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# SOME EFFECTS OF ADRENAL CORTICAL STEROIDS ON INTERMEDIARY METABOLISM*

BY

GEORGE W. THORN, M.D., A.M., LL.D., Sc.D.(Hon.)

*Hersey Professor of the Theory and Practice of Physic, Harvard Medical School; Physician-in-Chief, Peter Bent Brigham Hospital*

ALBERT E. RENOLD, M.D.

*Associate in Medicine, Harvard Medical School and the Peter Bent Brigham Hospital*

AND

ALBERT I. WINEGRAD, M.D.

*Research Fellow in Medicine, Harvard Medical School; Assistant in Medicine, Peter Bent Brigham Hospital; Post-doctoral Fellow of the United States Public Health Service*

The importance of the adrenal gland to the total economy of the individual was indicated by the classic description of Thomas Addison in 1855 and the subsequent experiments of Brown-Séquard (1856). It was not until 1908, however, that Bierry and Malloizel first described hypoglycaemia in adrenalectomized dogs. Porges in 1909 independently described a similar phenomenon in patients with Addison's disease, and in the following year first observed the occurrence of low levels of liver and muscle glycogen a few hours after adrenalectomizing fasted dogs. These observations were confirmed by Schwartz (1910) and Kahn and Starkenstein (1911).

Following the discovery of insulin, Maranon (1925) reported that patients with adrenal insufficiency exhibited marked sensitivity to administered insulin, and Cori and Cori (1927), extending the original observations of Porges, Schwartz, and Kahn and Starkenstein, showed that adrenalectomized rats fasted for 24 hours had practically no liver glycogen.

During this early period Britton and Silvette (1932) championed the theory that the "prepotent" function of the adrenal cortex was concerned with the regulation of carbohydrate metabolism. In summarizing their observations these investigators noted: "The effects of adrenalectomy on carbohydrate metabolism are profound in character and appear to be primarily responsible for the death of the animal. Blood glucose and liver glycogen . . . show marked reduction from the normal levels. There are also associated decreases in muscle glycogen and increases in blood lactate. The glycogen of the heart is not reduced. . . . Completely adrenalectomized animals show a markedly reduced ability to store liver glycogen. Normal animals who were injected with glucose stored eight to ten times as much glycogen as operated animals."

Controversies necessarily arose as different species of animals were used by different investigators and since the importance of the sodium and potassium content of the diet was not appreciated. In addition, the early preparations of adrenal extract showed wide and unpredictable variations in potency, so that standardization of dosage, permitting comparison by different investigators, was practically impossible.

In 1934 Hartman and Brownell observed that adrenalectomy significantly modified the severity of pancreatic diabetes in the cat, and that the diabetic syndrome was exacerbated by the administration of adrenal extract. Subsequently, in an extensive carefully controlled series of experiments, Long and Lukens (1934–5) established the effect of adrenalectomy on pancreatic diabetes.

In 1936 Evans demonstrated that fasted adrenalectomized animals excreted 25% less nitrogen than normal controls. The excellent paper by Long, Katzin, and Fry (1940) summarized the situation as of that time. In short, they noted that adrenalectomized mice and rats maintained on sodium salts retained practically normal carbohydrate levels when fed; however, when fasted, the levels declined more rapidly than in normal animals. Fasted adrenalectomized animals also excreted less urine nitrogen. The administration of cortical extract to either fasted-normal or adrenalectomized mice and rats was followed by large increases in liver glycogen and slight hyperglycaemia. The muscle glycogen was not affected. At the same time there was a significant increase in nitrogen excretion, suggesting that increased protein catabolism was the source of this newly formed carbohydrate. Moreover, they noted that in partially pancreatectomized rats adrenalectomy and maintenance on sodium salts was associated with decreased glucosuria; that the administration of adrenal extract and of certain crystalline steroids then available exacerbated the glucosuria; and that the ability to produce increased glucosuria was not shared to an equal degree by the crystalline steroids then obtainable.

