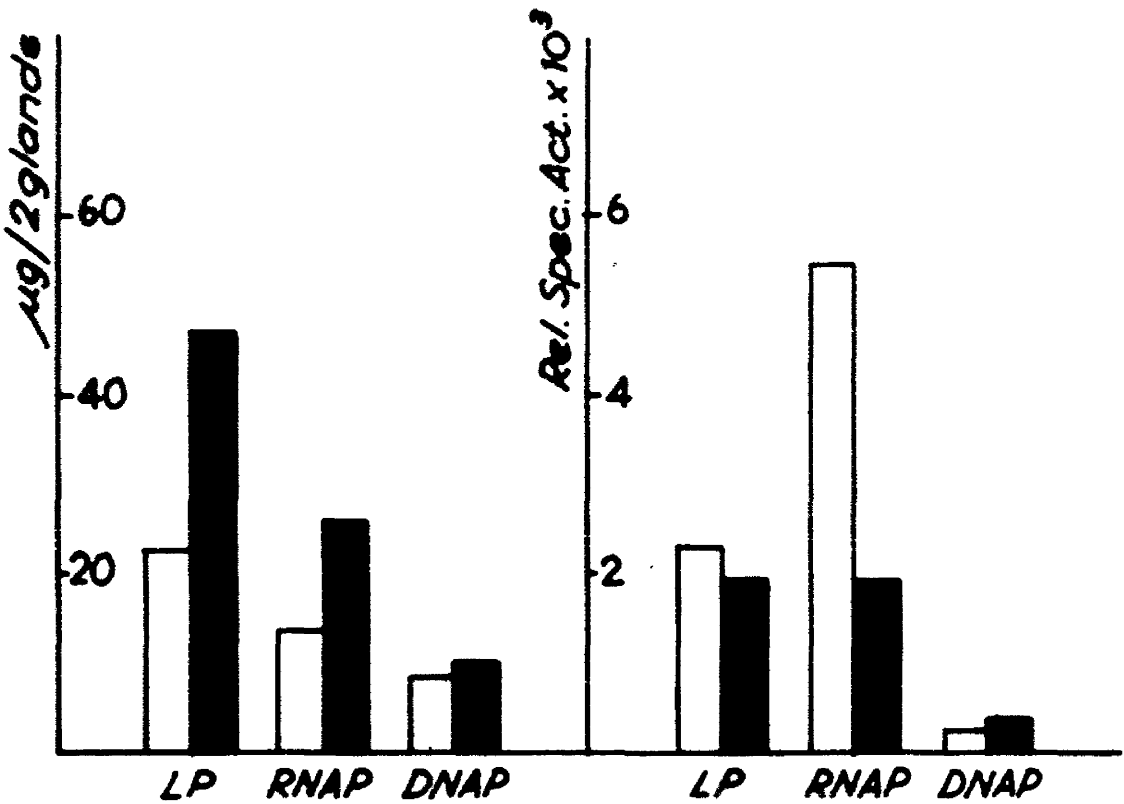


☐ - Control.
■ - 10 mg ACTH/day for 3 days before sacrifice.

Fig. 11

The effect of hypophysectomy on the adrenal glands of female August rats killed 10 days after operation.

Fig. II



□ – Control.
■ – 10mg ACTH/day for 3 days prior to sacrifice.

between the two strains of rat used in this experiment. In both cases the animals were of similar body weights. The gland weights do not show any significant differences between the two strains either in the ACTH-treated or untreated groups but significant differences are observed in the case of the other constituents. The amounts of LP and DNAP in the gland of the albino rat are higher than in that of the August rat irrespective of whether the animal has received ACTH after hyperphysectomy or not. The concentrations of these substances show similar differences. For RNAP no difference is observed in concentration and the picture in the case of the amount per gland is not clear cut, the August rat having a slightly higher content in the untreated group while the reverse occurs in the treated group.

Effect of cortisol on the rat adrenal gland.

Before embarking on any studies of the effect of ACTH on cortisol treated rats, it was first of all necessary to study how the administration of cortisol itself would effect the adrenal gland. In an initial experiment a batch of 40 rats was divided into 10 groups of 4 rats each (body wt. 140-160 g.). One of the groups was taken as the control and was given 0.9% saline injections.

Three levels of administration of cortisol (Hydrocortisyl), at 5, 10 and 15 mg. per day were chosen and treatment was continued for 1, 3 and 5 days respectively to different groups, making nine groups in all. The rats were killed on the day following the last injection and analyses were carried out on the pair of glands from each rat.

The results which are given in Table 17 show that administration of cortisol brings about a slight decrease after 1 day in the gland weight and in the amounts of LP and RNAP. The different dosage levels do not show any difference in the suppressive action, 15 mg. being no more effective than 5 mg. There is no observable change in the concentrations of any of the constituents but the RNAP/DNAP values for animals receiving 10 and 15 mg. are slightly lower than for those receiving 5 mg.

Continued administration of cortisol for 3 days brings about a very significant lowering in the gland weight and in the amounts of LP and RNAP as well as a slight decrease in the amount of DNAP. Here again there appears to be no marked difference in the effect of a daily dose of 5 mg. cortisol as compared with 10 and 15 mg. The concentrations of LP and RNAP are also lower than the control value and the concentration of DNAP in the groups receiving 10 and 15 mg. cortisol are significantly

Table 17

Effect of cortisol on rat adrenal glands

a) a study of different dosage levels

		controls	mg. cortisol/day	no. of days treatment 1	3	5
wt/2 glands (mg.)		49.6±4.0	5	41.7±2.8	37.7±3.9	31.2± 2.2
			10	43.2±2.0	34.9±2.4	36.6± 1.1
			15	44.9±4.2	34.2±1.2	30.9± 1.0
LP	µg/2 glands	83.8±4.9	5	70.0±4.3	51.9±6.3	43.9± 6.5
			10	70.5±3.6	55.8±5.0	54.5± 3.5
			15	77.1±7.7	54.0±4.1	41.8± 1.3
	µg/100 mg.	169.5±4.9	5	168.3±3.1	137.5±4.0	138.4±12.2
			10	163.9±6.9	159.4±6.2	148.1± 6.8
			15	172.0±5.1	157.9±6.5	138.1± 4.9
RNAP	µg/2 glands	29.3±2.2	5	23.8±2.6	17.6±2.5	18.0± 1.3
			10	22.8±0.8	17.1±1.8	18.8± 0.5
			15	24.5±1.9	17.2±0.7	14.8± 0.3
	µg/100 mg.	59.1±2.5	5	57.0±1.4	46.2±2.7	51.4± 1.3
			10	58.3±3.9	48.5±2.2	50.2± 0.4
			15	55.2±2.8	50.6±1.0	49.6± 1.9
DNAP	µg/2 glands	14.8±0.8	5	12.7±0.8	11.7±1.4	12.2± 0.2
			10	13.9±0.7	12.3±0.8	12.2± 0.1
			15	14.1±0.5	12.2±0.8	11.4± 0.7
	µg/100 mg.	30.0±0.8	5	31.5±2.5	31.1±1.3	39.7± 2.6
			10	32.1±0.2	35.3±0.01	38.8± 0.7
			15	29.6±2.8	35.8±2.0	37.7± 1.6
RNAP/DNAP		1.97	5	1.89	1.49	1.48
			10	1.70	1.38	1.29
			15	1.73	1.42	1.31
LP/DNAP		5.52	5	5.59	4.44	3.57
			10	5.11	4.52	3.85
			15	5.42	4.47	3.72

higher. The values of RNAP/DNAP and LP/DNAP representing the RNAP and LP content per cell are also very much lower.

Further administration of cortisol up to 5 days does not appear to bring about any greater decrease in the amounts of RNAP and DNAP even though LP shows a slight tendency to fall both in amount and in concentration. The concentration of RNAP remains unaffected compared with the 3 day treated groups, but that of DNAP increases. This is to be expected since the weight of the gland continues to show a decline.

So it is clear that the effect of cortisol which is only slight after treatment for 1 day becomes marked after 3 days. The level of cortisol administered does not appear to be of great importance. It is therefore possible that the hormone which is not very soluble in aqueous media is absorbed very slowly from the site of injection. In order to confirm this explanation it was decided to give rats a series of injections of cortisol and kill them at varying intervals after the last injection. If the action of the cortisol is prolonged due to slow absorption, one may expect that the suppressive action will continue for some time before normal production of endogenous ACTH can be resumed.

Six groups of 4 albino rats (body wt. 140-160 g.) were given 10 mg. cortisol (hydrocortisyl) daily for

3 days and the injections then stopped. The different groups were killed on the 4th, 5th, 6th, 7th, 9th and 11th days thus giving 0, 1, 2, 3, 5 and 7 days respectively for possible recovery after stopping cortisol administration. The control values for rats receiving saline injections are taken from the previous experiment (Table 17) for comparison since the animals were of the same body weight.

Analyses were done on the pair of glands from each animal and the results are shown in Table 18 and in Fig. 12.

It is obvious that even after stopping the administration of cortisol, pituitary secretion of ACTH remains suppressed and this lack of endogenous ACTH results in continued atrophy of the adrenal gland for a period as long as a week. The gland weight shows a steady decline as also do the amounts of LP, RNAP and DNAP. The concentration of LP tends to decrease whereas that of RNAP remains fairly steady at the control value after showing an initial decrease during the period of treatment with cortisol. The concentration of DNAP shows a steady increase except possibly in the last group. The values of $\frac{RNAP}{DNAP}$ and $\frac{LP}{DNAP}$ representing the content of RNAP and LP per cell show a significant lowering with continued suppression of the gland.

Table 18

EFFECT OF CORTISOL ON RAT ADRENAL GLANDS.

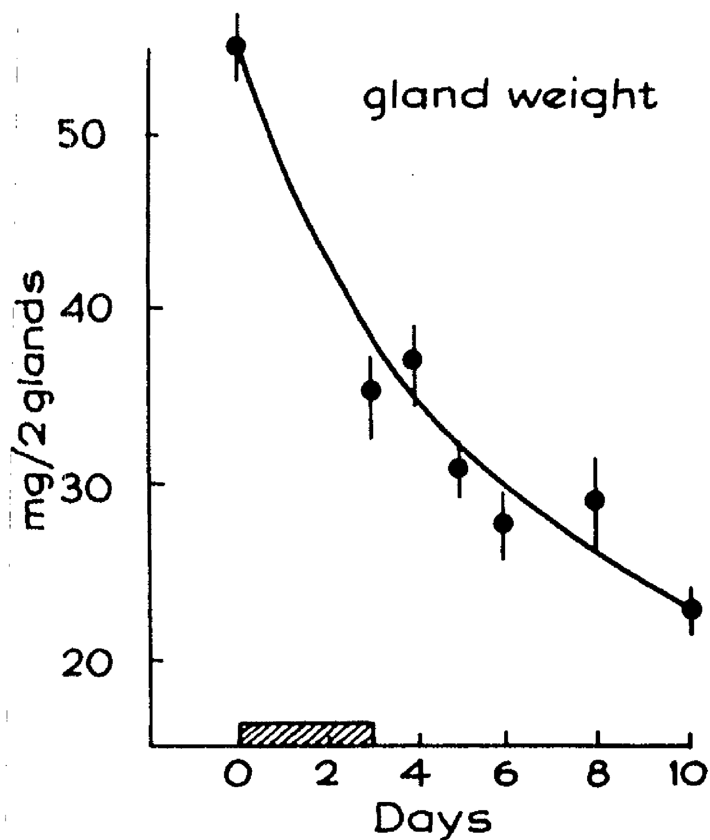
(b) Observations on continued action of Cortisol after cessation of Administration.

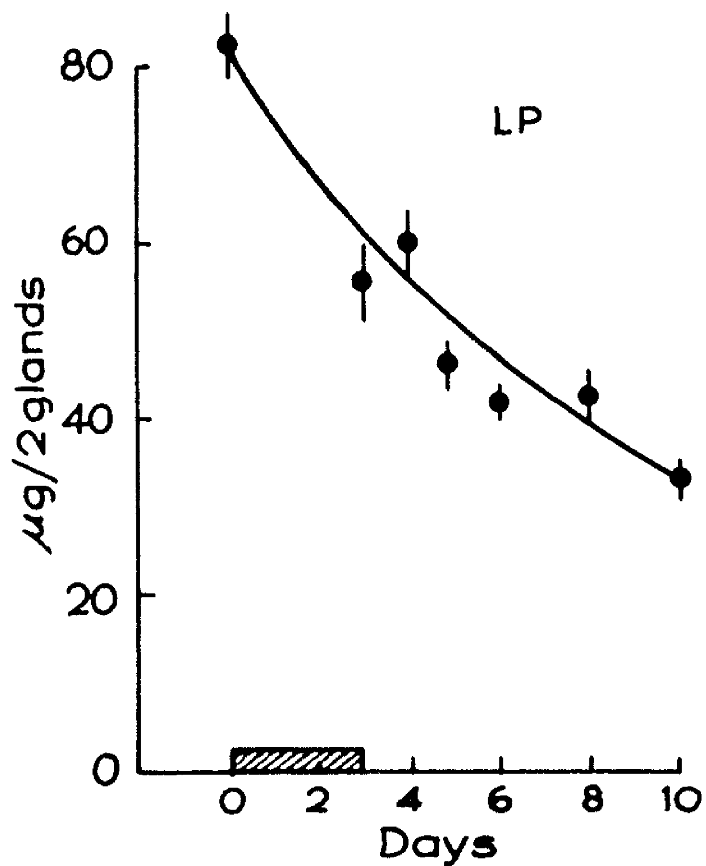
		Cortisol-treated Days after cessation of treatment					
	CONTROLS	0	1	2	3	5	7
Wt./2 glands (mg.)	49.6 ± 4.0	34.9 ± 2.3	37.3 ± 2.3	31.1 ± 1.5	27.6 ± 2.1	29.1 ± 3.7	22.6 ± 1.1
LP µg/2glands	83.8 ± 4.9	55.8 ± 5.0	60.9 ± 3.2	46.7 ± 1.3	42.0 ± 1.1	42.0 ± 3.7	33.1 ± 1.2
µg/100 mg.	169.5 ± 4.9	159.4 ± 6.2	163.7 ± 2.2	151.2 ± 7.2	152.3 ± 1.6	146.8 ± 7.2	147 ± 4.8
RNAP µg/2glands	29.3 ± 2.2	17.1 ± 1.8	19.7 ± 1.1	17.5 ± 1.6	15.6 ± 1.1	15.5 ± 1.6	13.1 ± 1.2
µg/100 mg.	59.1 ± 2.5	48.5 ± 2.2	53.1 ± 1.9	56.2 ± 2.6	55.6 ± 2.1	53.7 ± 1.5	57.6 ± 3.9
DNAP µg/2glands	14.8 ± 0.8	12.3 ± 0.8	12.8 ± 0.7	11.9 ± 0.7	11.2 ± 0.8	11.7 ± 0.8	9.6 ± 0.4
µg/100 mg.	30.0 ± 0.8	35.3 ± 0.01	34.4 ± 1.1	38.4 ± 0.4	40.7 ± 1.1	46.3 ± 4.0	42.5 ± 1.5
RNAP/DNAP	1.97	1.38	1.55	1.46	1.37	1.32	1.35
LP/DNAP	5.67	4.51	4.77	3.95	3.75	3.58	3.46

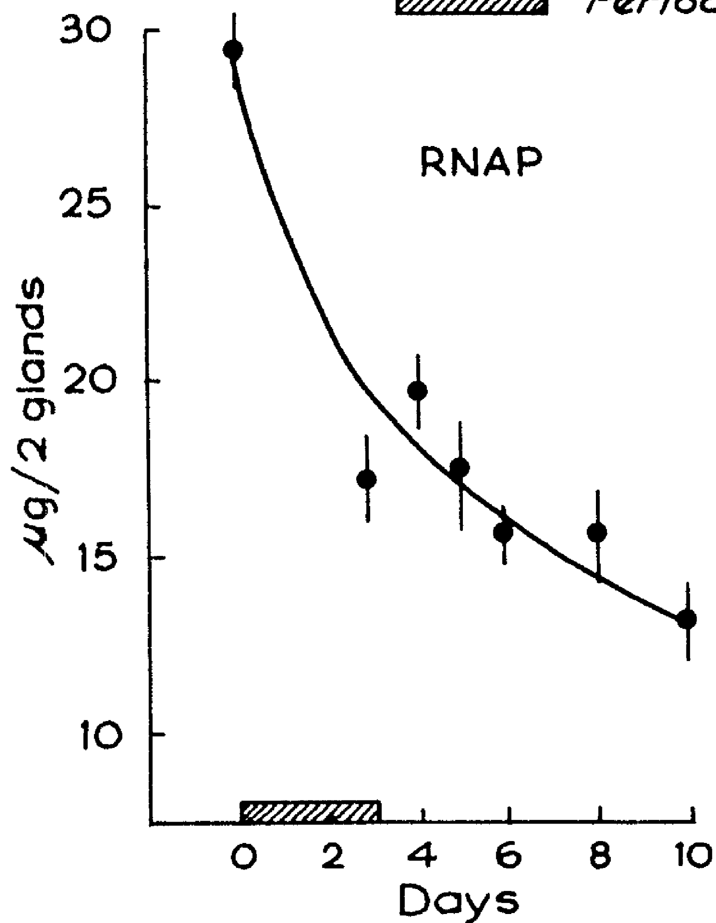
Fig. 12

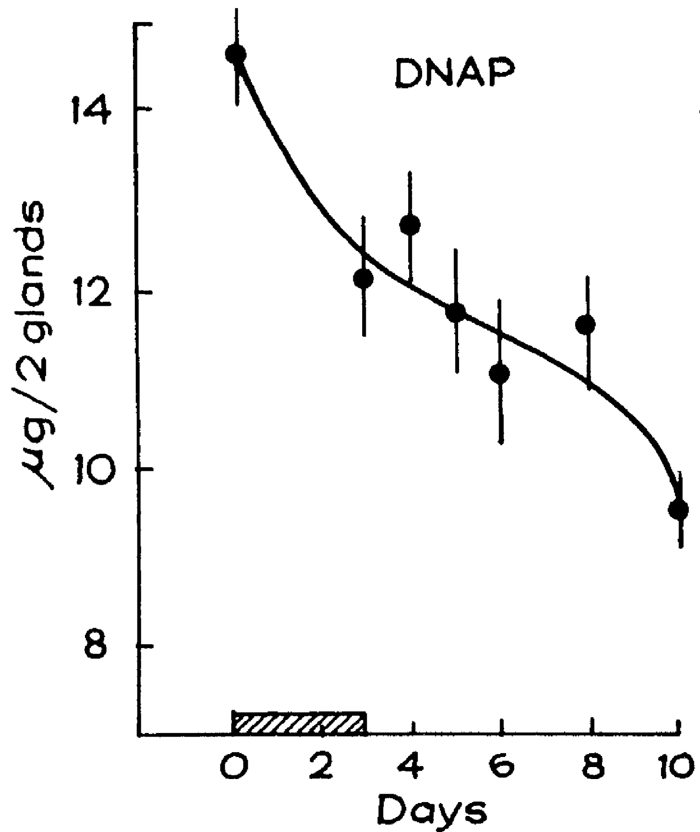
Continued action of cortisol on rat adrenal glands after cessation of administration.

