CIBA FOUNDATION STUDY GROUP No. 6

Metabolic Effects of Adrenal Hormones

CHURCHILL

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Metabolic Effects of Adrenal Hormones

in honour of
Prof. G. W. THORN

Editors for the Ciba Foundation
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The purpose of the Ciba Foundation, which is to promote international co-operation in medical and chemical research, is symbolized in the armorial bearings by five interlaced rings representing the continents, a sacrificial cock (emblem of Aesculapius) holding a medical caduceus, and three regular hexagons for chemistry. Its domicile in London is indicated by the sword of St. Paul and the British lion; the wyvern and the crozier, symbols associated with Basle, refer to the sponsoring firm located in this ancient Swiss town.

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METABOLIC EFFECTS OF ADRENAL HORMONES

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PREFACE

THE CIBA FOUNDATION, a unique international institution, owes its inception to the generosity of CIBA Limited, Basle. However, being established under British trust law, it enjoys complete independence in practice and policy.

Under the guidance of its distinguished Trustees, the Foundation offers accommodation to scientists from all over the world at its home in Portland Place. Foremost in its activities is the organization of small conferences, some lasting 3–4 days, others for one day only. The proceedings of the former are published in book form, and the latter in the manner of the present booklet. Each of these one-day study groups is being organized in honour and around the work of an eminent member of the Foundation's scientific advisory panel, on which there are 1–3 representatives for most of the countries of the world.

The Foundation convenes many other informal discussions between research workers of different disciplines and different nationalities and each year invites an outstanding authority to deliver a special lecture. An exchange programme between French and British postgraduates is conducted and a library service is available. Furthermore, the Ciba Foundation attempts in every other way possible to aid scientists, whether they be Nobel Laureates or young graduates making their first original contribution to research.

The publication of the first six study groups is regarded as experimental. Nevertheless with rapid production of the proceedings of both conferences and study groups, it is hoped that readers in more distant centres, away from easy reference to the latest literature, can feel a little more closely in touch with recent developments and may be stimulated to make their own topical contributions to international research.

List of those participating in or attending the Study Group on "Metabolic Effects of Adrenal Hormones"

15th July, 1960

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CHAIRMAN'S OPENING REMARKS

F. G. YOUNG

I CANNOT claim to be an expert on adrenal hormones, but I am very glad to have the opportunity to be here today to hear the discussion and to meet many old friends. It seems most appropriate to have a discussion on metabolic effects of adrenal hormones in 1960, since it marks the centenary of the death of Thomas Addison, the discoverer of the importance of the adrenal glands, and of course the discoverer of the disease which bears his name.

For this reason alone it would be suitable to hold this conference during the present year, but there is a much more compelling reason in the presence in Great Britain of Professor George Thorn, the guest of honour on this occasion. Professor Thorn and his colleagues have done so much in this field of investigation that there can be no doubt about the appropriateness of the subject we discuss today in his honour.

In considering the title of this conference I was struck by the need to use two plural nouns: "effects" and "hormones". Addison never really committed himself to any conclusion as to why the suprarenal capsules are so essential to health and life. There is some evidence that later in his life he tended to swim with the current of scientific and medical thought of the time and to lean to the view that the fatal effect of disease of the suprarenal capsules was not directly due to the disease of the glands themselves, but to the damage and disturbance that were thus induced in the neighbouring nerve trunks. At that time the nervous system was thought to be dominant and in fact almost alone in the co-ordination of functions in the body, and the idea that humoral factors might play a part was accepted with difficulty and only very slowly.

Although the modern idea of hormones starts with Addison's publications it can be regarded as having been formalized by the work of Bayliss and Starling early in this century, as the result of which the word "hormone" came to be coined. Derived from

the work of Bayliss and Starling and of others in the early years of this century, the idea seemed to be one hormone—one gland—one function. But the many different actions of adrenaline quickly disturbed the simplicity of the idea of a single function. Since, however, the presence of noradrenaline in the adrenal medulla was missed for many years, the idea that there might be one hormone—one gland seemed to be reasonably established until investigations on the anterior pituitary gland and, later, on the adrenal cortex, revealed a multiplicity of active substances in both glands. The isolation of many active adrenal steroids gave rise to the belief that no one of them could be the hormone of the adrenal cortex, and in the examination of the multiple effects of adrenal steroids, with respect to physiological action and clinical effect, Professor Thorn and his colleagues have been most effectively industrious. We owe it to them in particular that there is less confusion in this field than there might otherwise have been.