About this same time naturally occurring 11- and 11, 17-oxygenated steroids, in addition to synthetic 11-desoxycorticosterone (deoxycortone), became available in amounts which, though small, were sufficient for clinical trial. An attempt therefore was undertaken to extend the experimental studies to man.

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## Studies in Addison's Disease

In 1940 Thorn, Koepf, Lewis, and Olsen reported that the severe reactive hypoglycaemia observed in patients with Addison's disease after an intravenous infusion of glucose was not affected by the administration of deoxycortone acetate (30 mg.), but was abolished by the administration of an equivalent quantity of 17-hydroxy, 11-dehydrocorticosterone (compound E of Kendall, cortisone). Not only was the hypoglycaemia corrected by cortisone but a *delayed glucose disappearance curve and accompanying glucosuria* were noted (Fig. 1). Corticosterone (compound B of

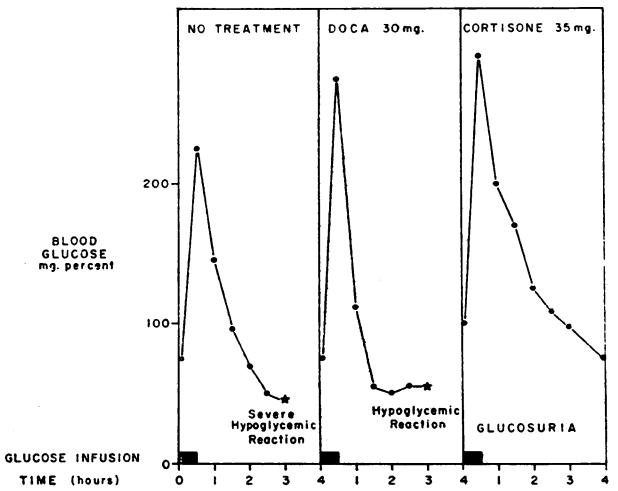


FIG. 1.—Intravenous glucose-tolerance tests in a woman (E. L.) aged 51 with Addison's disease. (From Thorn, Koepf, Lewis, and Olsen, 1940.)

Kendall) at the dose level of 85 mg. was also found to be effective in this regard. Comparison with adrenal cortical extract indicated that the most potent commercial extract then available contained less than 1 mg. of cortisone equivalent per millilitre.

That the increased fasting blood glucose level observed with corticosterone and cortisone was perhaps "diabetic" in nature was suggested by the observed fall in the fasting respiratory quotient (R.Q.) at the height of the blood glucose elevation induced by these substances (Fig. 2). The limitations set to the interpretation of overall R.Q. values are appreciated, but the remarkable changes noted in this well-trained patient maintained on a carefully controlled diet and metabolic regimen merit consideration. Furthermore, the lowering of the non-protein R.Q. was associated with an increase in basal nitrogen excretion and a rise in basal metabolic rate. These observations suggested at that early date that the 11- and 11,17-oxysteroids enhance gluconeogenesis and increase utilization of non-carbohydrate

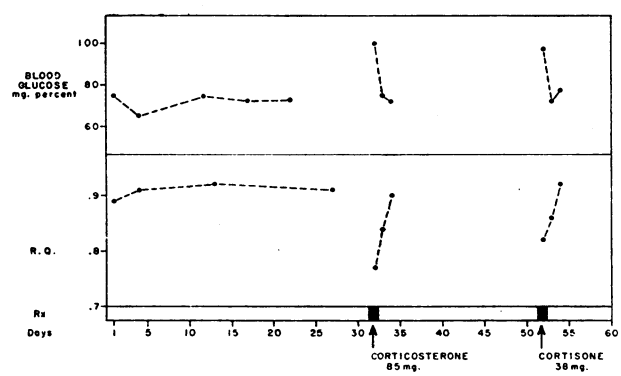


FIG. 2.—Fasting respiratory quotients and blood glucose levels in a woman (E. L.) aged 51 with Addison's disease. (From Thorn *et al.*, 1940.)

sources of energy. Studies such as this presaged the "diabetogenic" potential of these steroids when employed clinically in large doses.

