Some Further Studies On the Relationship Of Adrenal Cortical Hormones To Experimental Diabetes

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Many phases of organic metabolism are affected by either adrenal cortical insufficiency or excess. The diabetic animal or patient is especially sensitive to abnormal changes in the function of the adrenal cortex. Diabetes is ameliorated by adrenal cortical insufficiency and is exacerbated by the administration of an excess of cortisone, hydrocortisone or corticosterone. Diabetes is also exacerbated when hypercorticalism is produced by the administration of an excess of corticotropin. It is possible to administer these hormones in dosage sufficient to cause a temporary glycosuria in either normal laboratory animals or human subjects.

It has been imagined that these effects of adrenal cortical insufficiency and excess have made clear the normal role of the adrenal cortex in regulating carbohydrate metabolism. This is not true. Conclusions about the normal role of this quietly functioning organ in body economy represent inferences from observations made under the unusual conditions of experiment or disease. Our inability to make direct observations upon the basic mechanisms of hormone action should make us cautious and humble.

There is no doubt that carbohydrate metabolism is affected by adrenal cortical insufficiency and excess and there is no doubt that the normality of carbohydrate utilization is dependent upon the normality of adrenal cortical function. It is less certain that the adrenal cortex is a prepotent regulator of carbohydrate metabolism.

During the past twelve years I have been concerned with the following question: "What are the metabolic consequences of adrenal cortical activation during various forms of stress?" When the secretory activity of the adrenal cortices is stimulated by large doses of exogenous corticotropin signs of hypercorticalism ensue. During any type of stress the increased release of hypophyseal corticotropin stimulates the adrenal cortex to secrete more of its hormones. Does stress cause a state of hypercorticalism? If so, a stress should cause exacerbation of experimental diabetes due to activation of the adrenal cortices. This paper reviews the results of four experi-

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ments which have a bearing upon this question but which do not yet permit a satisfactory answer.

METHODS

Infection-free male rats of the Sprague-Dawley strain were partially depancreatized at a weight of approximately 275 gm. by the procedure described by Ingle and Griffith.[1] The experiments were conducted in an air-conditioned laboratory with the temperature maintained at 74° to 78° F. and the humidity at approximately 50 per cent of saturation. After the animals had recovered from the operation and had reached a weight of approximately 300 gm. they were placed in metabolism cages and were adapted to the forced feeding of a medium carbohydrate diet (Table I) by stomach tube each morning and late afternoon. The technique and diet were modified from those described by Reinecke, Ball and Samuels.[2] During the period of adaptation to forced feeding the amount of diet was increased gradually to prevent the development of food shock. The animals were brought to a full feeding of 26 cc. of diet per day on the 6th day. Twenty-four hour samples of urine were collected at the same hour each day (8:00 to 8:30 a.m.) and were preserved with toluene. Urine glucose was determined by the method of Shaffer and Williams.[3]

TABLE I—MEDIUM CARBOHYDRATE DIET

	grams
Cellu flour (Chicago Dietetic Supply House)	60
Osborne-Mendel salt mixture	40
Dried yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Mazola oil + 100 mg. vit. K	10
Mazola oil	190
Casein (Labco)	140
Starch	200
Dextrin	190
Sucrose	200
Water to total of	2000

EXPERIMENTS AND RESULTS

Experiment 1 was a repetition of an earlier study[4] on the relationship of the diabetogenic effect of diethylstilbestrol to the adrenal cortex in the rat. This compound and other synthetic and natural estrogens are diabetogenic in the forced-fed rat. Since certain of the adrenal cortical hormones are diabetogenic and since large doses of estrogen are among the noxious stimuli which cause the adrenal cortices to enlarge, it seemed reasonable to postulate that the diabetogenic effect of this and other estrogens is due to its stimulation of the anterior pituitary-adrenal cortex axis to produce a state of hypercorticalism. However, the following experiment shows that diethylstilbestrol exerts a diabetogenic effect which is not mediated by the adrenal cortex.