Fig. 12









Effect of ACTH on cortisol-treated rats.

A preliminary experiment was carried out administering ACTH to cortisol-treated rats to study the effect on gland composition and ^{32}P uptake. A daily dose of 10 mg. cortisol for 3 days was chosen to bring about an adequate suppression of the gland and in one group a test dose of 10 mg. ACTH was also given in an attempt to nullify the effect of cortisol.

Three groups of 12 rats (body wt. 145-160 g.) were used. One of the groups, taken as the control, was given 0.9% saline injections. The second group received 10 mg. cortisol (Hydrocortisyl) daily and the last group was given the same dose of cortisol along with 10 mg. Acthar-gel per day. The treatment was continued for 3 days and on the 4th day each rat was given 200 μc ^{32}P and killed 2 hours later.

Analyses were carried out on 6 pooled glands from 3 animals of each group and the results are shown in Tables 19 and 20 and in Figs. 13 and 14.

The suppression of the gland brought about by cortisol is more than reversed by 10 mg. of ACTH administered exogenously. In the ACTH-treated group the gland weight and the amounts of LP and RNAP are greater than the control values. No significant

Table 19

Effect of simultaneous administration of cortisol and ACTH on rat adrenals

		CONTROL	TREATED	
			cortisol	cortisol + ACTH
wt/2 glands (mg.)		46.1 ± 4.0	31.6 ± 2.2	54.6 ± 2.5
LP	µg/2 glands	77.1 ± 9.2	46.0 ± 4.0	97.5 ± 5.8
	µg/100 mg.	166 ± 9.4	145.6 ± 6.3	180 ± 18.1
RNAP	µg/2 glands	24 ± 2.4	15.1 ± 1.0	31.1 ± 2.3
	µg/100 mg.	51.8 ± 0.7	47.9 ± 1.0	56.6 ± 1.2
DNAP	µg/2 glands	12.5 ± 1.4	10.5 ± 0.5	10.5 ± 0.5
	µg/100 mg.	26.8 ± 1.4	33.3 ± 1.3	20.0 ± 0.5
RNAP/DNAP		1.92	1.44	2.96
LP/DNAP		6.3	4.38	9.3

Table 20

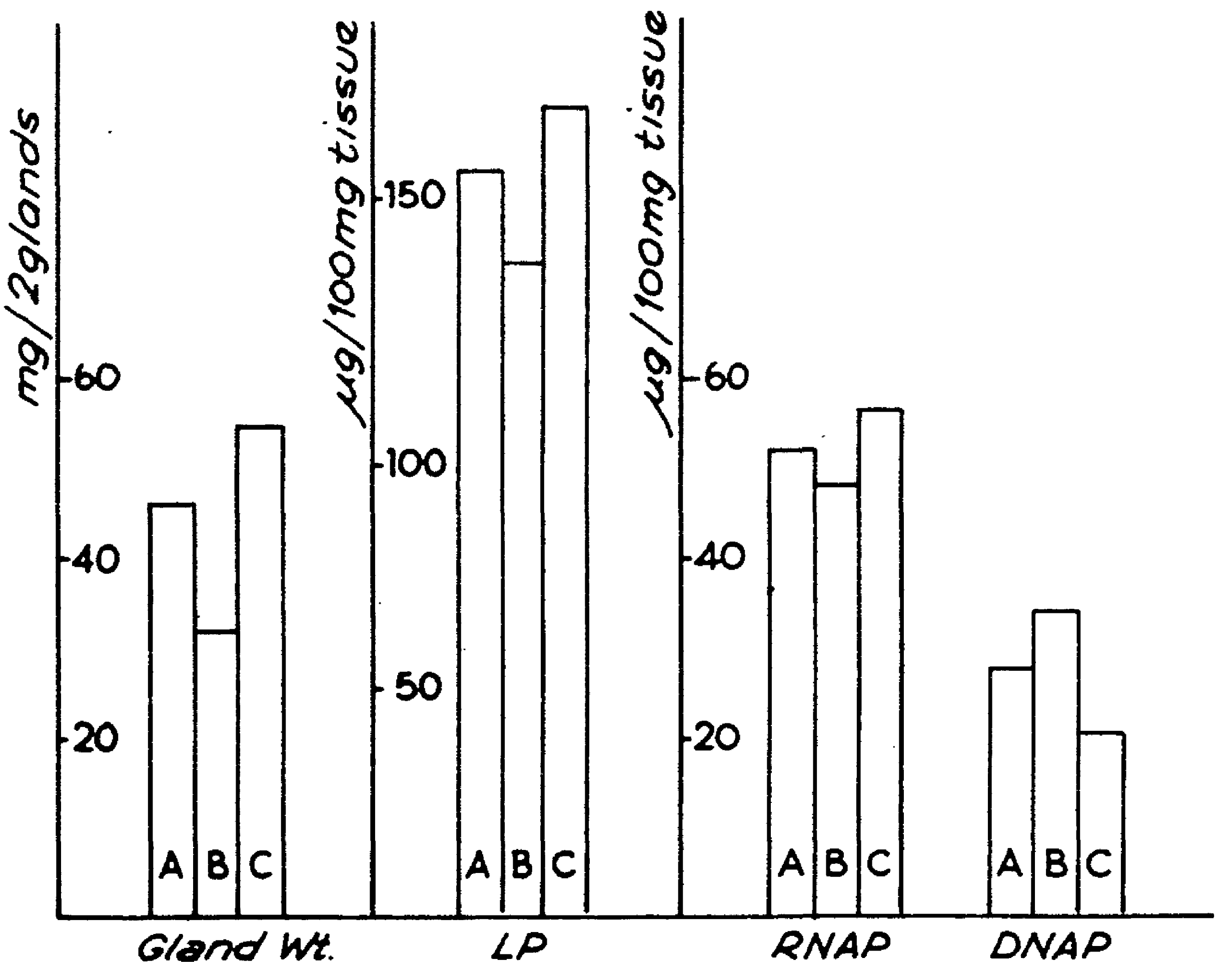
Specific activities (relative to tissue inorganic phosphate x 10^3) of LP, RNAP and DNAP from adrenals of rats receiving cortisol and ACTH

	Controls	Cortisol	Cortisol + ACTH
LP	6.2 ± 0.5	16.9 ± 3.1	32.5 ± 3.4
RNAP	22.0 ± 1.3	16.6 ± 1.6	14.9 ± 0.9
DNAP	2.7 ± 0.2	1.7 ± 0.5	3.4 ± 0.7

Fig. 13

The effect of ACTH on the adrenals of cortisol-treated rats.

Fig. 13

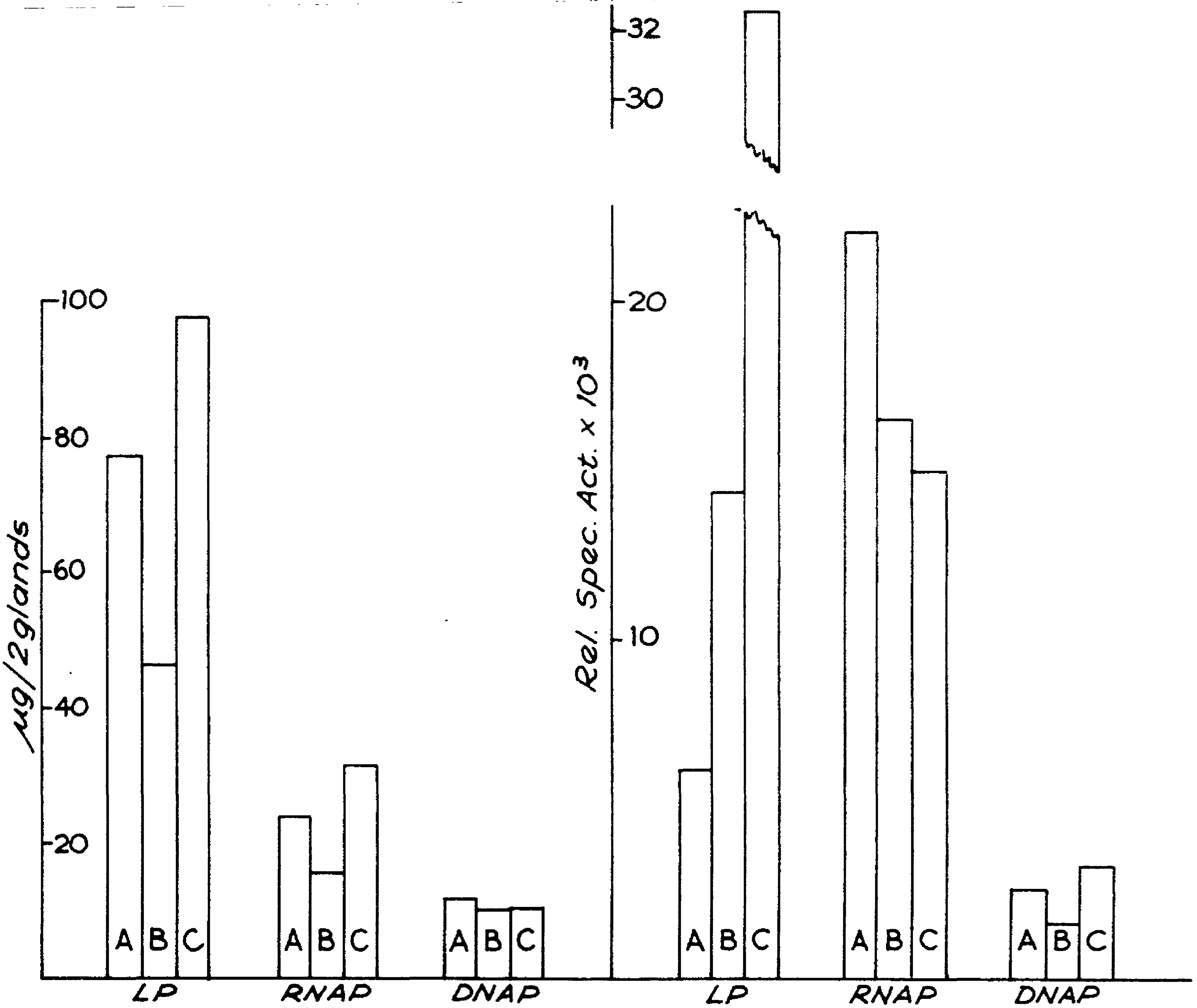


A- Control; saline injections
B - 10 mg Cortisol/day for 3 days
C - 10 mg Cortisol + 10 mg ACTH/day for 3 days

Fig. 14

The effect of ACTH on the adrenals of cortisol-treated rats.

Fig. 14



A — Control; saline injections.
B — 10 mg Cortisol/day for 3 days.
C — 10 mg Cortisol + 10 mg ACTH/day for 3 days.

differences, however, are seen in the concentrations of these constituents. The DNAP content of the gland is less than the control value in both the cortisol-treated groups irrespective of whether ACTH is given or not, so that the exogenous ACTH does not appear to produce any hyperplasia. The same pattern is seen also in the uptake of ^{32}P into DNA, the relative specific activity not showing any significant differences. On the other hand the relative specific activity of RNAP is less in the treated groups than in the control group. For LP, however, the value of relative specific activity in the treated groups is significantly greater than the control value.

Maintenance level of ACTH.

It was of interest to find the level of ACTH, which after cutting off the endogenous supply with cortisol, would maintain the rat at the normal level. In the following experiment, in which the rats supply of endogenous ACTH was cut off by a low level of cortisol (5 mg.) for 2 days, varying amounts of ACTH were administered for 3 days to discover which of them would bring the adrenal back to the normal level.

Five groups of 4 rats, (body wt. 140-160 g.) were

given 5 mg. cortisol (Hydro-Adresan) daily for 2 days. For the next 3 days the cortisol treatment was continued and four of the 5 groups received respectively 2, 3, 4 and 5 mg. Acthar-gel per day. The control values are the same as in Table 17 since the animals in this experiment were of similar body weight. The animals were killed on the 6th day and analyses were carried out on the pair of glands from each animal.

The results which are shown in Table 21 indicate that at the 2 mg. level of ACTH the glands do not recover sufficiently from the suppressive action of cortisol. The suppression, however, is completely reversed by administration of 3-5 mg. of ACTH per day. There appears to be no greater response to 5 mg. than is shown for 3 mg., since there are no significant differences between the groups receiving 3, 4 or 5 mg. Hence it was decided to choose the 4 mg. level of ACTH as the normal maintenance dose and to give the control group in the next experiment 4 mg. Acthar-gel daily after initial cortisol suppression.

Four groups of 12 rats (body wt. 130-150 g.) were given 5 mg. cortisol (Hydro-Adresan) daily for 2 days. Thereafter they received Acthar-gel in addition to the cortisol. The control group received 4 mg. as maintenance

Table 21

EFFECT OF ACTH ON THE ADRENALS OF CORTISOL-TREATED RATS

		Controls	Cortisol-treated mg. ACTH administered.				
			0	2	3	4	5
Wt./2 glands (mg)		49.6 ± 4.0	34.0 ± 3.9	42.8 ± 2.2	56.0 ± 4.2	54.2 ± 5.8	56.4 ± 6.3
LP	µg./2 glands	83.8 ± 4.9	50.9 ± 3.4	69.7 ± 5.9	95.9 ± 9.2	89.8 ± 10.8	85.4 ± 5.6
	µg./100 mg.	169 ± 4.9	152 ± 7.2	163 ± 8.0	163 ± 5.7	165 ± 3.4	163 ± 4.4
RNAP	µg./2 glands	29.3 ± 2.2	17.6 ± 1.0	25.1 ± 1.6	35.3 ± 3.7	34.2 ± 3.8	35.2 ± 3.2
	µg./100 mg.	59.1 ± 2.5	52.8 ± 3.0	58.8 ± 1.2	63.5 ± 2.2	62.5 ± 1.0	62.7 ± 2.5
DNAP	µg./2 glands	14.8 ± 0.8	13.1 ± 0.5	11.2 ± 0.3	14.8 ± 0.9	14.6 ± 3.4	13.9 ± 1.3
	µg./100 mg.	30.0 ± 1.0	39.4 ± 3.1	26.4 ± 0.8	26.6 ± 0.9	27.4 ± 1.2	27.4 ± 2.4
RNAP/DNAP		1.97	1.35	2.24	2.41	2.34	2.34
LP/DNAP		5.52	3.90	6.2	6.46	6.15	6.3

dose and the other groups 5, 10 and 15 mg. ACTH in excess of this dose. The ACTH administration was continued for 3 days and on the next day the rats were killed 2 hours after receiving an injection of 500 μc ^{32}P. Analyses were done on 6 pooled glands from 3 animals of each group and the results are given in Tables 22 and 23.

The gland weight shows a continual increase with increasing amounts of ACTH administered, accompanied also by a concomitant increase in the amounts of LP and RNAP without, however, any change in the concentration of these substances. The amount of DNAP does not show any increase, although the uptake of ^{32}P shows a slight increase in the groups receiving 10 and 15 mg. ACTH above the control group. The uptake of ^{32}P into LP shows no significant change, while there is a decrease into RNAP.

Effect of ACTH on young rats treated with cortisol.

Fiala *et al.* (1956) have reported considerably increased amounts of DNA in the adrenals of young Wistar rats which had received very large doses of ACTH. It was therefore deemed desirable to study whether young rats from our colony would respond to ACTH in a similar fashion. It was decided to examine the effect of the

Table 22

Effect of ACTH on the adrenal glands of rats treated with cortisol

		mg. of ACTH administered per day			
		4 (control)	9	14	19
wt/2 glands (mg.)		43.7±2.2.	51.0±3.8	56.0±2.2	608 ± 2.4
LP	µg/2 glands	70.2±1.9	84.3±5.6	86.2±4.6	92.9 ± 5.9
	µg/100 mg.	161 ±2.5	165 ±3.0	154 ±6.5	150 ± 6.5
RNAP	µg/2 glands	24.6±0.6	29.8±2.6	30.4±0.8	32.7 ± 2.0
	µg/100 mg.	56.4±2.1	53.2±3.2	54.5±2.0	53.7 ± 1.9
DNAP	µg/2 glands	11.3±0.6	12.5±1.1	10.9±0.5	12.0 ± 0.4
	µg/100 mg.	25.9±1.8	24.3±0.8	19.6±0.7	19.9 ± 0.7
RNAP/DNAP		2.18	2.38	2.79	2.73
LP/DNAP		6.21	6.75	7.90	7.75

Table 23

Specific activities (relative to tissue inorganic phosphate x 10^3) of LP, RNAP and DNAP from adrenals of cortisol-treated rats receiving ACTH

	mg. ACTH administered per day			
	4 (control)	9	14	19
LP	31.8 ± 0.6	35.5 ± 4.8	34.3 ± 2.7	34.6 ± 4.2
RNAP	22.5 ± 1.4	20.3 ± 1.0	18.1 ± 1.2	16.1 ± 1.6
DNAP	7.0 ± 0.8	7.2 ± 0.6	9.0 ± 0.9	9.1 ± 0.9

dose levels of ACTH being administered to older rats and also to study the incorporation of ^{32}P.