The susceptibility of patients with Addison's disease to fasting hypoglycaemia may be due in appreciable measure to the increased effectiveness of endogenous insulin in these patients and may explain some of their psychological difficulties. The changes observed over a period of years in a patient with Addison's disease who developed complicating diabetes mellitus between the years 1939 and 1942 support this concept (Fig. 3 and Table I) (Thorn and Clinton, 1943).

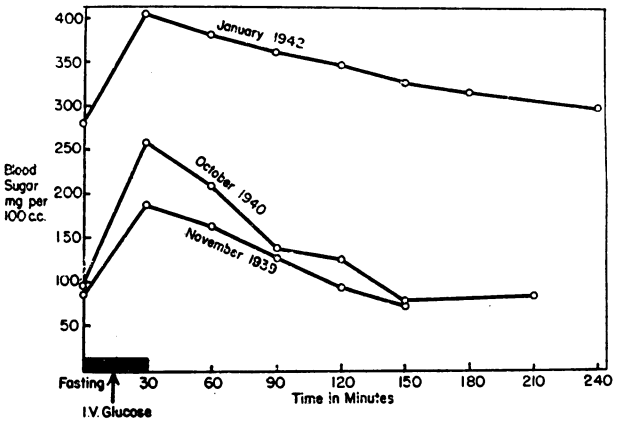


FIG. 3.—Intravenous glucose-tolerance tests in Addison's disease (patient J. H. H.), 0.5 g. of glucose per kg. of body weight. (From Thorn and Clinton, 1943.)

TABLE I.—*Observations in a Patient With Addison's Disease Who Developed Diabetes Mellitus*

1. Marked psychological improvement.
2. Fasting hyperglycaemia, minimal ketonuria.
3. Marked insulin sensitivity despite hyperglycaemia.
4. Acute, transient hypokalaemia with paralysis after insulin and glucose
5. Modest increase in nitrogen excretion following insulin withdrawal.
6. Persistently abnormal E.E.G. despite hyperglycaemia.
7. Marked hyperglycaemia and ketonuria after cortisone.

In conjunction with a slowly increasing fasting blood glucose level this patient lost his negativistic and almost catatonic attitude and became a warm, outgoing person. Until his diabetic state became flagrant there was also appreciable improvement in his overall activity, suggesting that major clinical manifestations of adrenal cortical deficiency were not limited to specific hormone depletion, but were in part due to the unopposed action of persistent hormonal secretions such as insulin. In this patient the elevation of blood glucose levels to higher-than-normal values did not in itself result in a correction of the abnormal electroencephalographic changes, thereby suggesting that glucocorticoid activity is more important than the absolute blood glucose level in restoring this indicator of brain metabolism to normal in patients with severe adrenocortical hypofunction. This observation confirms earlier studies of Engel and Margolin (1942) and Hoffman, Lewis, and Thorn (1942), who were unable to correct the abnormal electroencephalographic changes of patients with Addison's disease by the intravenous infusion of glucose.

At that time, calculation of the "caloric distribution" in the basal state served to emphasize some of the more subtle changes in metabolism which occurred in this patient as compared with an untreated Addisonian patient and a normal subject (Thorn and Clinton, 1943). Although the interpretation of these calculations is highly questionable, they do emphasize alterations of intermediary metabolism, and it was of interest to note an approximately normal "caloric distribution" under fasting conditions in this Addisonian-diabetic patient as compared with a patient with uncomplicated Addison's disease. The normal caloric distribution was, however, attained at the expense of a fasting blood glucose level of 280 mg. Following the administration of a single dose of cortisone, the untreated, uncomplicated

Addisonian patient attained a normal caloric distribution, whereas the normal subject, and particularly patient J. H. H., with Addison's disease and diabetes, increased greatly the proportion of fat being utilized according to the then accepted interpretation of the data.

These early studies on patients with Addison's disease, and in particular the unique opportunity afforded by the spontaneous development of diabetes mellitus in a patient with Addison's disease, confirmed in man the importance of specific adrenal steroids in the regulation of intermediary metabolism.