The fact that species differences are now known to exist in the proportions of the different adrenal steroids which are liberated into the blood is a matter that should not be neglected in the assessment of the function of the adrenal cortex in terms of experiments involving the effect of a single administered adrenal steroid. The permissive action of the adrenal steroids which was so elegantly described by Dr. Dwight Ingle is another complicating factor in the interpretation of simple experiments, and again this obviously will not be neglected by the members of this conference in the discussion today.

The striking effect of adrenal steroids in relieving the symptoms of rheumatoid diseases, described by Hench and his colleagues in 1949, naturally led to a boom in adrenal steroid research, but it is both chastening and disappointing that after eleven years of intensive research we are still not in a position to define the mechanism of action of steroids in this dramatic effect. The multiplicity of effects of adrenal steroids which we now recognize poses a challenge which the members of today's conference I am sure are ready to take up, and although progress may be slow advances are being made. Ten years ago there was a good deal of optimism about the significance of experiments in which endocrine glands were removed, or hormones administered to normal animals or to deficient animals, and the changes in the

measured enzyme activity in particular tissues was determined at intervals and under various conditions. It seemed very reasonable to suppose that this ultimately could lead to the pinpointing of the effect of a hormone on particular enzyme systems. This optimism has not been entirely justified, as things have turned out, although as we shall hear from Dr. Ashmore today there is more hope with respect to certain liver enzymes than there is elsewhere. On the whole the conference will probably be more concerned with the effects of steroids upon intact animals than might have been expected on the basis of our views ten years ago. Emphasis has perhaps shifted from enzymes themselves, and the effects of hormones thereon, to the availability of co-enzymes. We now realize full well that we have chains of enzymes, and cyclic processes, all parts of which may be affected, directly or indirectly, by hormones. The unravelling of the multi-enzyme systems which are involved has not yet led to an ability to pinpoint the initial effect in the process for which the hormone may be responsible. Whether adrenal steroids have a single point of action on the basis of which we may explain the multiple effects is a subject we shall have to consider, although again, the optimism that once reigned in this respect is not so evident now as might have been expected at one time.

It is unlikely that at the end of today's conference we shall be able to pinpoint any metabolic point as of focal interest in the action of adrenal steroids, but I hope we shall have made a few stabs at it.

I trust, Professor Thorn, that at the end of the day you will feel that we have, by our enthusiasm for research on adrenal hormones, honoured you in the way I am sure you would prefer to be honoured on an occasion such as this.

ACTIONS OF CORTISOL AND RELATED COMPOUNDS ON CARBOHYDRATE AND PROTEIN METABOLISM

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It is now well established that the adrenocortical hormones, of which cortisol may be considered a prototype, exert a considerable effect on the metabolism of carbohydrate and protein. The hypothesis that the effects on the metabolism of these substances are interrelated and are a consequence of an accelerated rate of protein catabolism is based on the following evidence. (a) Adrenalectomy reduces the glycosuria and increased nitrogen excretion of fasting totally depancreatized animals, while the administration of adrenocortical extract, cortisone or cortisol returns the glucose and nitrogen excretion to diabetic levels. (b) Adrenalectomy also reduces the nitrogen excretion and extra glucose formation associated with such conditions as phlorrhizinization or exposure of animals to reduced tensions of oxygen. (c) The administration of an excess of adrenal steroids, such as corticosterone or cortisone, to fasting normal, adrenalectomized or hypophysectomized animals increases, to a marked degree, the carbohydrate stores of the animals. At the same time there is an increased urinary nitrogen excretion sufficient to account for the newly formed carbohydrate in terms of the extra protein catabolism (Long and Lukens, 1936; Long, Katzin and Fry, 1940; Evans, 1936). Taken as a group these experiments appeared to furnish adequate support for the view that a primary function of the adrenal cortex is to accelerate tissue protein catabolism, thus rendering available to the organism a large and new source of carbohydrate to meet circumstances in which an adequate supply of this foodstuff is essential for existence.