Twenty four young female albino rats, 3-4 weeks old (body wt. 35-50 g.) were divided into 2 groups of 12 each. They were given daily 5 mg. cortisol (Hydro-Adresan) for 2 days. Thereafter, in addition to cortisol, the control group received daily 4 mg. Acthar-gel as maintenance dose and the test group 15 mg. ACTH in excess of the maintenance dose, for 3 days. On the 6th day the animals received 500 μc ^{32}P and were killed 2 hours later.

Six glands from 3 animals of each group were pooled and analysed. The results are shown in Tables 24 and 25.

It is seen that even though the gland weight increases markedly in response to ACTH administration, the constituents examined show less spectacular increases of only 56%, 42% and 15% for RNAP, LP and DNAP respectively. The relatively smaller increases are likewise reflected in the concentrations which show a significant lowering in groups receiving larger doses of ACTH.

The relative specific activity of DNAP is significantly increased, whereas that of RNAP decreases and no change is observed in the case of LP.

It is apparent that the response in young rats

Table 24

Effect of ACTH on the adrenals of very young rats after cortisol treatment.

		Cortisol + 4 mg. ACTH (A)	Cortisol + 19 mg. ACTH (B)	% increase ($\frac{B-A}{A}$ x 100)
wt/2 glands (mg.)		17.1 ± 0.7	30.1 ± 0.8	76
LP	µg/2 glands	28.2 ± 2.6	40.0 ± 0.9	42
	µg/100 mg.	165 ± 10.2	133 ± 2.5	
RNAP	µg/2 glands	13.4 ± 0.4	20.9 ± 0.2	56
	µg/100 mg.	79.1 ± 1.1	69.3 ± 1.7	
DNAP	µg/2 glands	7.1 ± 0.2	8.2 ± 0.4	15
	µg/100 mg.	42.1 ± 1.2	27.1 ± 0.6	
RNAP/DNAP		1.88	2.58	
LP/DNAP		3.94	4.92	

Table 25

Specific activities (relative to tissue inorganic phosphate x 10^3) of LP, RNAP, and DNAP from the adrenals of very young cortisol-treated rats receiving ACTH

	Cortisol + 4 mg. ACTH	Cortisol + 19 mg. ACTH
LP	54.1 ± 12.5	41.0 ± 5.8
RNAP	37.7 ± 4.2	24.3 ± 2.7
DNAP	15.7 ± 1.1	24.3 ± 2.6
Total radioactivity of RNAP *	505	507

* Total radioactivity = Amount(µg/2 glands) x Relative Specific Activity.

to the doses of ACTH given is better than that obtained in mature rats, but is still not so great as that reported by Fiala et al. (1956). It was, therefore, decided to carry out an experiment giving very large doses of ACTH similar to those given by these workers.

Four rats, 3-4 weeks old (body wt. 35-50 g.) were used in this experiment. Two acted as controls and were given 0.9% saline injections. Each of the other two received 75 mg. Acthar-gel daily. The rats showed a severe reaction to the high dose of ACTH administered and on the 4th day one of the rats, which had by then received 225 mg. ACTH, was given 150 uc ^{32}P and was killed 2 hours later, as it was suffering from severe diarrhoea with haemorrhages from nose and eyes, together with a distended abdomen. Treatment was continued for the other rat in an attempt to give a total of 450 mg. ACTH. This rat, however, died after 5 days when it had received 375 mg. ACTH. The glands from the dead rat were also removed and analysed. The control rats received 150 μc ^{32}P 2 hours before killing. The results are shown in Tables 26, 27 and 28 as well as in the form of a histogram in Fig. 15.

As compared with the control animals rat 1 shows

Table 26

Effect of very large doses of ACTH on young rats

		Control	ACTH-treated	
			rat 1 [(a)]	rat 2 [(b)]
wt/2 glands (mg.)		19.4	51.0	57.4
LP	µg/2 glands	31.6	53.0	44.0
	µg/100 mg.	162	104.7	76.6
RNAP	µg/2 glands	13.0	34.5	29.8
	µg/100 mg.	66	67	51
DNAP	µg/2 glands	8.0	11.7	10.8
	µg/100 mg.	40.6	22.8	18.8
Pr.N	µg/2 glands	305	740	860
	µg/100 mg.	1540	1450	1500
RNAP/DNAP		1.62	2.95	2.76
LP/DNAP		3.95	4.53	4.07

(a) Sacrificed on the 4th day after receiving 225 mg. ACTH.

(b) Died after receiving a total of 375 mg. ACTH.

Table 27

Specific activities (relative to inorganic phosphate $\times 10^3$) of LP, RNAP and DNAP from adrenals of young rats receiving very large doses of ACTH.

	Control	ACTH-treated (rat 1)
LP	102	23
RNAP	58	12
DNAP	3.8	11.8

Table 28

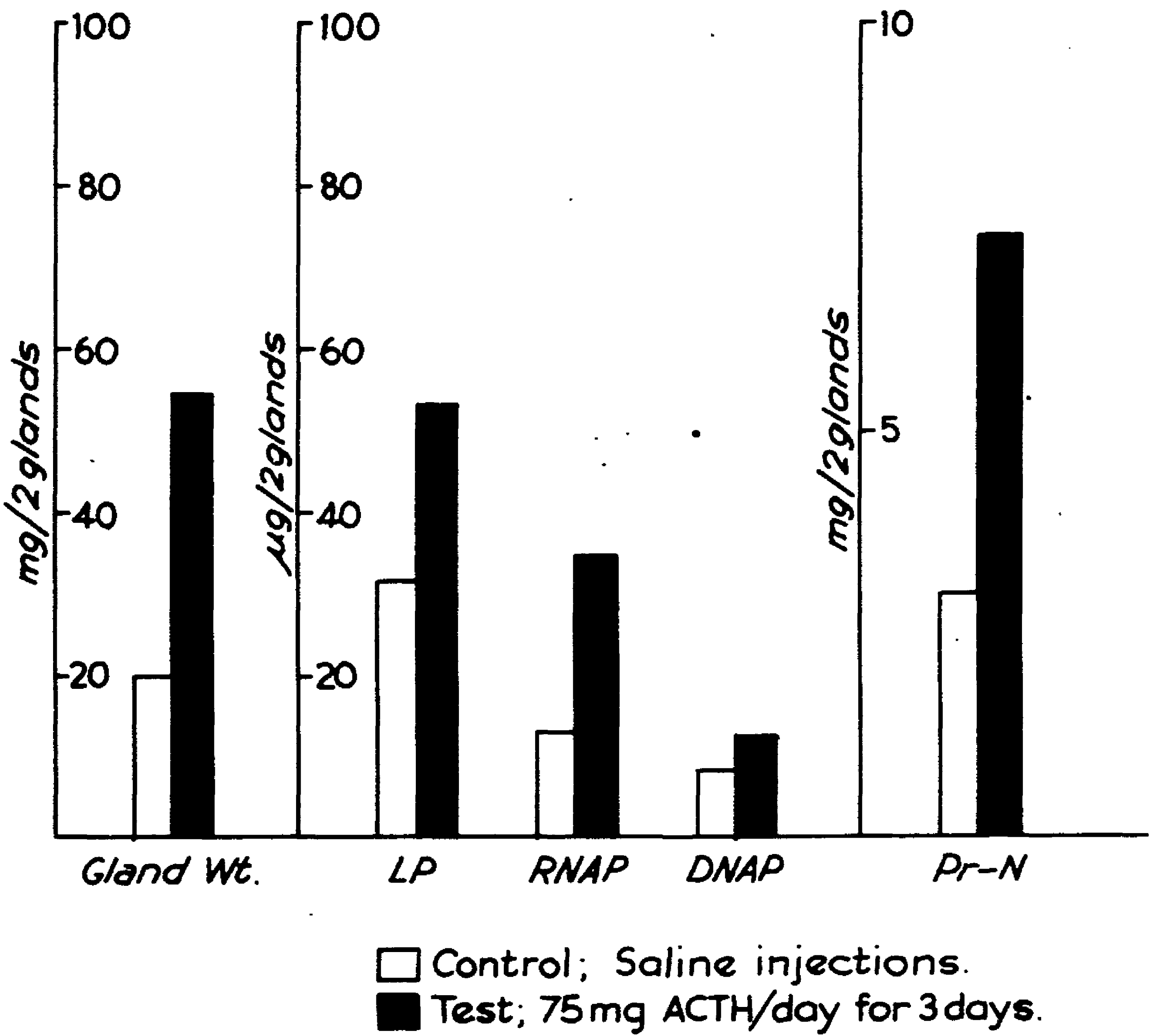
Analytical values (µg/2 glands) from Table 26 expressed as percentage increases over the control values.

	rat 1	rat 2
gland wt.	163	196
LP	68	39
RNAP	165	129
DNAP	46	35
PrN	142	182

Fig. 15

The effect of large doses of ACTH on the adrenals of immature rats.

Fig. 15



great response to the high dose of ACTH administered as indicated by the increase in gland weight (163%), which is reflected in similar increases in RNAP and in PrN (165% and 142% respectively). However, the increase in the amounts of DNAP and of LP is not so great and hence the concentration of these two substances shows a substantial lowering. The relative specific activity of DNAP shows a significant increase whereas the activities of RNAP and LP are considerably lowered.

It is interesting to note however, that in the case of rat 2, which died during treatment, even though the gland weight is much larger than that of rat 1 which also received ACTH, this increase is not reflected in other constituents since the amounts of LP and RNAP are considerably lower than in the ACTH-treated rat which survived, and even the amount of DNAP is slightly reduced.

Section 3.

The Action of ACTH on Normal, Adrenalectomised and Scorbutic Guinea-pigs.

Guinea-piIn the following experiments described in this se
animals for work on the adrenal glands since they are more difficult to deal with than rats and are also more easily subject to stress. However, in view of the fact that they have larger adrenal glands relative to body weight than rats and also because their glands respond better to administration of ACTH and to stressful stimuli they are in some ways peculiarly suited to the study of adrenal physiology. In some types of experiments such as those involving ascorbic acid deficiency where rats cannot be used because of their ability to synthesize this substance, guinea-pigs are the obvious choice.
this substance, guinea-pigs are the obvious choice. of the high concentration of ascorbic acid in the adrenal cortex.

In the following experiments described in this section an investigation has been made of the possible use of adrenocorticosteroids such as cortisone and cortisol to control the endogenous ACTH in guinea-pigs, of the effect of unilateral adrenalectomy on the nucleic acid metabolism in the remaining gland with and without exogenous ACTH, and of the effect of ascorbic acid deficiency on adrenal nucleic acid metabolism.

Experimental.

Unilateral adrenalectomy was carried out under ether anaesthesia using the ventral approach, the left gland being removed at operation.

In the ascorbic acid deficiency experiments the animals were given a diet consisting of equal parts of bran and oats. The control animals in these cases were given the same diet but received a supplement of 10 mg. ascorbic acid daily provided as a 1% ($^{w}/v$) solution which was given orally by means of a teat pipette. The animals were weighed every 3-4 days, and the diet was continued until the test group showed severe deficiency signs such as steady loss of body weight, haemorrhagic joints, etc.

In the experiments designed to suppress the endogenous ACTH, it was decided to use a cortico-steroid in view of the results which had been obtained with rats using cortisol. In order to discover whether other cortical hormones were also effective, use was made of cortisone which is known to be a biological derivative of cortisol.

An initial experiment was carried out with two groups of 3 guinea-pigs (body wt. 240-260 g.). The control group received 0.9% saline injections and the test group a daily dose of 10 mg. cortisone for 3 days. Each animal was given 500 μc ^{32}P on the 4th day and

killed 2 hours later. Since no significant changes were observed in this experiment, a further experiment including 6 animals in each group and designed on similar lines was carried out. The animals used in this second experiment were rather larger (body wt. 440-460 g.). The analyses were carried out on the pair of glands from each animal.

The results of both experiments are given in Table 29 and 30 and from these it is apparent that administration of cortisone to guinea-pigs at the level used in our experiments does not bring about any significant changes either in gland weight or in the constituents measured.

The incorporation of ^{32}P into any of the fractions, LP, RNAP or DNAP is totally unaffected by the administration of cortisone.

Symington and Davidson (1956) have studied the effect of a two stage bilateral adrenalectomy in human cases where, after the removal of the first gland, some time is allowed for the second gland to recover from the effects of the operation and ACTH then administered, in response to which the gland undergoes significant changes. The second gland is subsequently removed, and an analysis of the two glands shows the differences that have been

Table 29

Effect of cortisone administration on the guinea-pig adrenal gland.

		Expt. No.*	control	cortisone-treated
wt/2 glands (mg)		1	130 ± 4.5	140 ± 6.0
		2	220 ± 16	207 ± 23
LP	μg/2 glands	1	208 ± 7.7	204 ± 8.0
		2	343 ± 25	320 ± 40
	μg/100 mg.	1	161 ± 5	146 ± 1.5
		2	156 ± 2.5	153 ± 3.2
RNAP	μg/2 glands	1	63 ± 2.1	65 ± 1.9
		2	94.4 ± 8.0	90 ± 11
	μg/100 mg.	1	48 ± 0.2	47 ± 2.8
		2	43 ± 0.9	43 ± 1.2
DNAP	μg/2 glands	1	30 ± 2.6	36 ± 2.3
		2	45 ± 3.5	48 ± 4.7
	μg/100 mg.	1	23 ± 1.3	26 ± 2.2
		2	21 ± 0.4	23 ± 0.8
RNAP/DNAP		1	2.12	1.82
		2	2.07	1.86
LP/DNAP		1	6.97	5.69
		2	7.54	6.54

* The number of animals in each group in experiments 1 and 2 is 3 and 6 respectively.

brought about in the second gland by the administration of ACTH. Thus the first gland from the subject serves as a control for the second gland. It was decided to follow this procedure, and study the effect of unilateral adrenalectomy in the guinea-pig on the metabolism of the remaining gland.

In the guinea-pigs, however, the two adrenal glands are not of the same size, the left gland being significantly bigger than the right (Kojima, 1928), and it is usually assumed that no functional significance can be attributed to this difference (Jones, 1957). It was, however, desirable to establish that the total amount of nucleic acid in the two glands was directly proportional to the gland weight and that the incorporation of ^{32}P and the action of ACTH were similar.

The guinea-pigs used varied widely in body weight (300-750 g.) and were divided into two groups of corresponding individual weights. The control group was untreated and the test group received daily 10 mg. Acthar-gel for 3 days. On the 4th day 500 µc ^{32}P was administered to each animal 2 hours prior to killing. The right and the left adrenal glands from each animal were weighed and analysed separately. The results are shown in Tables 31, 32 and 33.

Table 30

Effect of Cortisone administration on the guinea-pig adrenal gland

Specific Activities

(relative to tissue inorganic phosphate x 10^3)

	Expt. No.	Control	Cortison-treated
LP	1	11.2 ± 1.8	10.5 ± 0.9
	2	18.0 ± 2.0	14.0 ± 2.8
RNAP	1	12.1 ± 1.6	10.1 ± 0.7
	2	13.4 ± 2.1	14.8 ± 1.6
DNAP	1	1.8 ± 0.2	2.1 ± 0.4
	2	6.1 ± 1.0	7.6 ± 1.4

Table 31

Composition of the individual adrenal gland in the guinea-pig before and after ACTH administration.

	Control			ACTH-treated		
	right gland(R)	left gland(L)	$\frac{L-R}{R}$ x 100	right gland(R)	left gland(L)	$\frac{L-R}{R}$x100
wt. (mg.)	106	121	14.2	141.3	161.8	14.6
LP (µg.)	192	208	8.3	234.7	242.7	3.5
RNAP (µg.)	43.2	50	15.8	80	91.7	14.6
DNAP (µg.)	20.5	24.4	19	23.3	28	20
RNAP/DNAP	2.11	2.05		3.44	3.28	
LP/DNAP	9.37	8.52		10.1	8.67	

(Note: Each number in the table is the mean of 4 values).

The weight of the gland and the levels of the constituents measured, i.e., LP, RNAP and DNAP are significantly smaller in the right gland than in the case of the left gland. When these differences are expressed as the percentage excess of the left gland over the right gland($\frac{L-R}{R}$ x 100) there is a 15-20% excess in weights,RNAP and DNAP. The LP, however, does not show this large difference. These differences are not observable when expressed as concentrations per 100 mg. weight of tissue (Table 32) and the uptake of ^{32}P into the right and the left gland is seen to be the same as shown by the relative specific activities of the LP, RNAP and DNAP.

Treatment with ACTH increases the size of both the glands to the same extent so that the percentage excess ($\frac{L-R}{R}$ x 100) continues to be the same, except in the case of LP which tends to decrease below the normal value. The concentrations of the constituents for the right and left glands are again the same, though different from normal values. The relative specific activities do not show differences between the two glands.

It is therefore justifiable in the case of the guinea-pig to adopt the procedure described by Symington and Davidson and to obtain the control and test glands

Table 32

Composition of the right and left adrenal glands of guinea-pigs, before and after ACTH administration, expressed as concentration per 100 mg. tissue.

	Control		ACTH- treated	
	R	L	R	L
LP (µg.)	182 ± 0.6	176 ± 3.5	167 ± 3.1	158 ± 8.5
RNAP (µg.)	41.4 ± 1.3	43.5 ± 1.2	58.9 ± 2.9	55.3 ± 2.9
DNAP (µg.)	19.8 ± 0.7	21.5 ± 1.2	17.5 ± 1.3	18.2 ± 1.4

Table 33

Specific Activities

(relative to tissue inorganic phosphate x 10^3)

of LP, RNAP, and DNAP from the right and left adrenal glands of guinea-pigs before and after ACTH administration.