Six partially depancreatized rats which were free from spontaneous glycosuria were used in these experiments. Each rat was given 0.1 mg. of diethylstilbestrol by subcutaneous injection once daily for 7 days. All of the rats developed glycosuria during the administration of the estrogen but became free from glycosuria when the injection of estrogen was stopped. At this time all of the rats were adrenalectomized and were treated with 3 cc. daily of beef adrenal extract (Upjohn) for 21 days. The animals remained free from glycosuria during the control period but developed marked glycosuria during the 7 day period that the estrogen was injected. When the administration of diethylstilbestrol was stopped the glycosuria disappeared.

The data of this experiment are shown in Figure 1. It is clear that diethylstilbestrol exerts an effect upon carbohydrate metabolism in the rat which is not mediated by the adrenal cortex, although, as shown in the earlier study[4] the presence of the adrenal cortical hormones is necessary for the full manifestation of the diabetogenic activity of the estrogen.

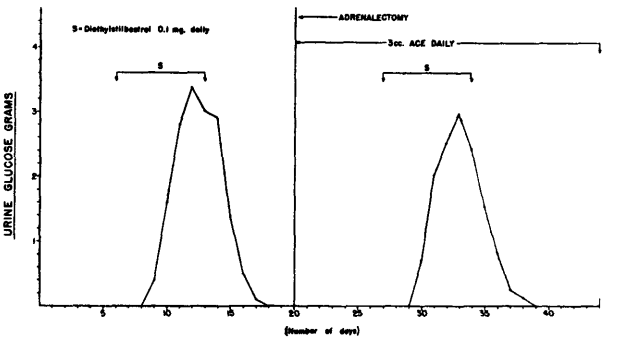

FIGURE 1 The effect of diethylstilbestrol upon the urinary glucose of 6 partially depancreatized rats before and after removal of the adrenal glands. Averages

Experiment 2 was a partial test of the hypothesis that the pharmacologic effects of aspirin are mediated by the release of increased amounts of corticotropin and hence of the adrenal cortical hormones. Hailman[5] has summarized the evidence that aspirin can cause a state of hypercorticalism via stimulation of the anterior pituitary-adrenal cortex axis. If aspirin does cause hypercorticalism it should exacerbate the diabetes of the partially depancreatized rat. The converse happens;[6] the diabetic state is ameliorated. We have compared partially depancreatized rats with adrenalectomized-partially

depancreatized rats in respect to the response to large doses of aspirin. The amount of adrenal cortical extract required to restore the glycosuria to its preadrenalectomy level (3 to 5 cc. per day) was established for each adrenalectomized rat and was then kept uniform throughout the remainder of the experiment. All of the rats showed amelioration of the diabetes during the administration of aspirin with marked exacerbation of the diabetes when aspirin was withdrawn. It was clear that this effect of aspirin can occur independently of any change in the secretory activity of the adrenal cortices. The data are shown in Figure 2.

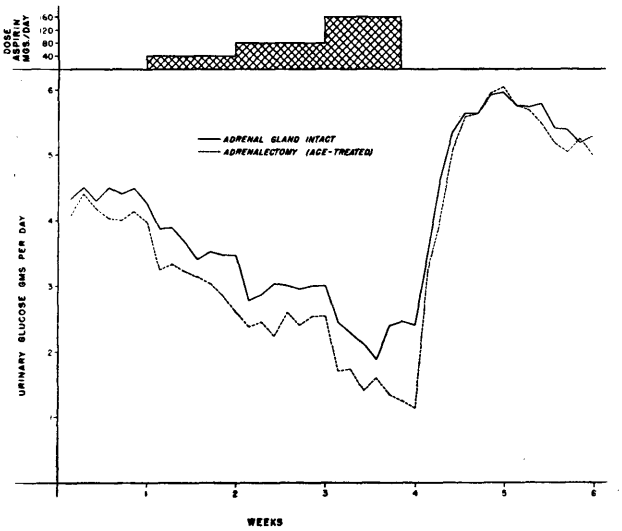

FIGURE 2 The effect of aspirin upon the glycosuria of 6 pairs of partially depancreatized and partially depancreatized-adrenalectomized rats. Averages