## Steroid Structure and Biological Activity

An attempt has been made to assign comparative values to the more generally available adrenal steroids in terms of their effectiveness in regulating intermediary metabolism in man. In general the criterion for this action is based upon the effectiveness of a specific hormone to restore to normal in patients with Addison's disease treated with deoxycortone those major abnormalities which persist despite this form of therapy. Some of these abnormalities are listed in Table II.

TABLE II —*Persisting Defects Observed in D.C.A.-treated Patients With Addison's Disease*

1. Fasting hypoglycaemia and hypersensitivity to insulin.
2. No prompt diuresis following water load.
3. Abnormal E.E.G. and E.C.G.
4. Persistent personality changes.
5. Pigmentation.
6. Frequent eosinophilia.
7. Febrile response to intravenous glucose.
8. Poor resistance to stress in any form.

The peculiar capacity of the glucocorticoids to cause a virtual disappearance of eosinophils from the circulating blood is well established, and in general closely parallels the overall carbohydrate-regulating potency of these steroids. The basis for the sensitivity of eosinophils to glucocorticoids has never been explained. Lymphocytes share this property to a limited degree, whereas the number of polymorphonuclear leucocytes usually rises with glucocorticoid therapy.

In addition to the actions of glucocorticoids which have been mentioned, these substances also exert a marked effect on such widely different bodily functions and reactions as gastric secretion, heart function, skin pigmentation, and response to inflammation. At this time none of these indices lends itself to short-term or precise quantitative analysis in man.

In our hands, the present most useful semi-quantitative assay method for 17-hydroxysteroid activity *in man* is the estimation of urinary glucose excretion following the intravenous infusion of steroid substances under standard conditions in normal subjects or in patients with Addison's disease in whom glucocorticoid therapy has been temporarily withdrawn. Since the glucosuria observed is quantitatively small, this procedure depends on the use of a specific method for urinary glucose determinations. The method employed by us utilizes the highly specific enzyme glucose oxidase (Froesch and Renold, 1956). The reliability of the assay is greatly increased by comparing in a single patient, if possible, all steroids under investigation. As an illustration, the relative carbohydrate-regulatory effects of hydrocortisone and prednisone are compared in patient R. A. (Fig. 4). The major changes which some alterations of the basic structure of progesterone effect in the biological activity of the parent compound are summarized in Fig. 5.

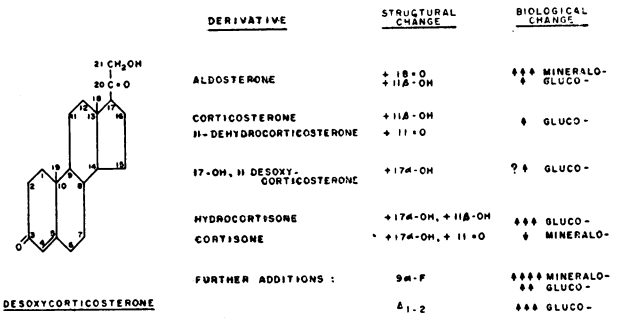


FIG. 5.

The synthetic steroid deoxycortone possesses little or no carbohydrate-regulating activity. The clinical improvement in patients with Addison's disease in whom this substance is the sole therapeutic agent can be largely accounted for by the restoration of electrolyte balance and of normal circulatory status with an attendant increase in appetite and overall activity. However, with continued deoxycortone therapy and without sodium chloride restriction or potassium supplementation, intermediary metabolism may be adversely affected (Kuhlmann, Ragan, Ferrebee, Atchley, and Loeb, 1939). Presumably such an untoward effect is brought about, in major part at least, by potassium deficiency. Clinically, one may observe hypertension and cardiac enlargement, impaired neuromuscular function, persistent abnormalities in the electroencephalogram and electrocardiogram, diminished gastric secretion, and changes in renal tubular function (polyuria resistant to "pitressin").