	Control		ACTH-treated	
	R	L	R	L
LP	8.2 ± 2.6	7.4 ± 0.4	14.0 ± 2.0	13.4 ± 1.0
RNAP	11.6 ± 1.2	13.6 ± 2.0	15.9 ± 3.0	16.7 ± 1.2
DNAP	4.6 ± 0.1	4.3 ± 0.1	10.7 ± 1.0	11.3 ± 0.5

from the same animal by removing the adrenals in two operations. During the first stage the larger of the two glands i.e. the left one is removed and the right gland obtained when the animal is killed some days later. During the post-operative period some of the animals received ACTH and in each case the results of compensatory hypertrophy plus ACTH or compensatory hypertrophy alone in the right gland could be compared with those for the corresponding normal left gland.

Due to the major nature of the surgery involved and the high mortality among the operated animals and also bewause the use of ^{32}P in this type of experiment is precluded, these studies were not pursued long enough to yield a representative set of data. However, such results as were obtained are of some interest and are shown in Tables 34 and 35.

Animal 1 was allowed to recover for 3 days after removal of the left gland and received no ACTH, so that any increase observed in this case represents compensatory hypertrophy. Animal 2 was also allowed a post-operative recovery period of 3 days but received during this period intramuscular injections of 10 mg. Acthar-gel per day. The remaining group of 3 animals was allowed

Table 34

Effect of unilateral adrenalectomy in guinea-pig on the metabolism of the remaining adrenal gland, with and without ACTH administered post-operatively.

Comparison of gland weights.

Animal No.	left gland (L) (mg.)	right gland (R) (mg.)	$\frac{R-L}{L}$ x 100		Treatment and remarks	
1	133	156	17.2		recovery for 3 days no ACTH	compensatory hypertrophy.
2	101	179	77		recovery for 3 days + ACTH	
3	136	217	60	73	recovery for 7 days + 3 days ACTH	
4	163	295	81			
5	78	140	79			

Table 35

Effect of unilateral adrenalectomy in the guinea-pig on the metabolism of the remaining gland, with and without ACTH administered post-operatively.

The total amount of the constituent in the right gland is expressed as percentage increase over the corresponding amount in the left gland i.e. $\frac{R-L}{L}$ x 100.

	animal 1	animal 2	animals 3,4 & 5 (mean)
LP	20	56	87
RNAP	31	110	103
DNAP	15	27	26

Notes: For the conditions of treatment applicable to each animal see Table 34).

to recover for 7 days and then received for the next 3 days 10 mg. ACTH daily before being killed on the 11th day.

In all cases the values obtained for the right gland are shown as the percentage increase over the corresponding value for the left gland from the same animal.

It is clear that even though the left gland is normally somewhat larger than the right gland nevertheless the size of the right gland is increased by compensatory hypertrophy following extirpation of the left gland and that ACTH produces a considerable further increase in size and this is reflected in the levels of the constituents determined. The administration of ACTH after 7 days of recuperation does not bring about any greater synthesis of RNAP and DNAP than its administration does during the first 3 days, but the amount of LP increases to a greater extent.

Experiments on scorbutic guinea-pigs.

As has already been stated in the General Introduction, the role of ascorbic acid in adrenocortical function is contradictory. It was therefore of interest to study whether this substance played any important role in the nucleic acid metabolism of the adrenal gland.

In a preliminary experiment 6 guinea-pigs were made scorbutic as described in the experimental part of this section. Three of the animals were given 10 mg. Acthar-gel daily for 3 days from the 15th day of feeding when the animals were exhibiting definite signs of deficiency. On the 18th day all animals were killed 2 hours after being given 500 μc ^{32}P. The analyses were carried out on the pair of glands from each animal. The results are given in Tables 36 and 37, and the results of the statistical analysis to determining the significance of differences between the mean values for the data per pair of glands are shown in Table 38.

The scorbutic guinea-pigs respond to administration of ACTH by showing highly significant differences in gland weight ($P < 0.01$) and RNAP ($P \ll 0.01$) and significant differences in LP and DNAP ($P < 0.02$). Likewise the amounts of RNAP and LP per cell represented by the ratios $\frac{RNAP}{DNAP}$ and $\frac{LP}{DNAP}$ also show increases in the group receiving ACTH. The increases in the amount of RNAP and DNAP are accompanied by significant increases in ^{32}P incorporation as shown by the values for the relative specific activities of these substances, whereas that of LP, though higher in the text group is perhaps not significant.

Table 36

Effect of ACTH on the adrenals of Scorbutic guinea-pigs.

		no ACTH	ACTH
wt/2 glands (mg.)		224 ± 5.6	373 ± 33
LP	µg/2 glands	412 ± 16	671 ± 63
	µg/100 mg.	184 ± 2.9	178 ± 4.5
RNAP	µg/2 glands	105 ± 4.2	220 ± 12
	µg/100 mg.	46.7 ± 1.5	59.7 ± 0.8
DNAP	µg/2 glands	44.2 ± 0.6	58.2 ± 2.7
	µg/100 mg.	19.7 ± 0.5	15.7 ± 0.5
RNAP/DNAP		2.37	3.78
LP/DNAP		9.32	11.5

Table 37

Specific activities (relative to tissue inorganic phosphate x 10^3) of LP, RNAP and DNAP from the adrenals of scorbutic guinea-pigs.

	no ACTH	ACTH
LP	13 ± 1.0	17 ± 2.5
RNAP	8.9 ± 0.2	12.2 ± 1.0
DNAP	1.7 ± 0.5	4.6 ± 0.5

Table 38

Statistical analysis of results in Table 36;

't' tests to determine the significance of differences in the mean values from groups with and without ACTH

	Degree of freedom	values of 't' found	corresponding values of 'p'
Gland wt.	4	4.34	< 0.01
LP	4	4.01	< 0.02
RNAP	4	7.14	$\ll 0.01$
DNAP	4	3.95	< 0.02

Table 39

Changes in mean body weights (g.) of guinea-pigs showing the effect of deficiency of ascorbic acid with and without ACTH administration.

	scorbutic diet + vit.c (5)*	scorbutic diet + vit. c + ACTH(6)	scorbutic diet(5)	scorbutic diet + ACTH (6)
Initial wt.	481 ± 11	490 ± 10	480 ±31	521 ± 22
Final wt. (24th day)	436 ± 16	433 ± 30	335 ±25	348 ± 30

* The number in brackets indicates the no. of animals in each group.

In a second experiment 4 groups of 6 guinea-pigs (body wt. 450-550 g.) were chosen such that the groups consisted of animals of matched body weight. They were all kept on the ascorbic acid deficient diet, 2 of the groups receiving a supplement of ascorbic acid. From the 21st day one scorbutic group and one supplemented group received 10 mg. ACTH (cortrophin-ZN) daily for 3 days and on the 24th day, the animals were killed 2 hours after 500 µc ^{32}P per animal.

The mean body weight of the animals in each group, at the start of the experiment and on the day of killing, is given in Table 39, and the analytical results in Tables 40 and 41, and in Fig. 16. The statistical analysis for determining the significance of differences in the mean values for gland weight and the amounts of constituents per pair of glands is given in Table 42.

Apart from confirming the results of the previous experiment (Table 36) of the pronounced stimulation of the gland by ACTH in scorbutic animals (Group D in this experiment), these results show that the onset of severe scurvy itself tends to bring about an effect similar to the administration of ACTH. The increases in gland weight, LP, RNAP and PrN in scorbutic animals is of the same order of magnitude as in the ACTH-treated group,

Table 40

Analytical values for the adrenal glands from the guinea-pigs whose body weights are given in Table 39.

		Scorbutic diet + vit. C.		Scorbutic Diet	
		no ACTH	ACTH	no ACTH	ACTH
wt/2 glands (mg.)		193 ± 14.6	239 ± 11.4	243.5 ± 15	357 ± 16
LP	µg/2 glands	358 ± 29	458 ± 28	445 ± 22	627 ± 35
	µg/100 mg.	185 ± 5.7	191 ± 7.1	185.6 ± 13.1	176.6 ± 5.2
RNAP	µg/2 glands	91.2 ± 6.4	135.5 ± 7.9	149.8 ± 20.2	208 ± 17.0
	µg/100 mg.	47.3 ± 0.9	56.6 ± 1.2	61.2 ± 3.4	57.9 ± 1.8
DNAP	µg/2 glands	37.0 ± 3.1	38.2 ± 3.0	44.0 ± 1.6	50.1 ± 2.9
	µg/100 mg.	19.3 ± 1.1	16.1 ± 0.9	18.4 ± 1.4	14.6 ± 0.9
PrN	mg/2 glands	3.21 ± 0.27	4.30 ± 0.22	4.15 ± 0.23	6.24 ± 0.47
	mg/100 mg.	1.67 ± 0.06	1.79 ± 0.06	1.68 ± 0.08	1.81 ± 0.05
RNAP/DNAP		2.46	3.55	3.40	4.15
LP/DNAP		9.67	12.0	10.10	12.5

Table 41

Specific activities (relative to tissue inorganic phosphate x 10^3) for LP, RNAP, and DNAP from the adrenal glands of guinea-pigs showing the effect of ascorbic acid deficiency with and without ACTH administration.

	Scorbutic diet + vit C no ACTH	+ ACTH	Scorbutic diet no ACTH	+ ACTH
LP	13.8 ± 1.3	20.6 ± 1.6	13.9 ± 4.3	20.2 ±1.7
RNAP	8.9 ± 1.2	25.2 ± 2.0	16.1 ± 1.7	29.0 ±1.9
DNAP	0	2.8 ± 0.7	1.4 ± 0.4	2.8 ±0.8

Table 42

Statistical analysis of data presented in Table 40; 't' tests to determine the significance of differences between the mean values of the different groups for the action of ACTH on guinea-pigs placed on a scorbutic diet with and without a supplement of ascorbic acid.

Measure tested	*Groups compared	Degrees of freedom	value of 't' found	corresponding value of 'p'
wt. of glands	A:B	9	2.51	< 0.05
	A:C	8	2.36	< 0.05
	A:D	9	7.63	< 0.01
	B:D	10	6.05	< 0.01

Table 42 (contd.)

measure tested	*Groups compared	Degrees of freedom	value of 't' found	corresponding value of 'p'
LP µg/2 glands	A:B	9	2.50	< 0.05
	A:C	8	2.38	< 0.05
	A:D	9	5.93	< 0.01
	B:D	10	3.7	< 0.01
RNAP µg/2 glands	A:D	9	4.35	< 0.01
	A:C	8	2.77	< 0.05
	A:D	9	6.44	< 0.01
	B:D	10	2.20	about 0.05
DNAP µg/2 glands	A:B	9	0.27	not significant
	A:C	8	2.0	0.1 not significant
	A:D	9	3.06	< 0.02
	B:D	10	1.81	not significant
PrN mg/2 glands	A:B	9	3.18	< 0.01
	A:C	8	2.65	< 0.05
	A:D	9	5.60	< 0.01
	B:D	10	3.99	< 0.01

* A = Diet + supplement of vitamin C

B = " " " + ACTH

C = Diet

D = Diet + ACTH.

Fig. 16

The effect of ascorbic acid deficiency and ACTH administration on the guinea-pig adrenal gland.

(The vertical lines on top of the columns show the standard error).

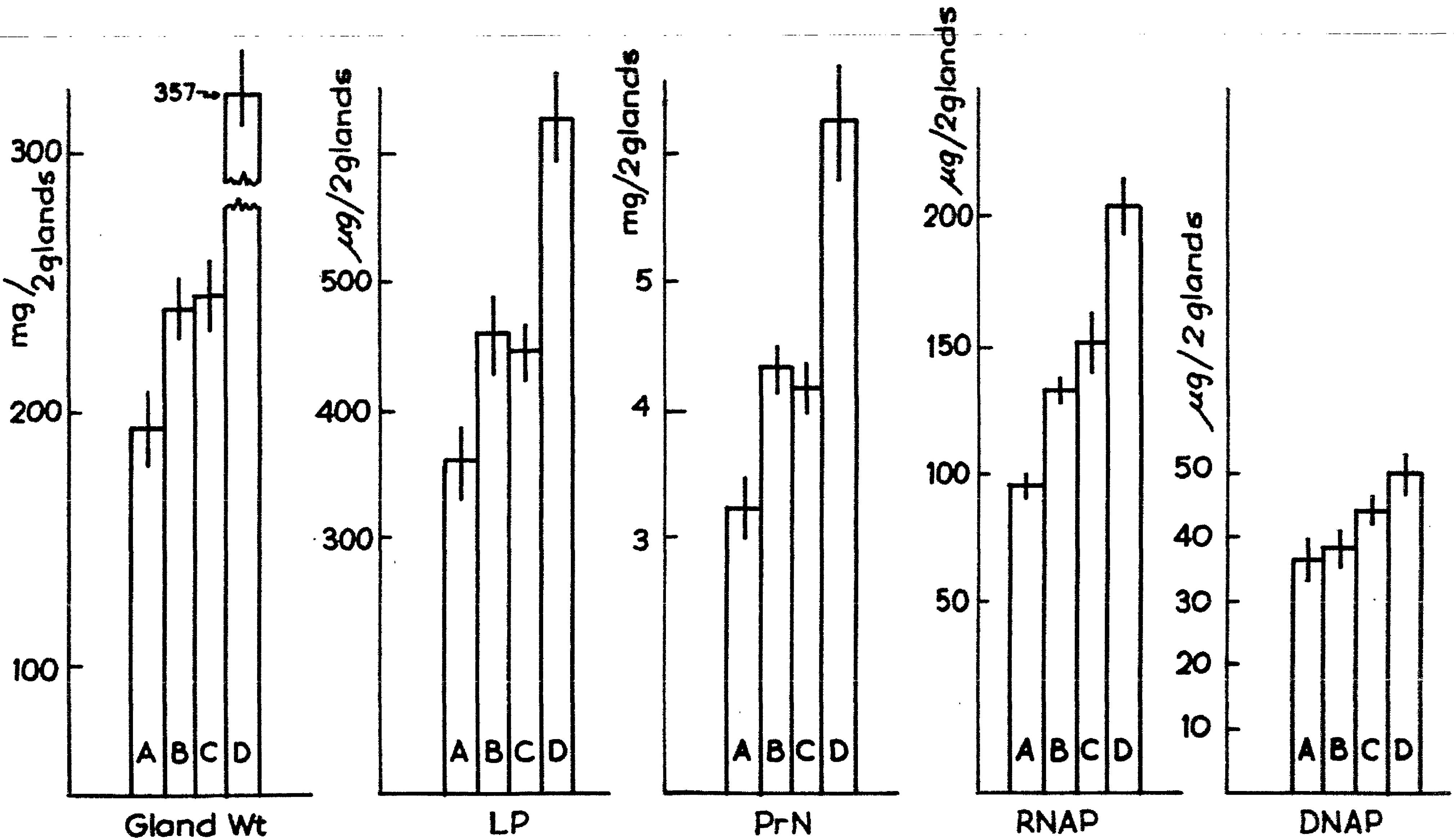


Fig 16
mg/2glands
300
200
100
357
A
B
C
D
Gland Wt
μg/2glands
500
400
300
LP
mg/2glands
5
4
3
PrN
μg/2glands
200
150
100
50
RNAP
μg/2 glands
50
40
30
20
10
DNAP
A: Diet + vit.C.
B: Diet + vit.C+ACTH.
C: Diet.
D: Diet + ACTH.

while the increase in DNAP is not significant. The only significant increase in DNAP is in the scorbutic group receiving ACTH (Group D, $P < 0.02$).

The relative specific activity of RNAP in the scorbutic group is higher than in the control, and in the two ACTH-treated groups is very much higher, though in these two groups it is of the same order (Groups B and D) in spite of the enormously increased amounts of RNAP in Group D compared to Group B. The DNAP, which in this experiment was purified by ionophoresis on paper, shows no incorporation of ^{32}P in the control group and among the other groups which do show incorporation, the ACTH-treated groups have higher activities than the scorbutic group. The relative specific activity of LP is the same for the scorbutic group as for the control group, and while both ACTH-treated groups show an increased incorporation into LP, in this case, too, there is no significant difference between them.

Effect of cortisol on scorbutic animals.

In view of the observation that the onset of scurvy brings about hypertrophy of the adrenal gland it was of interest to know whether the deficiency under the acute conditions employed in these experiments was itself acting

as a non-specific stress through the medium of the adenohypophysis causing the release of endogenous ACTH. One would expect that if this were the case, the administration of a corticoid to block the pituitary release of ACTH would be effective in preventing the increases found in the gland in scorbutic animals.

Since it was found earlier that cortisone administration to guinea-pigs did not have any marked effect on the composition of the adrenal, it was decided to use cortisol instead since this had proved effective in rats and also to administer the drug during the period when the scurvy was developing.

Four groups of 6 guinea-pigs (body wt. 300-400 g.) were fed as in the previous experiment. From the 10th to the 20th day inclusive, two of the groups, one with and another without the supplement of ascorbic acid, received 5 mg. cortisol (Hydro-Adresan) daily. All the animals were killed on the 21st day, 2 hours after being given 500 μc ^{32}P.

The initial and final mean body weights of animals for the different groups are given in Table 43, and the analytical results in Tables 44 and 45, as well as in Fig. 17. The statistical analysis for determining the significance of differences between the mean values of

Table 43

Effect of cortisol on normal and scorbutic guinea-pigs.

(a) Mean body weights of animals (g.)

	non-scorbutic		scorbutic	
	no cortisol (A)	cortisol (B)	no cortisol (C)	cortisol (D)
Initial	344 ± 11	338 ± 10	334 ± 14	333 ± 10
Final (21st day)	321 ± 17	321 ± 12	272 ± 13	266 ± 11

Table 44

Effect of cortisol on normal and scorbutic guinea-pigs.