Experiment 3 was intended to test the hypothesis that any nonspecific stress should cause exacerbation of the diabetic state in the partially depancreatized rat via the activation of the adrenal cortices by endogenous corticotropin. Adrenalectomized and nonadrenalectomized diabetic rats were compared in respect to the effect of a stress (injections of dilute solutions of formaldehyde) upon the level of glycosuria. These data have been published.[7] Fifteen mildly diabetic rats were adrenalectomized and treated with adrenal cortical extract in amounts which sustained the preadrenalectomy level of glycosuria. An equal number of nonadrenalectomized rats were studied in parallel. The subcutaneous injection of 1.5 per cent formaldehyde, in doses of 0.25, 0.5, and 1.0 cc. twice daily for 7 days, 5 rats per dose level, caused some decrease in the glycosuria of the nonadrenalectomized rats and a much more striking decrease in the glycosuria of the adrenalectomized rats. When the injections were stopped, the glycosuria was reestablished at its preinjection level. The data are shown in Figure 3.

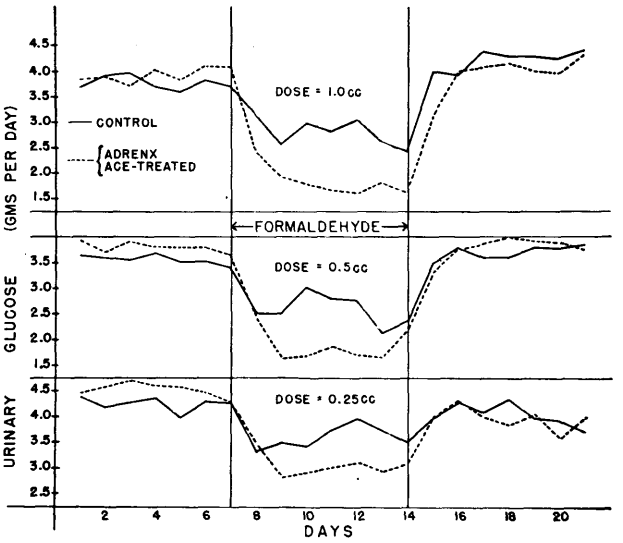

FIGURE 3 Effects of injections of 1.5 per cent formaldehyde on the glycosuria of partially depancreatized and partially depancreatized-adrenalectomized rats. Averages for 5 pairs of rats in each of 3 groups

It has been shown by unpublished experiments in this laboratory that the administration of ethylenediamine causes an increase in the excretion of glucose by the partially depancreatized rat. The adrenal cortices enlarge during the injection of ethylenediamine and it is assumed that there is an accompanying increase in the secretion of adrenal cortical hormones.

Experiment 4 demonstrated that the presence of the adrenal glands is required for the exacerbation of the diabetic state by ethylenediamine. Twelve mildly diabetic rats were used in the study. Six of the rats were adrenalectomized and treated with adrenal cortical extract in amounts which sustained the preadrenalectomy level of glycosuria. An equal number of nonadrenalectomized rats with a similar level of glycosuria were studied in parallel. Ethylenediamine was administered by subcutaneous injection in amounts of 0.05 cc. daily for 5 days and 0.1 cc. daily for 5 days. There was a striking increase in the glycosuria of the nonadrenalectomized rats but there was no increase in the glycosuria of the adrenalectomized animals. When the administration of ethylenediamine was stopped, the glycosuria of the nonadrenalectomized rats fell to the preinjection level whereas the glycosuria of the adrenalectomized rats fell below the preinjection level. The average values for urinary glucose are shown in Figure 4.