The quantity of *aldosterone* commercially available has not permitted extensive clinical observations, but it would appear that this naturally occurring mineralocorticoid is more active than deoxycortone with respect to its carbohydrate-regulating capacity. Certainly its "eosinopenic" effect is easily demonstrated, and represents a deviation from the usual close correlation between eosinopenic and carbohydrate-regulating activities observed in the case of the more active glucocorticoids (Thorn, Sheppard, Morse, Reddy, Beigelman, and Renold, 1955). Aldosterone is oxygenated in both carbon 11 and carbon 18. The biological effect of the oxidation of carbon 18 appears to result mainly in an enhanced electrolyte-regulating activity, whereas the addition of an oxygen atom in position 11 is probably the structural change responsible for its increased glucocorticoid activity.

The well-defined glucocorticoid activity of *corticosterone* and *11-dehydrocorticosterone* is in keeping with the latter hypothesis and demonstrates that the addition of an oxygen atom in position 11 is essential for significant carbohydrate-regulating activity. The associated and rather marked sodium-retaining activity of these substances has limited their use as the sole form of therapy in Addison's disease.

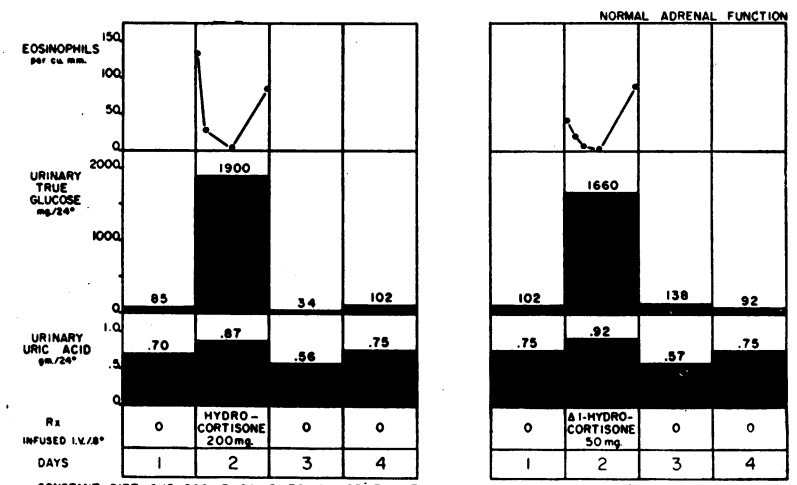


FIG. 4.—Comparative effects of hydrocortisone and delta-hydrocortisone (prednisolone) in a man (R. A.) aged 40. (From Thorn, Renold, Morse, Goldfien, and Reddy, 1955.)

In contrast to the importance of the oxygen atom in position 11, an oxygen atom in position 17 *alone* confers a much lesser degree of carbohydrate-regulating activity. Thus *11-desoxy, 17-hydroxycorticosterone* is a relatively inactive steroid. However, the addition of an oxygen atom in position 17 in compounds already oxidized in position 11 results in marked enhancement of glucocorticoid activity. Thus *cortisone* and *hydrocortisone* derive their physiological and pharmacological importance from their glucocorticoid potency and are the most active naturally occurring corticosteroids.

One of the most intriguing recent developments in this area of steroid-structure relationships has been the synthesis of derivatives possessing biological activity many times greater than that of the naturally occurring secretory products of the adrenal cortex. Among these fludrocortisone (9α-fluorohydrocortisone) and the delta-1 derivatives of cortisone and hydrocortisone (prednisone and prednisolone) have been extensively investigated (Fried and Sabo, 1955; Thorn, Renold, Morse, Goldfien, and Reddy, 1955). The glucocorticoid activity observed with prednisone is approximately four times that of cortisone with a relative decrease in sodium-retaining activity, thus making this preparation especially useful in situations in which "anti-inflammatory" activity without appreciable sodium retention is desired. The steroid fludrocortisone is highly potent, being approximately twenty times as active as its parent substance hydrocortisone with regard to glucocorticoid action. In this instance, however, there is a still greater increase in sodium-retaining activity, thereby diminishing the therapeutic usefulness of the compound. Interestingly enough, delta-1 fluorohydrocortisone does not appear to differ appreciably in its activity in man from the carbohydrate- and electrolyte-regulating activity of the parent compound, fluorohydrocortisone. It has recently been reported that the introduction of a hydroxyl group in position 16 obliterates the sodium-retaining effect of the 9α-fluoro substitution (Bernstein, Lenhard, Allen, Heller, Littell, Stolar, Feldman, and Blank, 1956).