(b) Analytical results.

		non-scorbutic		scorbutic	
		no cortisol (A)	cortisol (B)	no cortisol (C)	cortisol (D)
wt/2 glands(mg)		208 ± 5.3	195 ± 6.7	230 ± 4.5	197 ± 10.9
LP	µg/2 glands	361 ± 7.0	323 ± 8.2	385 ± 8.4	311 ± 12.1
	µg/100 mg.	173 ± 5.9	165 ± 4.5	168.7 ± 3.9	158 ± 6.1
RNAP	µg/2 glands	99.3 ± 4.7	87.2 ± 2.9	116.4 ± 2.5	92.4 ± 7.7
	µg/100 mg.	47.3 ± 1.2	45.0 ± 1.4	51.0 ± 1.7	46.4 ± 1.4
DNAP	µg/2 glands	40.0 ± 0.1	42.5 ± 1.4	43.0 ± 1.7	44.1 ± 2.5
	µg/100 mg.	19.5 ± 0.8	22.0 ± 1.1	19.2 ± 3.8	22.5 ± 1.1
PrN	mg/2 glands	3.50 ± 0.2	3.05 ± 0.09	3.99 ± 0.07	3.18 ± 0.25
	mg/100 mg.	1.71 ± 0.06	1.57 ± 0.05	1.67 ± 0.05	1.61 ± 0.13
RNAP/DNAP		2.47	2.06	2.69	2.10
LP/DNAP		9.38	7.61	8.90	7.13

Table 45

Effect of cortisol on normal and scorbutic guinea-pigs.

(c) Specific activities (relative to tissue inorganic phosphate x 10^3)

	non-scorbutic		scorbutic	
	no cortisol (A)	cortisol (B)	no cortisol (C)	cortisol (D)
LP	17.1 ± 2.3	21.4 ± 2.2	19.4 ± 3.0	19.3 ± 3.1
RNAP	14.4 ± 1.0	15.4 ± 2.3	19.3 ± 1.3	21.3 ± 4.3
DNAP	0	0	0	0

Table 46

Statistical analysis of data presented in Table 44; 't' tests to determine the significance of differences in the mean values for the different groups used to study the effect of cortisol on guinea-pigs kept on a scorbutic diet with and without a supplement of ascorbic acid.

measures tested	*Groups compound	Degrees of freedom	value of 't' found	corresponding value of 'p'
Wt. of glands	A:B	9	1.47	not significant
	A:C	9	3.00	< 0.02
	A:D	9	0.89	not significant
	C:D	10	2.80	< 0.02

Table 46 (contd.)

measures tested	*Groups compound	Degrees of freedom	value of 't' found	corresponding value of 'p'
LP µg/2 glands	A:B	9	3.54	< 0.01
	A:C	9	2.22	< 0.05
	A:D	9	3.57	< 0.01
	C:D	10	5.03	< 0.01
RNAP µg/2 glands	A:B	9	2.17	not significant
	A:C	9	2.20	< 0.05
	A:D	9	0.9	not significant
	C:D	10	2.96	< 0.02
DNAP µg/2 glands				not significant for any groups tested.
PrN mg/2 glands	A:B	9	2.04	not significant
	A:C	9	2.31	< 0.01
	A:D	9	1.00	not significant
	C:D	10	3.12	< 0.01

* A: Diet + Ascorbic acid supplement.

B: " " " " + cortisol.

C: Diet.

D: Diet + cortisol.

gland weight and the amounts of constituents per pair of glands is given in Table 46.

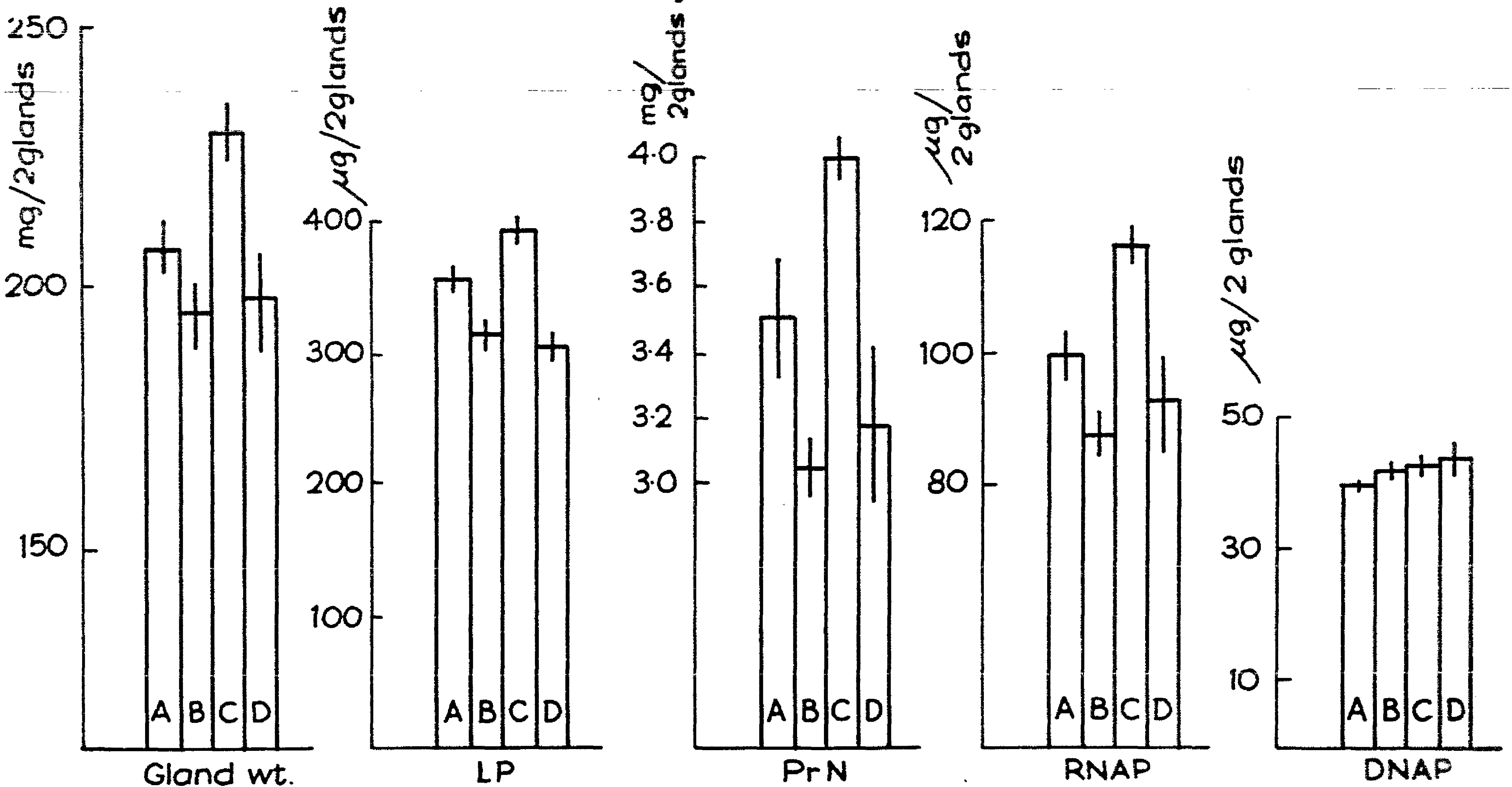
Administration of cortisol to guinea-pigs on an ascorbic acid deficient diet with or without a supplement of the vitamin tends to suppress the adrenal gland, but not to any significant extent, as is shown by the gland weight, RNAP and PrN content of the gland in groups A, B and D.

There is no incorporation of ^{32}P into DNA of any of the groups, nor is there any significant difference in the relative specific activities of LP. In the case of RNAP there is a slight increase in ^{32}P uptake by the two scorbutic groups.

Fig. 17

The effect of ascorbic acid deficiency and cortisol administration on the guinea-pig adrenal gland.

Fig. 17



A: Diet + vit.C. B: Diet + vit.C + cortisol. C: Diet. D: Diet + cortisol.

Section 4.

Effect of Dietary Changes on Nucleic Acid and Protein Metabolism in normal and Tumour-bearing Rats.

It is known that the administration of cortical hormones such as cortisone brings about a general loss in body protein, accompanied at the same time by an accummlation of protein in the liver (Tremolieres, Derache and Lowy, 1955) and a similar situation also obtains in tumour-bearing animals (Stewart and Begg, 1953). Begg (1958) has reported adrenocortical overactivity in tumour-bearing rats and Verzar and Vogt (1953) also consider that changes in the normal diet constitute a form of stress.

It was therefore of interest to examine the effect of diet on the nucleic acid and protein metabolism in the adrenal gland of rat.

Experimental: Female albino rats weighing 170-200 g., from the departmental colony were used in these experiments.

The tumour was the Walker carcinoma 256 which was propagated by transplantation.

Diet. The general dietary arrangements were modelled to provide different levels of protein and of energy (Munro and Naismith, 1953). At 9 a.m. 1g. of a vitamin-mineral-roughage mixture (Munro, 1949) was given to all animals. At 5 p.m. they ate a meal that provided all the protein of the diet. In the case of

the rat receiving an adequate protein intake the evening meal contained 2.4 g. of casein (unextracted grade, Glaxo), 1.2 g. carbohydrate (equal parts of glucose and potato starch) and 0.4 g. fat (margarine). The corresponding evening meal in the case of rats receiving protein-free diet had all the casein replaced by an isocaloric amount of carbohydrate.

At each of these two levels of protein the basic diet provided 21 Kcal./rat/day and carbohydrate was added to the morning meal to bring the energy intake up to any desired level. At first all rats were given a total energy intake of 33 Kcal/day (about 1200 Kcal./sq. m. body surface area) for a period of 5 days, in order that they might become accustomed to the diets. This energy intake was obtained by adding 2.5 g. starch and 0.5 g. glucose to the morning meal.

After this preliminary period of adjustment, the animals at each level of dietary protein were divided into a group receiving a high energy intake and a group receiving a low energy intake. Those on the high energy intake were given 5 g. starch and 1 g. glucose in the morning meal, the total daily energy content of the diet being 45 Kcal. (about 1800 Kcal./sq. m.).

In the case of animals receiving a low energy intake the supplementary carbohydrate was withdrawn from the morning meal and they were thus reduced to the 21 K cal. provided by the basic diets (about 850 Kcal./sq. m.). Administration of the various diets was continued for six days. At the end of the period a batch of rats in a fasting condition was killed in the morning at 10 a.m., 17 hours after the last protein meal and another batch killed at 5 p.m. 8 hours after the last energy meal. In the case of experiments involving ^{32}P incorporation 500 μc ^{32}P was given to each rat intramuscularly 2 hours prior to killing.

The results of the quantitative analysis are shown in Fig. 18 statistical analyses to determine the significance of differences in the mean values for the different groups of treatment are given in Tables 47-51 for gland weight, PrN, LP, RNAP and DNAP.

The time of killing does not appear to affect either the gland weight or the amount of any of the constituents measured and has not been further considered.

Changes in the protein intake alter the PrN and the LP content of the adrenal glands significantly, an adequate protein diet causing an increase in the amounts of these components in the gland. Although on the

Fig. 18

The effect of dietary changes on the metabolism of the adrenals of normal female rats.

Fig. 18

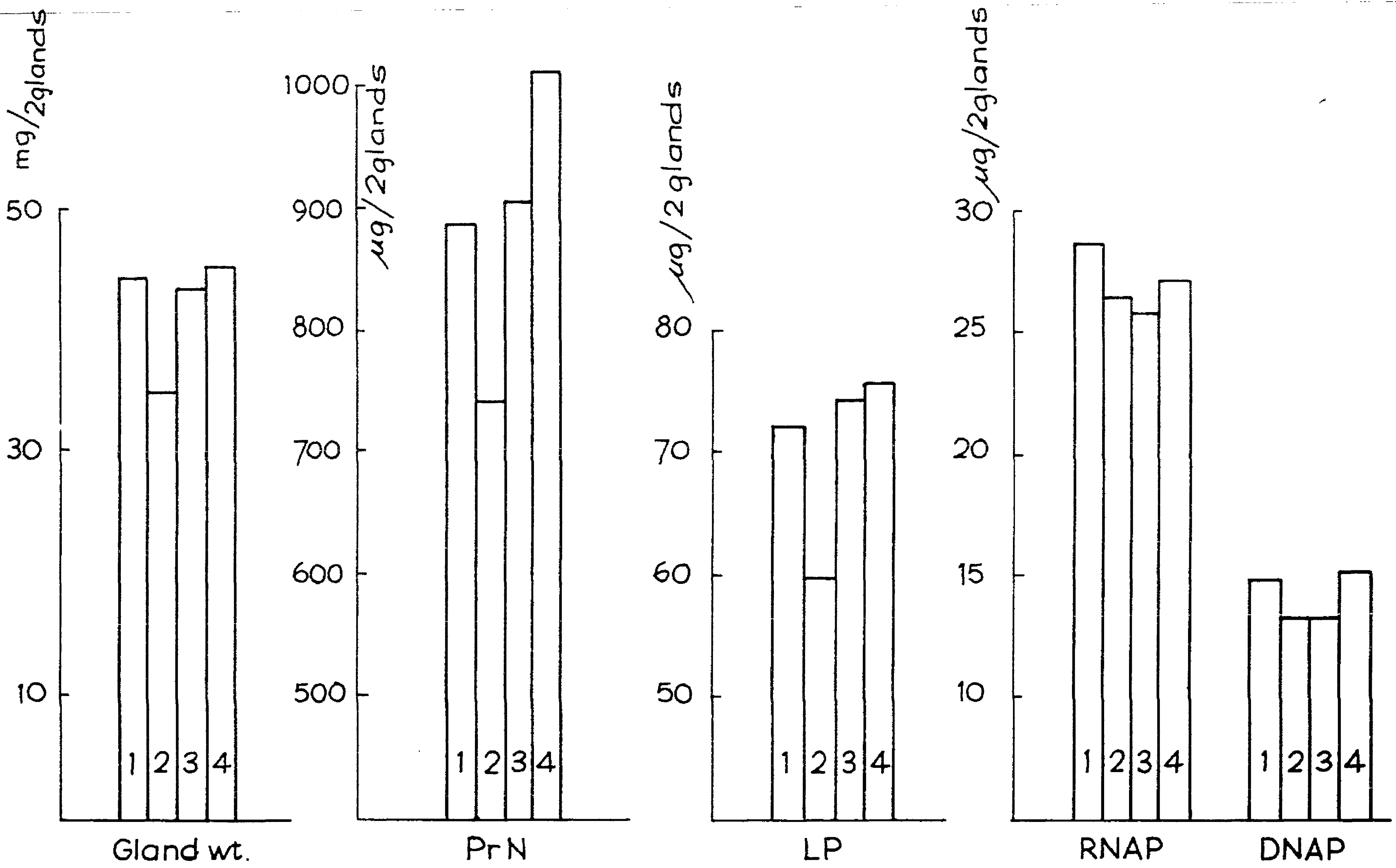


Table 47

Analysis of variance to test the significance of differences between the mean values for gland weight among groups of rats fed with different diets.

(data of Fig. 18)

source of variation	degrees of freedom	sum of squares	mean squares	variance ratio, F
Total	15	458		
subclasses	7	288		
Energy levels, E	1	56	56	2.64
Protein levels, P	1	90	90	4.25
Time of killing, T	1	0	0	
Interaction, ExP.	1	133	133	6.27*
" PxT.	1	3	3	0.14
" ExT.	1	7	7	0.33
Residual	8	170	21.2	

For $n_1 = 1$, $n_2 = 8$ the value of $F = 5.32$ at the 5% significant level.

There is therefore a significant interaction between energy intake and protein content of the diet.

Table 48

Analysis of variance to test the significance of differences between the mean values for PrN among groups of rats fed with different diets

(data of Fig. 18)

source of variation	degrees of freedom	sum of squares	mean squares	variance ratio, F.
Total	15	252,544		
subclasses	7	174,315		
Energy levels, E	1	1,444	1,444	
Protein levels, P	1	84,390	84,390	8.68*
Time of Killing, T	1	21,609	21,609	2.2
Interaction, ExP.	1	63,504	63,504	6.50*
" PxT.	1	490	490	
" ExT.	1	1,980	1,980	
Residual	8	78,229	9,779	

For $n_1 = 1$, and $n_2 = 8$, the value of F = 5.32 at the 5% significant level.

Therefore, dietary changes in the protein bring about significant differences in the protein content of the gland and also changes in energy intake show a significant interaction with protein.

Table 49

Analysis of variance to test the significance of differences between the mean values for LP among groups of rats fed with different diets

(data of Fig. 18).

source of variation	degrees of freedom	sum of squares	mean squares	variance ratio, F
Total	15	1,104		
subclasses	7	813		
Energy levels, E	1	126	126	3.46
Protein levels, P	1	333	333	9.15*
Time of Killing, T	1	3	3	
Interaction, ExP.	1	189	189	5.2
" PxT.	1	97	97	
" ExT.	1	28	28	
Residual	8	291	36.4	

For $n_1 = 1$ and $n_2 = 8$, the value of $F = 5.32$ at the 5% significant level.

Therefore, changes in the protein content of the diet show a significant effect on the LP content of the adrenal gland and the interaction between energy and protein content of the diet is significant nearly at the 5% level.

Table 50

Analysis of variance to test the significance of differences between the mean values for RNAP among groups of rats fed with different diets

(data of Fig. 18)

source of variation	degrees of freedom	sum of squares	mean squares	variance ratio, F
Total	15	478		
Subclasses	7	146		
Energy levels, E	1	1	1	0.02
Protein levels, P	1	5	5	0.1
Time of killing, T	1	13	13	0.2
Interaction ExP.	1	11	11	0.2
" PxT.	1	20	20	0.5
" ExT.	1	48	48	1.2
Residual	8	332	40.2	

For $n_1 = 1$, and $n_2 = 8$, the value of $F = 5.32$ at the 5% significant level.

There is therefore no significant effect on the RNAP content of the adrenal gland by any of the changes in the diet.

Table 51

Analysis of variance to test the significance of differences between mean values for DNAP among groups of rats fed with different diets

(data of Fig. 18)

source of variation	degree of freedom	sum of squares	mean squares	variance ratio, F
Total	15	42.6		
subclasses	7	18.5		
Energy levels, E	1	0.3	0.3	0.01
Protein levels, P	1	0.6	0.6	0.02
Time of killing, T	1	4.0	4.0	0.15
Interaction ExP.	1	9.3	9.3	0.4
" PxT.	1	2.5	2.5	0.1
" ExT.	1	0.4	0.4	0.01
Residual	8	24.1	24.1	

For $n_1 = 1$ and $n_2 = 8$, the value of $F = 5.32$ at the 5% significant level.