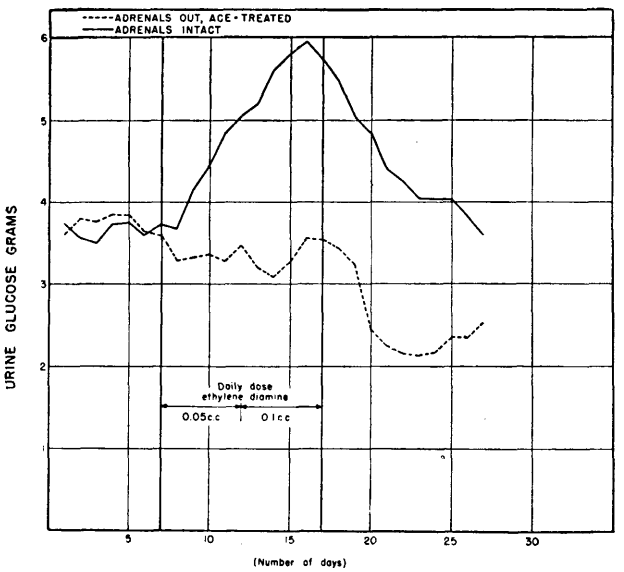

FIGURE 4 Effects of ethylenediamine upon the glycosuria of 6 pairs of partially depancreatized and partially depancreatized-adrenalectomized rats. Averages

COMMENT

In experiments 1 and 2 it was shown that diethylstilbestrol and aspirin have effects upon carbohydrate metabolism which are not mediated by a change in the secretory activity of the adrenal cortices. Although large doses of either of these drugs can cause an increase in the secretory activity of the adrenal cortices there is no satisfactory evidence that the metabolic consequences of this response represent a state of hypercorticalism.

In experiment 3 the injection of toxic doses of formaldehyde was used as a stress to cause activation of the adrenal cortices. It is probable that the increased secretion of adrenal cortical hormones during this stress did have a positive effect upon the level of urinary glucose in that it tended to prevent the marked decrease in glycosuria which occurred in rats without adrenal glands. These adrenalectomized rats which were on a fixed intake of adrenal cortical extract — an amount which represented adequate replacement therapy during non-stress conditions — developed a state of relative adrenal cortical insufficiency during stress and the glycosuria fell sharply as is characteristic of adrenal cortical insufficiency. The consequence of the adrenal response to the stress was to maintain the status quo as far as the level of glycosuria was concerned and not to exacerbate the diabetes and cause other signs of hypercorticalism.

During the past twelve years we have tested for the possible effect of a number of toxic compounds and stressful situations upon the glycosuria of the mildly diabetic forced-fed rat. Among them are phenol, carbon tetrachloride, methadone hydrochloride, lobeline sulfate, dihydrocodeinene bitartrate, nikethamide, furacin, quinine, quinidine sulfate, tartronic acid, cadmium chloride, copper sulfate, sodium arsenate, aluminum chloride, caffeine, benzyl benzoate, betaphenyl-ethylamine, pyrogens, water intoxication and low temperature. Each form of noxious stimulus was gradually increased in severity until the animal died, yet in no case was there any significant rise in the level of glycosuria.

From the results of experiments 1, 2 and 3 and from the negative results obtained by testing the several noxious agents listed above we have drawn the tentative conclusion that the increased secretory activity of the adrenal cortices during stress tends to maintain homeostasis rather than to disturb it. The increased secretion of adrenal cortical hormones serves to meet an increased need for these hormones and does not cause a state of hypercorticalism such as develops when the titer of these hormones is increased artificially in the absence of a need. The results of experiment 4 represent a challenge to this generalization. The exacerbation of glycosuria by ethylenediamine is dependent upon the presence of the adrenal glands and presumably upon the response of the adrenal cortices to this form of stress. The response does not appear in similar animals which have been adrenalectomized and are maintained on a fixed intake of adrenal cortical extract.

Does the injection of ethylenediamine cause exacerbation of glycosuria in the partially depancreatized rat solely because it causes a state of hypercorticalism? This is a possible explanation but it is not the only interpretation that can be made of the data. An alternative hypothesis is that ethylenediamine has some diabetogenic activity of its own but by virtue of also being toxic and stressful the diabetogenic activity is masked by the concomitant production of a state of adrenal cortical insufficiency in the adrenalectomized rats whose intake of exogenous hormone is fixed. Similar animals having intact adrenal glands secrete more hormone during the stress and maintain a state of eucorticalism (not hypercorticalism) thereby permitting the manifestation of the diabetogenic action of ethylenediamine. Thus the response of the adrenal cortex may possibly serve as a supporting cause of the metabolic response without becoming the exciting cause.