Undoubtedly some modification will take place in the order in which these substances are classified as additional evidence becomes available regarding their anti-inflammatory action, the efficacy with which they suppress corticotrophin stimulation, and their effect on other body functions such as the gastro-intestinal tract. It is interesting to note that until fluorohydrocortisone was synthesized an increase in glucocorticoid activity was almost invariably associated with reduced mineralocorticoid activity. The mechanism of the spectacular potentiation of glucocorticoid activity by apparently small structural changes is, of course, as unknown as the precise mode of action of these hormones. It appears likely that their increased effectiveness cannot be entirely explained on the basis of a delayed rate of conjugation, degradation, or inactivation.

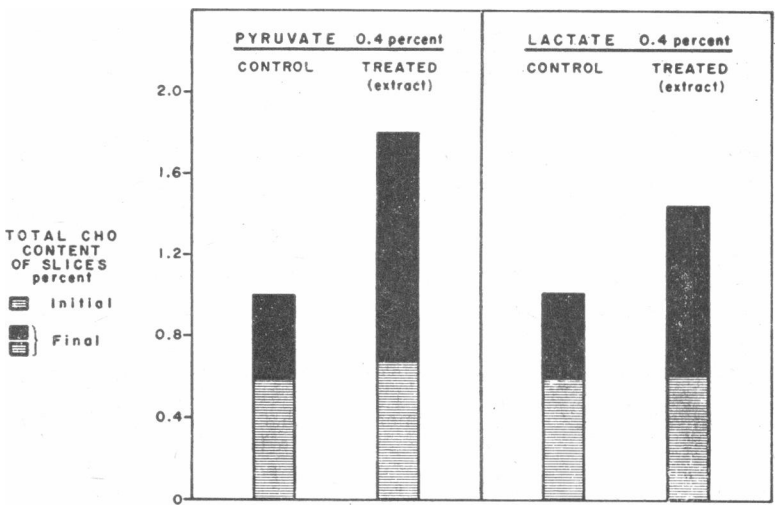


FIG. 6.—Synthesis of total carbohydrate by liver slices from treated and untreated adrenalectomized rats (incubated in Krebs-Ringer phosphate buffer). (From Koepf *et al.*, 1941.)

## Some Specific Glucocorticoid Effects

### Studies in Vitro

In 1941 Koepf, Horn, Gemmill, and Thorn showed in rat-liver slices that prior administration of adrenal cortical extract to adrenalectomized animals led to increased synthesis of carbohydrate from pyruvate and lactate (Fig. 6). This finding was then interpreted as suggesting increased hepatic gluconeogenesis as a result of adrenal cortical extract action. Similarly it has been shown since with C-14-labelled compounds that adrenalectomy restores to normal the excessive synthesis of glucose from pyruvate by liver slices from diabetic rats (Renold, Teng, Nesbett, and Hastings, 1953) and that the administration of cortisone to adrenalectomized diabetic animals promptly results in marked acceleration of glucose synthesis from pyruvate (Ashmore, Hastings, Nesbett, and Renold, 1956), as illustrated in Fig. 7. Renold and Hastings (1953) demonstrated

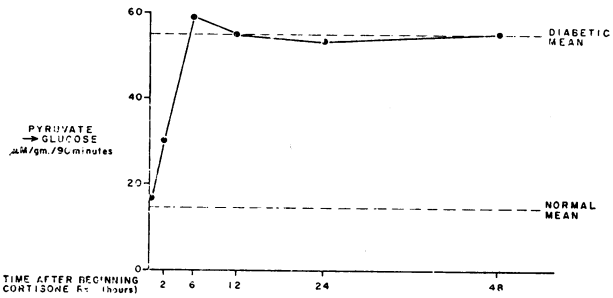


FIG. 7.—Synthesis of glucose from pyruvate by liver slices from diabetic adrenalectomized rats sacrificed 0 to 48 hours after cortisone administration. (From Ashmore *et al.*, 1956.)

increased glucose production but normal glucose utilization by liver slices from normal rats pretreated with large doses of cortisone for several days (Fig. 8).