However, this view of the nature of the essential effect of the adrenocortical hormones on carbohydrate and protein metabolism

has required modification since it was observed by Russell (1939), Long, Katzin and Fry (1940) and Ingle (1941) that adrenocortical extracts or cortisone, administered to rats fed with glucose or a high carbohydrate diet by stomach tube, altered the pattern of glucose utilization itself. This conclusion was necessary since in these experiments the changes in glucose utilization were not associated with corresponding or equivalent changes in protein metabolism. The experiments by Ingle (1941) were carried on over several days of cortisone injection. By this time the animals had developed marked hyperglycaemia and glycosuria, a condition now generally referred to as "steroid diabetes". At this time the glycosuria was greatly in excess of the amount that could be attributed to the increased protein catabolism.

However, long-continued injection of cortical hormones is not necessary to demonstrate their capacity to alter the pattern of glucose utilization itself. Long, Katzin and Fry (1940) showed that animals treated with cortical extract over a four-hour period have a smaller utilization and a greater retention of glucose, as liver and muscle glycogen, than do untreated animals. A similar depression of glucose utilization, at least as judged from the respiratory quotient, was observed in man by Thorn and coworkers (1940).

It would appear then that, depending on the experimental conditions used, it can be concluded that the primary effect of cortisol and allied substances is either to accelerate tissue protein catabolism or to decrease the utilization of carbohydrate. Since it is well known that decreased carbohydrate utilization, such as follows insulin deficiency, is also associated with a loss of tissue protein, it becomes of some importance to decide which of these two possible modes of action is responsible for the effects of excess or deficiency of adrenocortical hormones on the metabolism of carbohydrate and protein.

This paper is to report some further experiments designed in an attempt to distinguish between these two possible modes of action.

Carbohydrate metabolism of fasting rats given an excess of cortisol

When adrenalectomized rats, previously fasted for 18 to 24 hours, are injected subcutaneously with 10 mg. of cortisol there

occurs over the next 48 hours a continuous and remarkable accumulation of carbohydrate in the bodies of the animals (Table I). The features of this effect are (a) a rise in blood glucose of about 40–50 mg. per 100 ml. which is evident from one to two hours after the injection, and which is sustained at this level for 48 hours; (b) an elevation of the liver glycogen, which begins somewhat later than the rise in blood glucose, but is continued for at least 24 hours and is maintained at a very high level (150–200 mg. per 100 g. body weight) for another 24 hours; (c) a much slower rise in muscle glycogen which is not significant until 8 to 12 hours after the injection of cortisol; (d) a total accumulation of carbohydrate in the body that is equal to the amount present in the animal at the time of injection, and which increases the total carbohydrate content of the body to levels usually found in glucose-fed normal animals.

Table I

FASTED ADRENALECTOMIZED RATS, SUBCUTANEOUSLY INJECTED WITH 10 MG. CORTISOL

All values mg. per 100 g. body weight

	Glucose†	*Liver glycogen*	*Muscle glycogen*‡	*Total carbohydrate*	*Increase*
Controls	14	2	196	212	—
Cortisol 1 hour	16*	1*	210*	227	+ 15
,, 2 hours	18	3*	205*	236	+ 24
,, 3 ,,	24	14	234*	272	+ 60
,, 4 ,,	23	18	198*	239	+ 27
,, 6 ,,	22	32	215*	269	+ 57
,, 12 ,,	24	82	255	361	+ 159
,, 24 ,,	25	179	284	488	+ 276
,, 48 ,,	25	200	286	511	+ 299
Fed normal rats	21	238	368	627	—

* Not significant.
† Calculated on glucose space of 25 per cent of body weight.
‡ Calculated on muscle mass of 50 per cent of body weight.