The following considerations give some support to the above hypothesis: 1. The fact that the several other forms of stress studied by us have failed to cause exacerbation of diabetes indicates that ethylenediamine

has some effect not possessed by nonspecific damaging agents. 2. It is known that diabetes is ameliorated by adrenal cortical insufficiency. In experiment 3, the relative state of adrenal cortical insufficiency caused by the injection of toxic doses of formaldehyde did cause a decrease in the glycosuria of the adrenalectomized rats which were on a fixed intake of adrenal cortical extract. A similar response could have been expected during the injection of ethylenediamine to the adrenalectomized rats of experiment 4 but it did not occur, possibly because ethylenediamine had some extra-adrenal effect upon the glycosuria. 3. Following the withdrawal of ethylenediamine the glycosuria did fall below its initial level in the adrenalectomized rats which indicates that the compound may have had a positive effect upon glycosuria during its administration. Animals so injected have a considerable amount of necrosis of the skin and therefore remain under stress after the injection of ethylenediamine is stopped.

At the present time we have not excluded the possibility that ethylenediamine affects glycosuria via the adrenal medulla rather than the adrenal cortex.

This paper is a progress report and our interpretations of the data are listed as hypotheses, not conclusions. We expect to continue our studies on the metabolic consequences of adrenal cortex activation during stress.

SUMMARY

These studies were intended to test the hypothesis that nonspecific noxious stimuli can cause exacerbation of a diabetic state via activation of the anterior pituitary-adrenal cortex axis. Partially depancreatized male rats were forced fed a fluid medium carbohydrate diet by stomach tube. In experiment 1, the injection of diethylstilbestrol caused exacerbation of the glycosuria in 6 nonadrenalectomized rats and induced a similar response in the same animals after they were adrenalectomized and kept on a fixed intake of adrenal cortical extract. In experiments 2, 3 and 4 half of the animals were adrenalectomized and treated with amounts of adrenal cortical extract which sustained the preadrenalectomy level of glycosuria during the control periods. In experiment 2 the injection of large doses of aspirin caused amelioration of the glycosuria in both nonadrenalectomized and adrenalectomized rats. In experiment 3 the injection of dilute solutions of formaldehyde caused a small decrease in the glycosuria of the nonadrenalectomized rats and a marked decrease in the glycosuria of the adrenalectomized rats. The results of these three experiments fail to support the hypothesis under test. In experiment 4 the injection of toxic amounts of ethylenediamine caused exacerbation of the diabetes in the presence of the adrenal glands but not in their absence. These results suggest that this stress caused a state of hypercorticalism.

A second possible explanation is that ethylenediamine has a specific extra-adrenal diabetogenic action which is masked by the development of adrenal cortical insufficiency in those adrenalectomized rats whose intake of exogenous hormones was adequate to meet the needs of non-stressful conditions only.

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DISCUSSION

DR. GEORGE W. THORN *(Boston):* In a field as complicated as that dealing with the relationship between the adrenal cortical hormones and insulin secretion, all of us appreciate the beautifully controlled experiments which Dr. Ingle has been able to carry out over a period of several years. It is unfortunate that in our studies of the mechanism of diabetes in man we cannot establish the same rigid control.

There is one point in Dr. Ingle's presentation which I am certain he would like emphasized. Throughout his discussion it has been tacitly assumed that changes in urinary glucose content automatically reflect overall changes in the diabetic state of his animals. With the particular substances which he employed, many of which are known toxic agents, changes in renal threshold may easily occur. Such changes could lead to more or less glucose being present in the urine without necessarily reflecting aggravation or amelioration of the underlying diabetic state.