There is therefore no significant effect on the DNAP content of the adrenal gland by any of the dietary changes.

average of the two levels of protein intake the energy content of the diet does not have a significant effect, the action of the extra dietary carbohydrate at the low level of protein intake was to reduce the gland weight, PrN and LP whereas at the higher level of protein intake it tended to cause the opposite effect on each constituent. This difference of action at the two protein levels is significant ($P < 0.05$).

It is interesting to note, however, that at the same time, none of these changes in the protein or energy content of the diet affects the nucleic acids the amounts of RNAP and DNAP remaining unaltered.

Experiments with tumour-bearing rats.

The rats with the Walker carcinoma were put on the same diet regimen as the normal rats and were given the protein-free and high protein diets. As before, each of the dietary levels of protein was associated with either a low energy diet or a high energy diet, giving four different diets in all.

Table 52 contains the representative data for the weight of adrenal glands of normal and tumour-bearing rats given the various diets. It was apparent that while the normal rats do respond to dietary changes by showing changes in the gland, the tumour-bearing rats show a general tendency for enlargement of the gland without

Table 52

Paired gland weights (mg.) of adrenal glands from normal and tumour-bearing rats on different diet.

Diet	Normal	Tumour-bearing
LP, LE	48	48
LP, HE	34	49
HP, LE	38	50
HP, HE	50	62
Mean	42.5	52.3

LE = Low Energy intake

HE = High energy intake

LP = Protein-free diet

HP = High protein diet.

significant differences between the various diets. It is therefore desirable to present the pooled data for all the tumour bearing animals as compared with similar data for the normal animals also on the same dietary treatment.

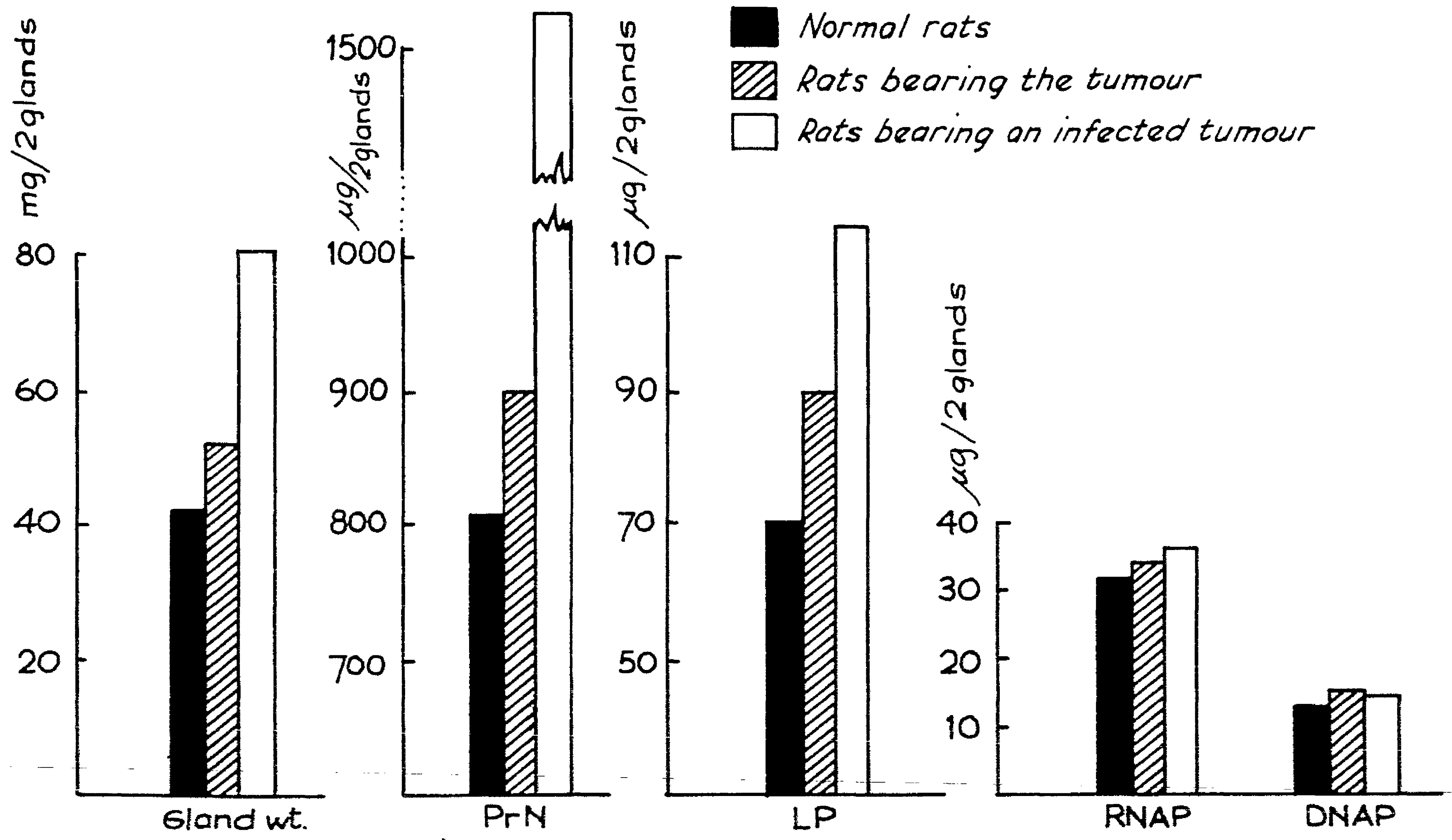
(Note: In a sunsequent experiment with tumour bearing rats it was discovered that there was a pyogenic infection of the tumour. The glands, however, were taken out as usual and analysed and the results are shown separately).

The results for the normal animals on various diets have been averaged and compared with those of tumour-bearing and infected tumour-bearing animals in Fig. 19. These results show that the gland weight as well as the content of LP and of PrN show a significant increase in the case of the tumour-bearing rats as compared with the normal rats and in the case of animals with an infected tumour this increase is very pronounced. Though the amount of RNAP in the two groups of tumour-bearing rats is slightly higher than in the normal group this increase is not significant, nor is there any significant difference between the two tumour-bearing groups. DNAP shows a slight increase in the case of tumour-bearing rats. The infected tumour group, in

Fig. 19

The effect of the presence of the Walker carcinoma on the rat adrenal.

Fig. 19



spite of the greatly increased gland weight, has a mean DNAP content less than that found in the non-infected tumour group, and it was also observed that there was a greater variation between individual values of DNAP in this infected group than in any other group. The incorporation of ^{32}P into the adrenal glands of normal and tumour-bearing rats placed on various diets was studied. The specific activity of the acid-soluble fraction (A_1s) is shown in Fig. 20.

In the case of the normal rats the specific activity of A_1S is significantly less in the high energy groups for both protein-free and high protein diets. When the energy content of the diet is low as in groups 1 and 3, the difference in protein supply does not appear to alter the radioactivity of the acid-soluble fraction.

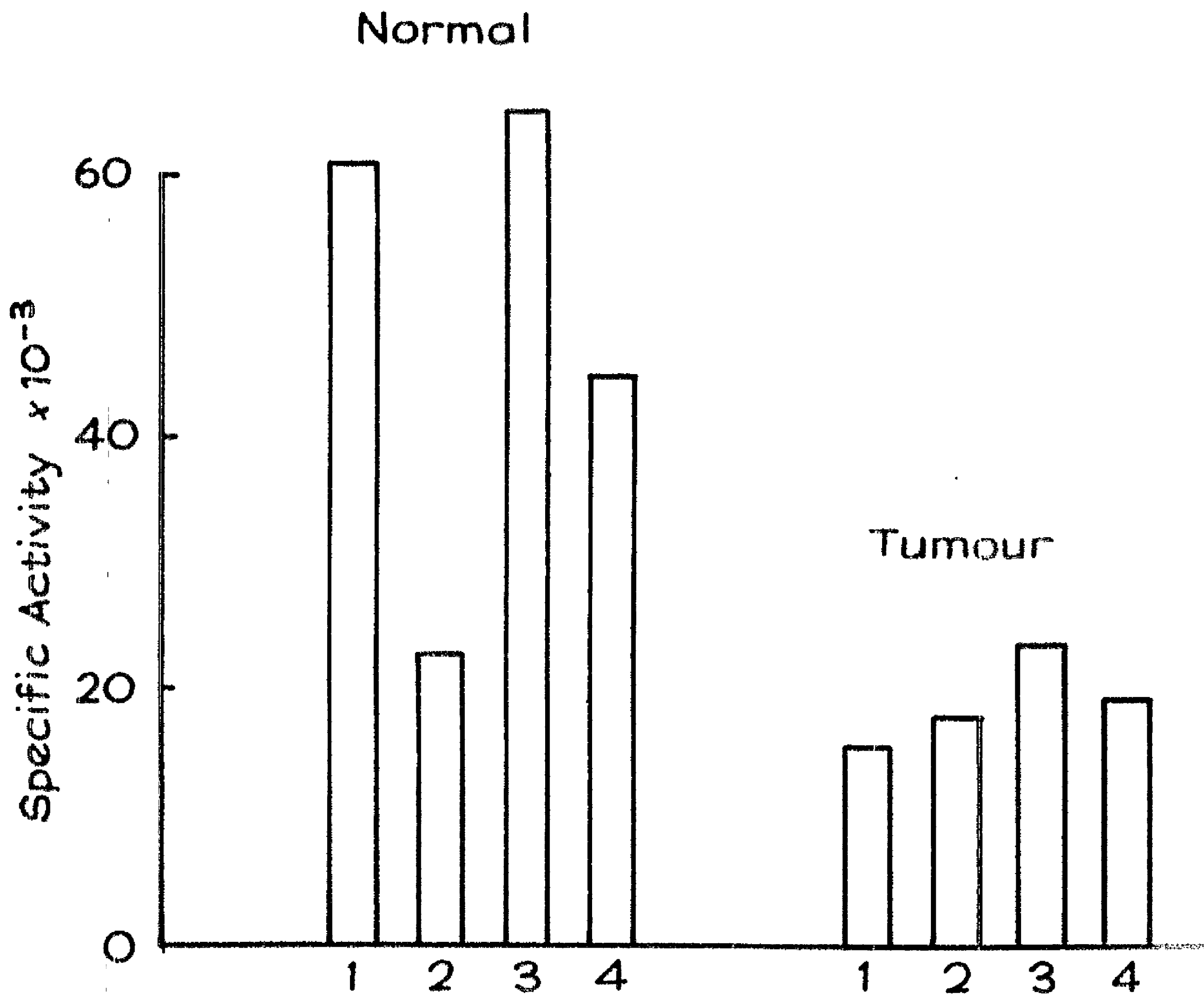
These variations in the specific activity of the acid-soluble fraction due to changes in diet observed in the normal rats are completely abolished in the tumour-bearing rats in which the incorporation is very much lower than that found in normal animals.

Fig. 21 shows the values for the specific activities of the A_1s, LP, and RNAP fractions for normal rats receiving various diets killed either in the morning

Fig. 20

The effect of dietary changes on the specific activity of the acid soluble fraction (A_1S) of the adrenals in normal and tumour-bearing rats.

Fig. 20



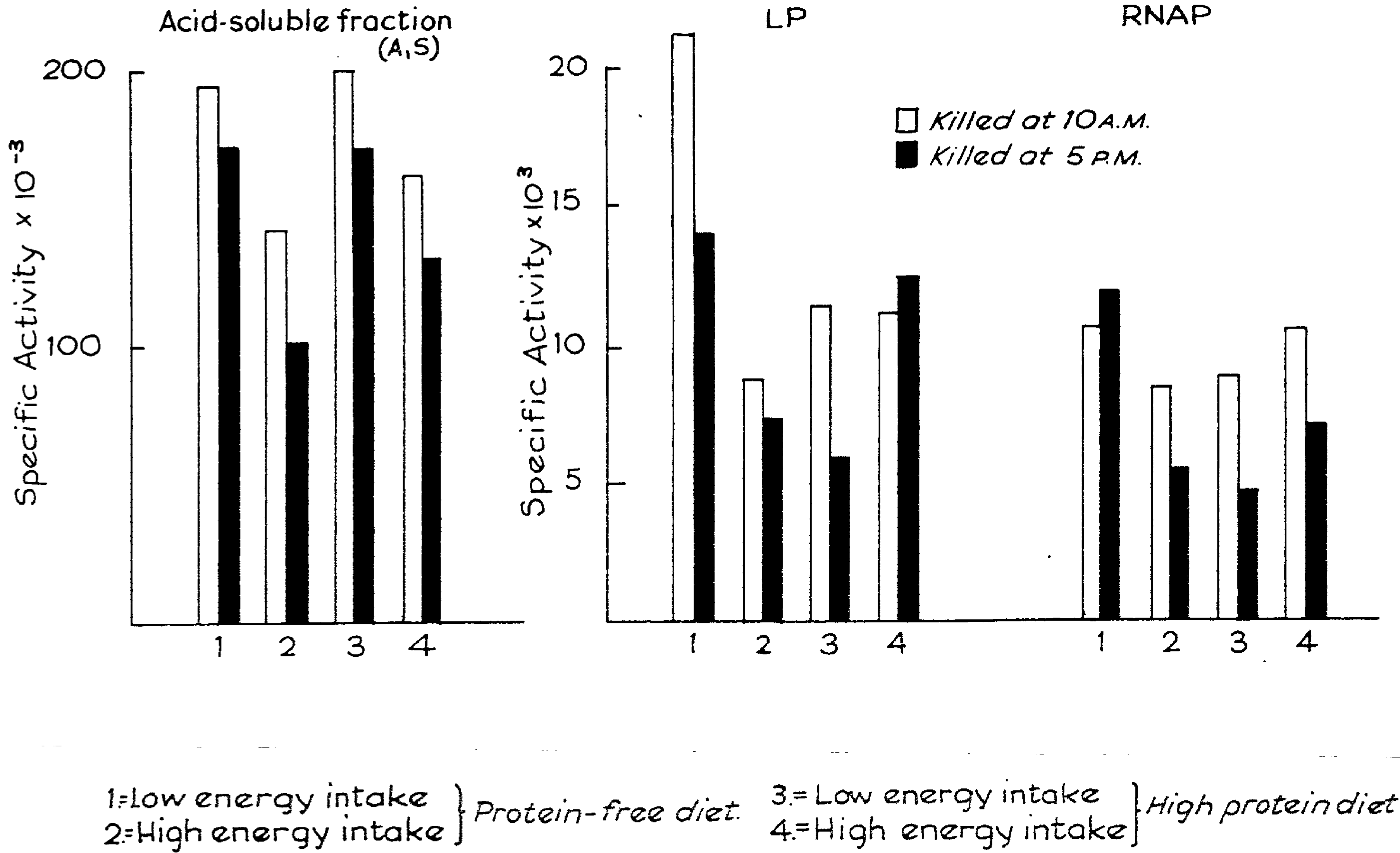
Effect of dietary changes on the specific activity of the acid-soluble fraction (A,S) of the adrenals in normal and tumour-bearing rats.

1 = Low energy intake } *Protein-free diet*
2 = High energy intake }
3 = Low energy intake } *High protein diet*
4 = High energy intake }

Fig. 21

The effect of diet and time of killing on the specific activities of the Acid soluble fraction, LP and RNAP of the adrenals of normal rats.

Fig. 21



1=Low energy intake
2=High energy intake } Protein-free diet.

3.= Low energy intake
4.=High energy intake } High protein diet

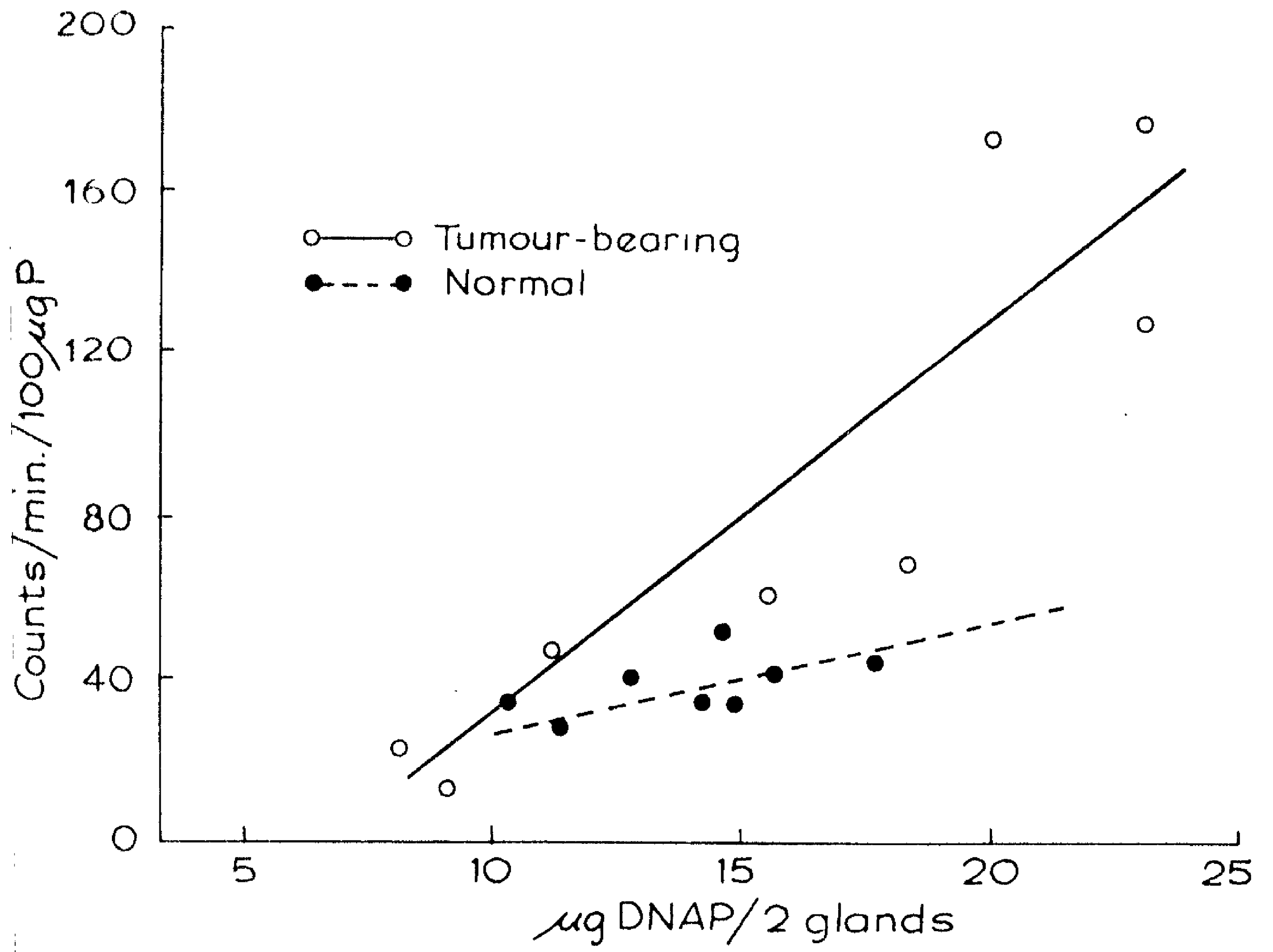
or in the evening. The rats killed in the morning tend to show generally greater incorporation into all fractions irrespective of the dietary changes. However, in group 4 for LP and group 1 for RNAP, the incorporation appears to be about the same both in the morning and evening.