Whereas the observations made on isolated liver which have just been reviewed are all compatible with increased hepatic gluconeogenesis as a major glucocorticoid effect, further effects on lipid and protein metabolism have also been established. Brady, Lukens, and Gurin (1951) observed that pretreatment with cortisone greatly reduced the incorporation of labelled acetate into fatty acids by liver slices. Furthermore, the impaired lipogenesis observed in liver tissue of diabetic animals was restored toward normal by adrenalectomy. Kline (1949) reported that adrenalectomy decreased the release of amino-nitrogen by isolated rat diaphragm and that prior treatment of the animal with adrenal cortical extract restored the rate of nitrogen release to normal.

In leucocytes, cortisone and hydrocortisone decrease lactic acid production (Martin, McKinney, and Green, 1955) and corticotrophin has been shown to increase the glycogen content of these cells (Robineaux, Bazin, Delbarre, and Delaunay, 1951). In mammary gland tissue of the rat before parturition or after weaning, incubation with cortical hormones has been reported to decrease lipogenesis and to prevent the lipogenetic effect of insulin when both were added to the incubation medium (Balmain, Folley, Glascock, and McNaught, 1954). The adrenal steroids have a less specific effect on brain tissue. Although a decrease in Q $O_2$ has been shown, this appears to correlate with the anaesthetic action of these steroids rather than their carbohydrate-regulating capacity (Gordan, 1956). Cortisone as well

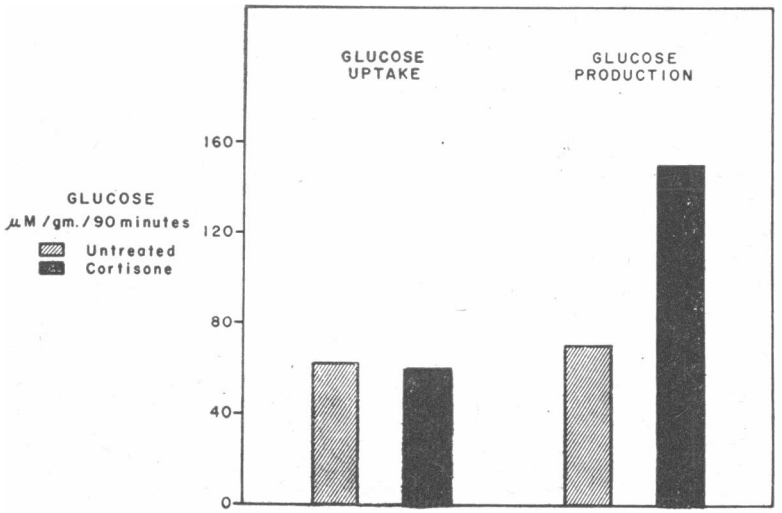


FIG. 8.—Uptake and production of glucose by liver slices from untreated and cortisone-treated rats (incubated) in Hastings buffer K = 110 mEq/l., with glucose 20 mM/l. and pyruvate 40 mM/l. as substrates). (From Renold and Hastings, 1953.)

as deoxycortone decreases the respiration of adrenal slices (Sourkes and Heneage, 1952), whereas corticotrophin is known to increase the respiration of this tissue (Reiss, Brummel, Halkerston, Badrick, and Fenwick, 1953). Some of these glucocorticoid effects resulting from studies *in vitro* have been summarized in Table III.