I should also like to discuss an aspect of Dr. Ingle's work which interests me particularly. This might be

termed a comparison between the flexibility of the intact human to deal successfully with wide changes in carbohydrate metabolism, in contrast to the rigidity or inflexibility of the patient without adrenals or with limited capacity to secrete insulin. My reason for discussing this point at this time is the striking experience many of us have had in administering large doses of ACTH or gluconeogenic adrenal steroids without observing the development of diabetes. On the other hand, occasional patients may develop frank or latent diabetes under such a program. It appears at present that the load imposed by a course of ACTH or cortisone may be one of the most effective means of detecting latent diabetes mellitus and I hope that over the next few years this hypothesis will be tested in a large enough population to establish its validity. Certainly if I were faced today with the necessity of determining the capacity of a patient to produce insulin, I would use first the serum phosphorus response to intravenously administered glucose and follow this by a course of ACTH or cortisone with a subsequent glucose tolerance test carried out before the course of adrenal hormone administration had been discontinued. When glucose tolerance tests are carried out in patients receiving ACTH or cortisone, one must not misinterpret the elevation of the glucose tolerance curve which reflects increased deposition of glycogen in the liver. Under these circumstances, the elevated glucose curve should be accompanied by a normal fall in serum inorganic phosphorus in contrast to the true diabetic state in which this does not occur without insulin. Refined tests of this type are only necessary when the usual glucose tolerance test is negative or when there is a strong family history of diabetes mellitus.

In contrast, the patient with diabetes mellitus and Addison's disease may have his diabetes mellitus markedly aggravated by a daily dose of 25 mg. of cortisone or may become severely hypoglycemic with 5 units of insulin when not receiving a maintenance dose of cortisone. These experiments in man, of course, merely confirm the animal experiments of Long and Lukens in pancreatectomized-adrenalectomized animals.

In our laboratory, Dr. Dalton Jenkins has accumulated evidence that the glucogenic corticoids do not appear to act directly or specifically in antagonizing the action of insulin. In other words, the metabolic changes induced by a given dose of insulin may occur despite the administration of very large quantities of cortisone. These observations, while needing confirmation, do support the thesis which Dr. Ingle has presented, namely: that the role of the adrenal hormones in the genesis of diabetes mellitus is not as specific as formerly considered, but occupies more the position of providing a tissue factor essential for the development of diabetes.

Finally, I should like to say that in our own experiments in man we have not been able to confirm the idea that the additional glucose accumulated with glucogenic steroids arises predominantly from protein sources. Certainly the degree of negative nitrogen balance or increase in total nitrogen excretion is often so small as to make this hypothesis untenable unless associated with some as yet unknown rearrangement in nitrogen metabolism. Certainly the evidence is suggestive that a large factor is the transition to fat utilization with perhaps gluconeogenesis being increased from fat sources.

DR. JEROME W. CONN *(Ann Arbor, Michigan):* I think that someone should emphasize the point that in diabetic humans the stress of major surgery usually increases the severity of the diabetes, at least in the immediate postoperative period.

Although we do recognize evidence of increased adrenal-cortical activity under conditions of stress, the total response to stressful circumstances involves other endocrine activities which have not yet been clearly delineated. It is likely, for example, that there occurs increased activity of the R. Q. depressing factor recently described by Recant.

The point I wish to make is that the human diabetic, exposed to a very stressful situation, does not diminish his glycosuria. Dr. Ingle's well controlled experiments in rats probably do not include all of the factors which are involved when the diabetic patient responds to an "alarming stimulus."

DR. DWIGHT J. INGLE (Closing): The changes in glycosuria that I described to you are accompanied by a marked hyperglycemia, which shows we were not dealing with changes in the renal threshold.

The diabetogenic activity of the adrenal steroids and of corticotropin in experimental animals is similar to the effects of these hormones upon carbohydrate metabolism in man as described by Dr. Thorn. It requires enormous amounts — much larger in proportion than is being used for therapeutic purposes — to cause glycosuria in normal animals. It can be done but it requires 5 or 10 mg. of cortisone per rat per day, for example. Even though a severe insulin-resistant type of diabetes is induced, it does not persist but fades out in time.

We have never been able to sustain steroid diabetes in normal rats, even during the time that we continued to give the hormone, and, of course, it disappears whenever the hormone is withdrawn.