Examination of the results for the uptake of ^{32}P into the DNA of infected tumour-bearing animals shows that there is a great deal of variation in the specific activity from animal to animal, the animals with larger DNA content showing relatively high incorporation. When the specific activity of DNA is plotted as a function of the corresponding amount of DNAP per pair of glands in both the tumour-bearing and normal rats (Fig. 22) a clear tendency in the tumour-bearing animals for greater incorporation of ^{32}P as the amount of DNA increases is observed.

Fig. 22

The effect of the presence of Walker carcinoma on the uptake of ^{32}P into the DNA of the adrenals in the rat.

Fig. 22



PART IV

DISCUSSION.

4.1. General effects of administration of ACTH.

From the results presented it is clear that the changes in the adrenal gland brought about by stimulation or suppression are rendered more obvious when the analytical figures are given as amounts per whole gland than when they are expressed as concentrations per unit weight of tissue as was done by Symington and Davidson (1956). In the experiments reported here with rats, the effect of stimulation with ACTH is to produce hypertrophy as shown by the amount of RNAP per pair of glands which shows increases of up to 30%, while the concentration is hardly affected because the gland weight increases correspondingly (Table 11). In the immature rats which were given 15 mg. ACTH for 3 days (Table 24) there is an increase of 55% in total RNAP, but expressed as µg. per 100 mg. there is a significant lowering perhaps because other constituents of the gland are increasing more rapidly. Even when there is an overall increase of 165% in RNAP content as was found in the rats given massive doses of ACTH (Table 26), this is not reflected in the concentrations.

The guinea-pig on the other hand, when treated with ACTH shows significant increases in RNAP even when the results are expressed as concentration per 100 mg. weight

of tissue (Tables 1, 3, 7 and 36). When expressed as the total amount per pair of glands, these changes are shown up more prominently.

There is generally a considerable increase in total LP content per gland both in the guinea-pig and in the rat on treatment with ACTH. If these results are expressed as µg. per 100 mg. tissue, however, there is no change in the case of the guinea-pig (Table 7) while there is a reduction in the rat (Table 24). In general the LP closely resembles the RNAP in its response to ACTH, except in cases where dietary factors also play a prominent part, since it has been observed that dietary changes bring about significant alterations in the LP content of the gland (Table 49).

Hutchison *et al*. (1958) have recently reported that in guinea-pigs the adrenal cells are essentially diploid and remain so after stimulation by ACTH. In view of this finding it is reasonable to think that any increase which occurs in DNA is not due to polyploidy but represents a true increase in cell number. Hence the results when expressed as the ratios RNAP/DNAP and LP/DNAP represent the content of RNAP and LP per cell and increases in these ratios can be taken to represent hypertrophy. ACTH generally tends to bring about an increase in both RNAP

and LP per cell when administered either to the guinea-pig or to the rat.

Stimulation by ACTH in the guinea-pig.

There is a considerable lag period in the action of ACTH in inducing measurable changes in the nucleic acid composition of the adrenal gland. The duration of this lag period depends on the nature of the ACTH used. With Acthar-gel the glands show slight hypertrophy after 1 day with no increase in the DNAP content or in the incorporation of ^{32}P into DNA, so it is reasonable to suppose that no increase in mitotic activity in the adrenal cortex occurs after treatment for 1 day with Acthar-gel. Thereafter there is a continuous increase in the DNAP content up to 5 days and the incorporation of ^{32}P rises to about 275% of the normal value during this period (Fig. 8), indicating marked hyperplasia. The RNAP content rapidly increases per gland as well as per call indicating simultaneous hypertrophy. The LP content per cell which shows an initial increase has returned to the normal level by the 5th day of ACTH administration (Tables 1 and 3). This may be related to the depletion of neutral fat, which is demonstrable histochemically, and which may be, partially at any rate, converted to phospholipid. The initial effect would therefore be to increase the

LP per cell but as the number of cells increases and the total LP per gland is distributed among a larger number of cells, there could be a tendency for the amount of LP in each cell to be reduced.

The slight decrease in general response to ACTH observed in one of the experiments (Table 3) in the 5 day test group as compared to the 3 day group is perhaps not a true picture because of the large variation between the two individual animals included in this group.

The use of lyophilised ACTH to cause quicker stimulation of the gland in the guinea-pig does not bring about any significant increases up to 16 hours either in the gland weight or in the constituents measured which in this case include PrN (Table 9). There is, in fact, a slight tendency for these to decline over 4 hours, even though this decline is not statistically significant. So far as incorporation of ^{32}P is concerned, there is in general no increased uptake into RNAP, DNAP or LP. The group killed 8 hours after initial injection in this experiment (Table 10) does show a higher relative specific activity for RNA than the other groups, but this may have been due to the fact that this group was killed in the evening at 4 p.m. while all the other groups were killed at noon. Such variation in incorporation due to

differences in the time of killing has been noticed in other experiments also (Fig. 21), an observation which agrees with the finding of Barnum, Jardetzky and Halberg (1958) that there is a diurnal variation in ^{32}P incorporation into various fractions of mouse liver. It seems therefore that lyophilised ACTH does not cause any stimulation in the adrenal gland of the guinea-pig up to 16 hours.

When a combination of lyophilised ACTH and Acthar-gel is employed for stimulation, the stimulation appears to be so strong that there is a great increase in DNAP content by 24 hours (Table 7) and apparently no further increase after that up to 48 hours. As regards incorporation of ^{32}P, increases to about 400% of the normal value are obtained after 24 hours and continued stimulation shows no further increase (Table 8; Fig. 8). It is theoretically impossible, if the stability of DNA is accepted, for both the specific activity and the amount of DNA to remain constant under these conditions. One would expect some further increase in amount during the second day and the fact that this is not observed may be due to the difficulty of estimating DNA with accuracy because of errors in the method and losses during the separation.

Since it has been seen that Acthar-gel alone does

not cause any increase in DNAP content or ^{32}P incorporation into DNA with a 24 hour period of stimulation, it appears that Acthar-gel in conjunction with lyophilised ACTH, which is likely to be absorbed more readily into the circulation, will bring about a pronounced increase in response. It is also to be noted that while the rate of DNA synthesis does not show any increase after 1 day the content of RNAP, LP and PrN continues to increase. Further, even though the amount of RNAP increases after 1 day, the relative specific activity which shows a rapid rise at 24 hours, remains at this level at 48 hours (Fig. 8). The significance of these findings is discussed later (see 4.6).

The radioactivity of LP is very variable, showing a decline at 1 day and an increase at 2 days (Table 8). This variability which is so often found with LP may be due to the fact that the analyses were carried out on the lipid extract of the tissue obtained after the initial treatment with TCA. Even a very small amount of the highly active acid soluble fraction contaminating the lipid fraction would vitiate the comparatively low values for the radioactivity of LP. Studies of LP metabolism using ^{32}P therefore call for greater attention to purification than has been given in these experiments.

More useful results might be obtained if the lipid extract were evaporated to dryness and re-extracted with chloroform as was done by Davidson et al. (1951).

The results presented in Tables 34 and 35 show that unilateral adrenalectomy in the guinea-pig acts as a considerable stress bringing about 'compensatory hypertrophy' within three days after operation. This results in an increase in RNAP, LP as well as in DNAP, so that 'compensatory hyperplasia' must also be taking place. In the guinea-pig, therefore, the need for the adrenal corticoids cannot be met by the remaining gland after unilateral adrenalectomy without compensatory hypertrophy and hyperplasia. If in addition, ACTH is given to these animals, the effect is more pponounced resulting in a very large increase in RNAP and DNAP and this additional stimulation by exogenous ACTH appears to be the same whether the administration follows soon after operation or after a wekk.

Stimulation by ACTH in the rat.

The response to ACTH administration in the rat is not only slower but also very much less pronounced than that in the guinea-pig, even though 2-3 times more ACTH is given to the rat per unit body weight than is

administered to the guinea-pig. When Acthar-gel is given there is no noticeable change after 1 day either in gland weight or the components measured (Table 11). At the end of 2 days there is a significant increase in RNAP and LP and thereafter no further changes are seen. The maximal increases are of the order of 30% above the normal values. There is no measurable increase in DNAP up to 5 days. The only indication of increases mitotic activity is the increased uptake of ^{32}P into DNA and here again the effect is not as pronouced as in guinea-pigs. Hypertrophy of the gland is shown by increases in the RNAP/DNAP ratio.

As high a dose as 15 mg. Acthar-gel daily given to adult rats (Tables 22 and 23) after controlling their endogenous ACTH secretion with cortisol produces considerable hypertrophy but hardly causes any increased incorporation of ^{32}P into the DNA, so that it can reasonably be concluded that there is no increased mitotic rate. A similar dose of Acthar-gel given to immature rats whose ACTH secretion is suppressed by cortisol administration (Tables 24 and 25) causes an increase in DNAP and a marked increase in ^{32}P incorporation into DNA, so that immature rats respond to ACTH by showing adrenal hyperplasia. In addition

very pronounced hypertrophy of the gland takes place.

When massive doses of ACTH are administered, as was done by Fiala *et al*. (1956), the glands, which appear gorged with blood, show a great increase in all the constituents measured (LP, RNAP, PrN and DNAP) (Table 26). It is important, however, to remember that stimulation as severe as that used in this experiment may result in haemorrhagic breakdown of the cortex (Ingle, 1951). The reason for the large differences in response observed by Fiala *et al*. (1956) and by the author may be the fact that the American group used Wistar rats which are a different strain from those used in the Glasgow laboratory. Differences in the composition of the adrenal gland due to variation in strain, such as between August rats and albino rats have been observed and are reported in Table 15.

Effects of withdrawal of circulating ACTH on the rat adrenal gland.

Adrenal cortical atrophy induced in the rat by administration of cortical extracts or cortical hormones is very well known (Ingle *et al*. 1938). Sayers (1950) proposed the hypothesis whereby he envisaged that the level of circulating cortical hormones effectively controls the pituitary secretion of ACTH, an increased

level tending to inhibit this secretion whereas a rapid utilization of these hormones by tissues as occurs under conditions of stress decreases their blood level and therefore induces greater ACTH secretion.

The results given in Tables 17 and 18 show that the effect of cortisol is to cause atrophy of the adrenal gland. The earliest changes observable are in the LP and RNAP content of the gland. These show a decline which is slight at 1 day, very marked at 3 days, and thereafter slow and continuous. The DNAP content of the gland also shows a decrease from the normal value (Fig. 12) and this may perhaps indicate that some degeneration of the cell nuclei is involved in the atrophic process.

It is interesting to note that the level of cortisol administered is of no great significance in causing the atrophy of the gland, 5 mg. per day being just about as effective as 15 mg. It would appear therefore that there is a minimal level of cortisol in the blood which causes complete suppression of ACTH production by the pituitary. However, the fact that a 10 mg. dose of cortisol administered for 3 days continues to be effective in suppressing pituitary activity for a further week (Fig. 12) indicates that the hormone, which is

water-insoluble, is slowly absorbed into the circulation from the site of injection and this will tend to limit the blood level of cortisol attainable with higher doses. A comparison of the effect of the doses of cortisol used with the effect of hypophysectomy, where there is no question of ACTH secretion, indicates that the amount of cortisol absorbed from the site of injection is adequate to suppress completely the animals endogenous ACTH secretion. The appropriate data are presented for albino rats of comparable size in Tables 15 and 18 which show that the two methods produce similar decreases in gland weight, and in the constituents determined.

Since the surgical removal of the source of ACTH involves the withdrawal of other hormones of the pituitary besides ACTH, including somatotrophic hormone, and also because of the difficulty of removing the entire pituitary at operation, it is perhaps more satisfactory to use cortisol to control the secretion of ACTH, though it should be remembered that cortisol also brings about other metabolic changes.

At the end of the 10 day period of the withdrawal of ACTH (Table 18), the decline in gland weight, in LP and in RNAP is about 55% and in DNAP about 35% of the normal values which are all highly significant. Since

these losses occur only in the zonae fasciculata and reticularis of the cortex, leaving the zona glomerulosa and the medulla almost unaffected (Deane and Greep, 1946) it appears that the damage to the cortical cells in these regions is extensive, perhaps also involving the nuclei.

Effect of withdrawal of Andogenous ACTH in guinea-pigs.

Atrophy of the guinea-pig adrenal cortex induced by cortisone administration has been reported, and hypophysectomy brings about similar changes in the zonae fasciculata and reticularis (Schweizer and Long, 1950). So it is reasonable to assume that the withdrawal of ACTH can be brought about either by the removal of the pituitary or by suppressing its action by corticoids.

In our experiments, however, administration of 10 mg. cortisone daily for 3 days does not produce any significant change either in gland weight or in the components measured (Table 29). The uptake of ^{32}P into LP, RNAP and DNAP is also essentially unchanged (Table 30). These observations are in agreement with those of Prunty et al. (1955) who administered cortisone to scorbutic guinea-pigs and observed that the gland weight in cortisone-treated guinea-pigs were the same

as in normal guinea-pigs.

Burstein and Dorfman (1955) have identified the urinary excretion products of cortisol metabolism in guinea-pig and have failed to find any cortisone. Therefore cortisone may not be a normal metabolic product, though there is histochemical evidence that it does, in some circumstances, have a suppressive action on the adrenal cortex. Further attempts to suppress the pituitary function with cortisol given over a period of 10 days (Table 44; Fig. 17) brought about only a slight drop in the weight of the gland, and its constituents, but the decrease was not statistically significant (except in the case of LP content). It is therefore concluded that cortisol, and perhaps cortisone also, do inhibit the pituitary function in the guinea-pig but that the effect of this inhibition on the adrenal is not so pronounced in the guinea-pig as in the rat.

Differences in the responses to ACTH between the rat and the guinea-pig.

The relatively large size of the guinea-pig adrenal gland is noteworthy. While in the rat the adrenal weight per 100 g. body weight is about 30 mg., the corresponding figure for the guinea-pig is about 100 mg. and it is of interest to note that the value for man is

only about 10-15 mg. In the rat the secretion of corticoids by the adrenal is higher than in the guinea-pig (Bush, 1951) and it therefore appears that the rat adrenal gland, even though small by comparison to that of the guinea-pig, is secreting its cortical hormones under greater stimulation. On the basis of this comparison it is possible that the guinea-pig, because it lacks strong stimulation by ACTH, needs a relatively large gland to synthesise its requirements of cortical steroids.

The differences in response to exogenous ACTH are also very pronounced. With a dose 2-3 times that given to the guinea-pig in terms of body weight, the rat shows only hypertrophy. The guinea-pig, on the other hand, responds more quickly and shows more pronounced hypertrophy and hyperplasia. These differences may possibly be due to either (i) the rapid inactivation of the ACTH administered in the rat but not in the guinea-pig, or (ii) to the fact that the rat adrenal normally functions under the influence of a higher level of endogenous ACTH than is the case in the guinea-pig so that the addition of more ACTH by an exogenous route has a relatively small effect in the rat but causes considerable stimulation

in the guinea-pig. The first possibility seems unlikely since the ACTH used was stabilised in the form of a gel to prevent its inactivation by rapid destruction, and it therefore appears more likely that there is a great difference between the amount of circulating ACTH in the rat and in the guinea-pig.

Differences observed in the effect of withdrawal of endogenous ACTH by corticoid administration or otherwise, point to the same conclusion. The suppression of ACTH secretion for as short a time as 3 days causes marked atrophy in the rat adrenal gland and by 10 days extensive degeneration has set in involving even the cell nuclei. In the guinea-pig the atrophy is only slight. The rat adrenal, being apparently maintained under the influence of a high internal secretion of ACTH, rapidly collapses when this is cut off, but the guinea-pig adrenal, since it is not normally so strongly stimulated by ACTH does not degenerate so markedly when this stimulation is withdrawn. It is possible that the guinea-pig may make greater use than the rat of pathways of corticoid biosynthesis which are not ACTH-dependent.

4.2. Post mortem changes in nucleic acids.

The administration of massive doses of ACTH to the rat which died after 5 days treatment simulates extreme

conditions of stress resulting ultimately in death. The gland in this animal, as is to be expected, is much bigger than in the rat which servived similar treatment with ACTH for 3 days (Tables 26 and 28) but the amounts of RNAP and of LP, and perhaps also of DNAP, are lower in the dead rat, indicating that within a few hours after death, autolytic changes bring about a considerable decline in the RNA and phospho lipids. This observation emphasizes the need to accept the post mortem data on human adrenal glands with caution.

4.3 Effect of ascorbic acid deficiency on the guinea-pig adrenal gland.

The adrenal glands undergo considerable enlargement showing a significant increase in weight during the development of acute scurvy (Table 40). This was first observed by Rodony (1919) and has since been confirmed by many other workers (Meyer and McCormic, 1928; Gergely, 1943; Stepto *et al.*, 1951; and Prunty *et al.*, 1955).