These striking increases in the carbohydrate content are accompanied by equally remarkable increases in urinary urea excretion (Fig. 1), the excretion being doubled over the 24-hour period. When the extra urea excretion is converted into the

amounts of extra protein catabolized it will be seen that about 60 to 70 per cent of the amino acids released have been retained as carbohydrate in the liver, muscles and blood, and that only a small portion of the large amounts made available by the action of this excess of cortisol has been utilized. When it is considered that these animals have received no food for 48 or 72 hours the paradox between the abundance of their carbohydrate stores and its non-utilization for their energy needs is apparent. In other

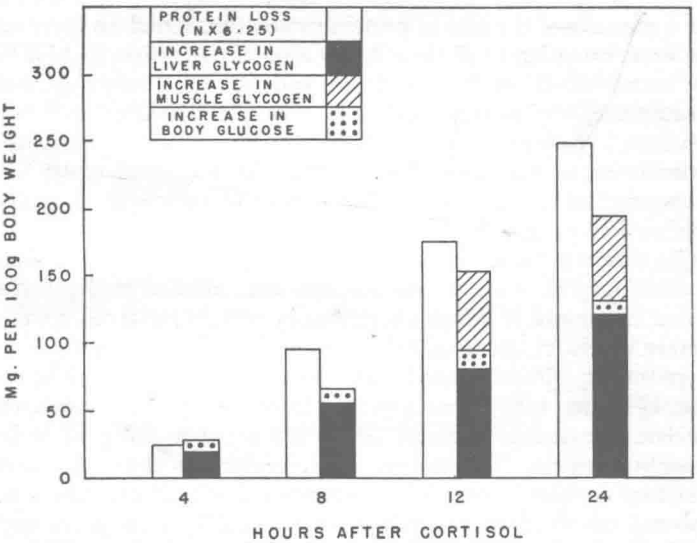

FIG. 1. The effect of cortisol (10 mg. subcutaneously) on the carbohydrate and protein metabolism of fasting adrenalectomized rats.

words, in spite of the provision of an excess of carbohydrate from the protein stores there does not appear to be a corresponding increase in carbohydrate utilization. The absence of any increase in utilization is also borne out by data derived from the respiratory exchange.

Consequently, it would appear that regardless of the source of the carbohydrate, that is whether it is derived from exogenous or endogenous sources, the administration of cortisol depresses glucose utilization and at the same time increases the levels of carbohydrate in the liver, muscles and body fluids.

Experiments that indicate an effect of cortisol on carbohydrate metabolism independent of changes in protein metabolism

It was pointed out above that Long, Katzin and Fry (1940) found an effect of cortical extracts, without any change in the nitrogen excretion, on the disposition of fed glucose in normal rats. In a converse type of experiment Engel (1950), using the rate of accumulation of urea in the blood of nephrectomized rats as a measure of the rate of protein catabolism, found no increase in urea formation until three hours after the injection of ACTH or cortisone. Since Boucat, Guild and Merrill (1958) observed that acute nephrectomy did not alter the disposition of fed glucose, it may be concluded from Engel's experiments that the stimulation of protein catabolism was either delayed under his experimental conditions or that a change in carbohydrate metabolism had preceded it.

In recent experiments in this laboratory the effects of cortical extract on carbohydrate metabolism were studied under three other conditions in which alterations in protein metabolism were either absent or occurred only to a minimal degree. In the first type of experiment, fasted adrenalectomized rats were infused for 1½ hours with either glucose, fructose, glycerol, lactate or malate in amounts of about 120 to 150 mg. per 100 g. of body weight an hour. The amount of liver glycogen deposition and the rise in blood glucose were compared with those found in normal rats or adrenalectomized rats injected 3½ hours previously with 5 mg. of cortisol (Winternitz, Dintzis and Long, 1957).

These results showed that in all the glycogen precursors studied, adrenalectomy reduced, while prior cortisol injection restored, the proportion of the precursor deposited as liver glycogen. The same investigators also observed that the subcutaneous injection of adrenaline, which in normal fasted rats is followed by a rapid deposition of liver glycogen as a consequence of the accelerated release of lactate from muscle, failed to cause significant liver glycogen deposition in fasted adrenalectomized rats even though comparable increases in blood glucose and lactate occurred. However, normal liver glycogen deposition occurred when the adrenalectomized animals were treated with adrenocortical extract or cortisone. Evidently a normal Cori