An analysis of the gland shows significant increases in the RNAP, LP, and PrN content of the gland and these changes are comparable with those produced by administration of 10 mg. ACTH for 3 days (Table 40; Fig. 16). Further, these changes can be prevented from occurring in the

gland by administration of cortisol which interferes with the pituitary secretion of ACTH (Table 44; Fig. 17). Hence it is concluded that the changes occurring in late scurvy represent true hypertrophy of the gland, brought about by increased pituitary secretion of ACTH due to the stress caused by the conditions of acute deficiency.

Guinea-pigs during the late stages of scurvy respond to administration of ACTH showing a very pronounced increase in gland weight. (Tables 36 and 40; Fig. 16). This observation is in agreement with that of Prunty et. al. (1955).

Our results indicate marked hyperplasia together with hypertrophy, since, in addition to an increase in the DNAP, the RNAP and the LP per cell also show increases. The increases in RNAP and LP per gland, though marked, are still not so great as to indicate a specific effect of the removal of ascorbic acid since administration of ACTH for 3 days to non-scorbutic guinea-pigs has shown similar increases (Tables 2 and 4). There is no evidence that the removal of ascorbic acid sensitizes the adrenal cortex to the action of ACTH and the effects of ascorbic acid deficiency without administration of exogenous ACTH, since they are

reversible by cortisol, are apparently due to an increased secretion of ACTH by the animals pituitary as a result of the stress arising from the scurvy. These results, therefore, do not support the idea of Hayano et al. (1956) and of Jones et al. (1958) that ascorbic acid acts as a restraining factor in the biosynthesis of adrenocorticoids.

4.4 Influence of dietary changes on the adrenal gland.

Our observations point to the fact that dietary changes have an important effect on the general metabolism of the adrenal gland. A supply of dietary protein and therefore an adequate amino acid level in the blood plays a prominent role in the biosynthesis of protein and LP in the gland. In the absence of an adequate supply of amino acids, a large energy intake brings about a significant fall in the PrN and LP content of the gland, while with a liberal supply of amino acids together with a high energy intake PrN and LP are increased (Fig. 18). These changes in diet do not bring about similar changes either in the RNAP or in the DNAP content of the gland. Hence protein synthesis takes place without increase in the amount of RNA.

The close association between RNA and protein

metabolism has already been discussed in the Introduction to this thesis, but it is perhaps worth recalling that recent evidence indicates that protein synthesis can take place without *de novo* formation of RNA. The inhibition of amino acid incorporation into proteins by ribonuclease observed by Gale and Folkes (1953) and others is not always observed in all systems. Rat skeletal muscle mitochondria, which contain RNA, can incorporate labelled leucine into their proteins, a process which is stimulated by ribonuclease according to Simpson and McLean (1957) and rapid synthesis of proteins by phosphate-starved yeast cells which do not synthesise new RNA has been reported by Liss (1958). Such formation of new protein without *de novo* synthesis of RNA is suggested by the results on the rat adrenal.

According to the scheme proposed by Munro (1956) the blood pool of amino acids enters the protein synthetic sequence in various organs, depending on the energy supply provided in the diet. The amino acid level can be increased either by catabolism of muscle protein under the influence of cortical hormones (Goodlad and Munro, 1959) or by direct provision through dietary intake of protein, as is done in the experiments described here. These

amino acids would supply the building units for protein synthesis in the adrenal gland.

Dietary changes appear to have an effect on the incorporation of ^{32}P into the acid-soluble fraction (A_1S) of adrenal tissue (Fig. 20) and also into the nucleic acids and other fractions (Fig. 21). The supply of extra energy in the diet reduces the incorporation into A_1S significantly, the effect being observed to a greater degree when the protein content of the diet, and therefore the supply of amino acids is cut off. This reduced incorporation of ^{32}P into the acid-soluble nucleotides and other compounds, is perhaps an indirect influence of the increased metabolic activity of the liver resulting from the provision of energy.

It is also noteworthy that the growth of a tumour similarly reduces the incorporation of ^{32}P into the acid-soluble fraction of the adrenal gland (Fig. 20) again indicating that increased metabolic activity in other parts of the body reduces the incorporation in the adrenal.

4.5. Metabolism of DNA.

A large number of studies involving the use of isotope incorporation into DNA (Furst and Brown, 1951; Smellie *et al.*, 1955) have shown that in normal animals

incorporation into DNA is low in tissues which have few cell divisions, but is higher in tissues like bone marrow and intestinal mucosa which have greater mitotic activity. The present position has been reviewed by Davidson (1957). Further it has also been shown that the synthesis of DNA is irreversible (Nygaard and Rusch, 1955). Since DNA constitutes the chromosomal material and the DNA content per set of chromosomes is fairly constant for any given species, it is suggested that DNA is not in dynamic equilibrium with other constituents of the cell, but that DNA is formed during mitosis and, once formed, remains intact throughout the life of the cell (Furst and Brown, 1951).

In our investigations, attempts have been made to obtain DNA from the adrenal gland in a fairly pure condition. In all cases the relative specific activity of the DNA is very small in the normal guinea-pig or rat. When the amount of DNA available permits a further purification by ionophoresis on paper no observable incorporation of ^{32}P is demonstrable (Tables 41 and 45). In the case of RNA it has been emphasized (Barnum et al., 1950; Davidson et al., 1951) that nucleotides obtained by simple hydrolytic methods are likely to be heavily contaminated with non-nucleotide compounds and therefore Davidson and Smellie (1952) devised the ionophoretic technique which gives a

fairly satisfactory purification of these nucleotides. It is likely that the methods of DNA purification by the usual precipitation procedure do not remove the last traces of protein which may include some radioactive phospho-protein. Further, the precipitate of DNA may have adsorbed on its surface other radioactive compounds which cannot readily be washed out with acids even though Daoust and Stevens Hooper (1957) have claimed that this is possible. Such radioactive contamination may raise considerably the specific activity of DNA. These impurities can, however, be easily separated by subjecting the DNA to ionophoresis. When this is done the radioactivity decreases markedly and the amount of phosphorus corresponding to DNAP is also considerably reduced.

When due precautions are taken to obtain pure DNA, ^{32}P is an extremely sensitive indicator for detecting new DNA synthesis in the adrenal gland. In the case of the guinea-pig treated with lyophilised ACTH there is no increased uptake of ^{32}P up to 16 hours (Table 10). With Acthar-gel there is no increased uptake even at 1 day, so that These stimulations do not cause any increase in mitotic rate, but a rapid and continual rise as apparent when treatment with Acthar-gel is continued beyond 1 day.

As has already been discussed, the use of lyophilised ACTH plus Acthar-gel acts as a great stimulant inducing very rapid mitotic activity which remains constant after reaching the maximum value at about 24 hours, at which time there is an increase by about 400% in ^{32}P incorporation into DNA.

In the case of rats also the increased incorporation of ^{32}P into DNA under the action of Acthar-gel occurs only at 2 days and thereafter is constant (Table 12; Fig. 9).

Increased uptake of ^{32}P takes place into the DNA of the adrenal glands of scorbutic guinea-pigs (Table 41) but this observation is not always reproducible (Table 45). Hence it is doubtful whether scurvy always induces synthesis of new DNA. In this experiment on scorbutic guinea-pigs (Tables 40 and 41) it was further noted that the two ACTH-treated groups with and without a supplement of ascorbic acid show the same extent of incorporation although the DNAP content of the scorbutic group receiving ACTH is significantly greater than that of the other group. The reason for this anomaly is, however, not clear.

Hypophysectomised rats show very little incorporation of ^{32}P into DNA, and stimulation with ACTH for 3 days still does not result in any pronounced increase in ^{32}P uptake (Table 16; Fig. 11). It is therefore reasonable to suppose

that the dose of ACTH given does not cause any significant increase in mitotic activity in the hypophysectomised rat, but causes only hypertrophy. Similarly, administration of cortisol to normal rats reduces the incorporation into DNA (Table 20) and simultaneous treatment with 10 mg. Acthar-gel daily, although inducing hypertrophy of the gland, does not increase the incorporation of ^{32}P into DNA above the normal value. It seems, therefore, that when the internal secretion of ACTH is suppressed an exogenous supply of 10 mg. daily to adult rats does not cause hyperplasia.

On the other hand, significant increases of ^{32}P uptake into the adrenal DNA of cortisol-treated immature rats are observed when these are treated with 15 mg. Acthar-gel daily (Table 25) and also when much larger doses of ACTH are given to immature rats which had not received cortisol (Table 26). In the latter case there is, in addition, a measurable increase in the amount of DNAP. It would seem that hyperplasia can be produced in the rat adrenal by very large doses of ACTH, though, of course the response in the young rat may be different from that which occurs in the mature animal.

The role of DNA in adrenocortical function is not fully understood. DNA has sometimes been implicated

in protein metabolism and the position has been reviewed by Wilkins (1957), but whether it does play any important role in similar processes in the adrenal is not clear from our experiments. It is possible that an increased demand for corticosteroids such as would occur under mild stress is met by hypertrophy of the gland, but when the demand is greater than can be accommodated by simple hypertrophy, an increase in the cell number becomes necessary, resulting in further synthesis of RNA and perhaps also of protein. It is also to be remembered that in conditions of overactivity of the gland, such as Cushing's syndrome, marked hyperplasia of the gland is found. Very great stimulation of the gland with ACTH or the application of a severe stress perhaps simulate such conditions of overactivity in causing hyperplasia.

4.6. Metabolism of RNA.

Administration of ACTH causes hypertrophy of the adrenal gland resulting in an increase in the RNAP content not only of the gland as a whole but also of each cell. In the rat this increase in amount is, however, accompanied by a net lowering in the relative specific activity. This finding which is consistently reproducible under different conditions of stimulation in the experiments with rats reported here is apparently

in contrast with that of Nicholls *et al.* (1956 b), who find that exposure of rats to cold for 16 hours brings about an increase in the incorporation of ^{32}P into RNA nucleotides. Since exposure to cold is presumed to act as a stress on the pituitary releasing more endogenous ACTH, the reason for this difference in the incorporation into RNA is not clear. The experiments described in this thesis mostly involve 3 days of ACTH administration with a 2-hour ^{32}P incorporation at the end of this period whereas Nicholls *et al.* subjected the rats to a stress lasting for 16 hours, ^{32}P incorporation occurring during this entire period. Further they used Sprague-Dawley rats a different strain from those used in Glasgow. It is possible that these differences in experimental conditions may in part be responsible for different incorporation patterns observed.

The net lowering in the relative specific activity of RNA indicates that the pool size of RNA is increasing while the incorporation is not keeping pace with it. Further the total radioactivity of RNA, the product of relative specific activity and RNAP content per pair of glands (Tables 12 and 25), remains fairly steady before and after ACTH treatment. It appears, then, that the rate of synthesis of RNA, as measured by the

uptake of ^{32}P, is not affected by stimulations with ACTH but that the entire increase is due to an enlargement of the pool size of RNA caused by restraint on the breakdown of RNA. Similar findings have been reported by Munro _et al._ (1953) in their studies on the effect of dietary changes on the RNA content of rat liver and the renewal of RNAP. They have found that by providing an energy rich diet having a high protein content, the amount of liver RNA is increased without any significant change in the proportion of phosphorus atoms replaced in a given time. Hence it is concluded that administration of ACTH interferes with the normal mechanism of breakdown of RNA in the adrenal of the rat and the increase in RNA content is mainly due to this.

Withdrawal of endogenous ACTH from rats through the administration of cortisol has been found to bring about a rapid decline in RNA content of the adrenal gland. Since the administration of exogenous ACTH restrains the breakdown of RNA it is possible to envisage the situation where complete lack of ACTH causes a rapid and unhindered breakdown. Administration of ^{32}P to the cortisol-treated rat reveals a decreased incorporation into RNA (Table 20). This may be due to decreased synthesis of RNA or it may be a secondary effect due to reduced incorporation into RNA precursors

in, for example, the acid-soluble fraction, as a result of the general disturbance of metabolism by cortisol.

The metabolism of RNA in the guinea-pig is different from that seen in the rat. Administration of ACTH brings about a more rapid and more pronounced increase in the RNA content of the gland and the uptake of ^{32}P shows an increase with stimulation in contrast to the rat. This fact indicates that the rate of synthesis of RNA is increased in the ACTH-treated guinea-pig. Also, unlike in the rat, the withdrawal of endogenous ACTH does not cause breakdown of RNA to any significant extent in the guinea-pig, so that the effect of ACTH on the metabolism of RNA in the guinea-pig appears to be somewhat different from that in the rat.

It has been shown that protein synthesis in the adrenal gland can be induced to take place without increase in the amount of RNA by providing an energy rich diet containing adequate protein. It must, however, be pointed out this sequence of biosynthesis of protein in the adrenal gland is not necessarily the same as that which takes place under ACTH stimulation. A close parallelism between the amounts of RNA and protein is observed when increased synthesis takes place due to stimulation by ACTH.

Symington, Duguid and Davidson (1956) point out that under stimulation with ACTH, the content of enzyme phosphatase increases in the adrenal gland, indicating increased protein synthesis at the same time as the RNA content is rising. In the experiments reported in this thesis similar close association has been observed. Administration of lyophilised ACTH to guinea-pigs which does not induce any increase in the RNA content, up to 16 hours does not produce any increase in the protein content (Table 9). When a rapid stimulation is caused by administering lyophilised ACTH and Acthar-gel, parallel increases are observed in protein and RNA (Table 7). In the scorbutic guinea-pigs in which the adrenals undergo considerable hypertrophy both RNA and protein undergo comparable increases.

There is no doubt that RNA plays a very prominent part in the adrenal cortical function, both under normal conditions and under conditions of stress even though its role is still not clearly understood. It is possible that it is intimately connected with the synthesis of proteins, especially the enzyme proteins whose activity increases with stimulation of the gland. It is noteworthy that the withdrawal of endogenous ACTH in rats, which rapidly decreases cortical activity,

probably by the loss of enzymes, also brings about a decrease in the RNA. Stimulation, which increases corticoid output, likewise increases RNA content. Such a role of RNA in steroid biosynthesis may not be direct, but only secondary, as is indicated by the fact that ACTH stimulation brings about an increase in steroid output within a few minutes while the increase in the RNA does not take place for several hours.

4.7. Role of LP in adrenocortical activity.

It is also worth noting at this stage that the LP content of the adrenal gland closely follows the RNAP and the PrN content in its changes under stimulation by ACTH and is related to the protein pattern in its changes following dietary variations. It appears therefore that LP is more directly related to the protein than to the RNA. The recent report of Hendler (1958) that lecithinase A and lysolecithin inhibit the incorporation of isotopically labelled phenylalanine into proteins of hen oviduct minces, gives support to the possibility of phospholipids taking part in the biogenesis of proteins.

SUMMARY.

1. The effect of administration of ACTH on the metabolism of nucleic acid, phospholipid and protein in the adrenal gland has been studied in the guinea-pig and in the rat.

2. It has been shown that ACTH does not cause changes in nucleic acid and protein content of the gland until several hours after administration.

3. ACTH causes hypertrophy as evidenced by an increase in RNAP content per cell, and hyperplasia as shown by an increase in DNAP content of the gland. The response in the guinea-pig is more rapid and more pronounced than in the rat. In the guinea-pig increases ranging from 85-110% over the control values are obtainable for RNAP by stimulation with Acthar-gel for 3-5 days, or by stimulation with a combination of Acthar-gel and lyophilised ACTH for 24 hours. The increase in DNAP amounts to about 30% over the control value. In the rat the maximal increase obtained in RNAP is about 30% while no measurable increase occurs in DNAP.

4. The suppression of endogenous ACTH by administration of cortisol to the rat is shown to be as effective as hypophysectomy. Such treatment results in rapid and extensive damage to the cortex, as

shown by the decrease in nucleic acid content. A similar suppression of endogenous ACTH in the guinea-pig by cortisol administration does not cause a severe decline in the nucleic acid content of the gland. The suggestion is made, in order to explain these differences in the two species, that the adrenal in the rat is functioning under greater stimulation of endogenous ACTH, the lack of which causes rapid degeneration, while in the guinea-pig the stimulation by circulating ACTH is comparatively small.

5. Studies on ^{32}P incorporation into RNA indicate that in the rat the increase in RNA content of the adrenal gland under ACTH stimulation is caused by the cessation of RNA breakdown, while in the guinea-pig the stimulation causes increased rate of synthesis of RNA, so that ACTH appears to control RNA metabolism in different ways in the two species.

6. Incorporation of ^{32}P into DNA has shown that synthesis of DNA in the adrenal gland of the normal animal is negligible, while ACTH stimulation causes a large increase in incorporation in the guinea-pig and a smaller increase in the rat.

7. The effect of severe ascorbic acid deficiency on adrenal nucleic acid metabolism has been studied

in the guinea-pig. It has been shown that the hypertrophy of the gland which occurs in acute scurvy can be prevented by simultaneous administration of cortisol, so that the stress caused by the disease presumably results in enhanced pituitary secretion of ACTH.

8. Dietary changes have been shown to cause significant changes in the LP and the PrN content of the rat adrenal, without change in nucleic acid content and to alter the pattern of ^{32}P incorporation into various fractions of adrenal tissue. A protein-free diet with a high energy intake causes a decrease in LP and PrN, while an adequate protein diet also providing a high energy intake, causes an increase in LP and PrN. An energy-rich diet decreases the incorporation of ^{32}P into the acid soluble fraction, this decrease being more pronounced when the diet is protein-free.

9. The presence of a tumour in the body produces pronounced changes in the adrenal of the rat, tending to increase the LP and PrN content of the gland without, however, any significant change in the nucleic acid content. There is a marked suppression of incorporation of ^{32}P into the acid soluble

fraction of the adrenal. Increased synthesis of DNA is indicated by an increase in the specific activity of the adrenal DNA of rats bearing an infected tumour.

10. The significance of these changes as well as the possible role RNA, DNA and LP in adreno-cortical function is discussed.

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