TABLE III.—*Some Effects of Glucocorticoids on Tissues*

1. Increased glycogen deposition (liver, leucocytes).
2. Increased glucose output (liver).
3. Increased glucose synthesis from pyruvate (liver).
4. Increased release of amino-acids (muscle, liver).
5. Decreased lipogenesis (liver, mammary gland, carcass).
6. Decreased oxygen uptake (brain).

### Studies in Experimental Animals

Long *et al.* (1940) showed that the administration of adrenal cortical extract or crystalline glucocorticoid preparations to fasted normal or adrenalectomized rats was followed by a large increase in liver glycogen with an associated increase in nitrogen excretion. In the same year Lewis, Kuhlman, Delbue, Koepf, and Thorn (1940) demonstrated that cortisone increased greatly the renal excretion of glucose and nitrogen in adrenalectomized, phlorizinized rats (Fig. 9). In contrast to an initial glucose to nitrogen ratio of 2.5 in the untreated adrenalectomized group (a ratio which did not change appreciably during the 48-hour period of phlorizinization), the hormone-treated animals displayed a higher initial ratio—that is, 2.8—and a progressive increase to a level of 3.3 during the experimental period. Furthermore, when a lactate load was administered to phlorizinized normal, adrenalectomized, and cortisone-treated rats, urinary glucose excretion accounted for 71% of the administered lactate in the normal group, for 80% in the hormone-treated group, and for only 26% in the untreated adrenalectomized-phlorizinized group (Fig. 10). In a similar type of experiment, adrenal cortical extract restored the capacity of phlorizinized-adrenalectomized rats to form glucose from alanine.

Direct demonstration of increased hepatic gluconeogenesis in intact animals had to await the development of adequate isotopic labelling techniques. In 1952 Welt, Stetten, Ingle, and Morley, employing a continuous intravenous infusion of carbon-14-labelled glucose in anaesthetized rats, demonstrated a sevenfold increase over normal in the rate of gluconeogenesis in rats pretreated with large doses of cortisone. The rate of glucose oxidation, on the other hand, was not greatly affected by pretreatment with the hormone. The experiments of these investigators also indicated a relatively low specific activity of liver glycogen in the presence of a high liver glycogen content, suggesting that under these circumstances a preponderance of the glycogen originated from non-isotopic—that is, non-glucose—precursors.

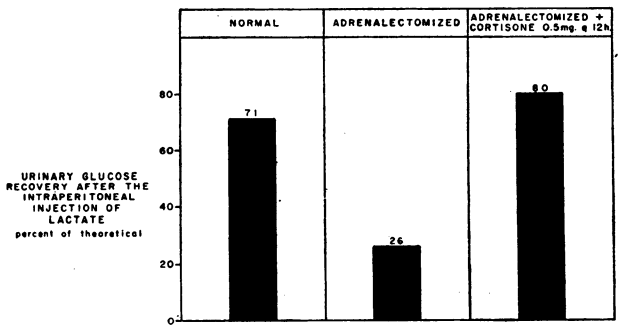


FIG. 10.—Urinary glucose excretion in phlorizin-treated normal, adrenalectomized, and cortisone-treated adrenalectomized rats, after administration of lactate. (From Lewis *et al.*, 1940.)

It also has been established that adrenalectomy decreases, and that administration of adrenal extracts increases, the amino-acid concentration in the serum of hepatectomized animals (Ingle, Prestrud, and Nezamis, 1948 ; Bondy, 1949), thus establishing that the effect of glucocorticoids is not limited to increasing hepatic gluconeogenesis. Indeed, it is entirely possible that the as yet unknown primary site of glucocorticoid action will be found in the extrahepatic tissues. Studies of this type emphasize the fact that adrenal cortical action and, more generally, hormonal action are conditioned by the chemical configuration of the hormone molecule on the one hand and by the specific nature of the responsive tissue on the other hand. This is further illustrated by the strikingly different response of the two organs known to be sites of significant gluconeogenesis—that is, liver and kidney. As shown in Fig. 11 the liver of fasting adrenalectomized rats responds to cortisone administration with a marked deposition of glycogen, whereas the kidney fails to do so (Froesch, Ashmore, and Renold, 1957).

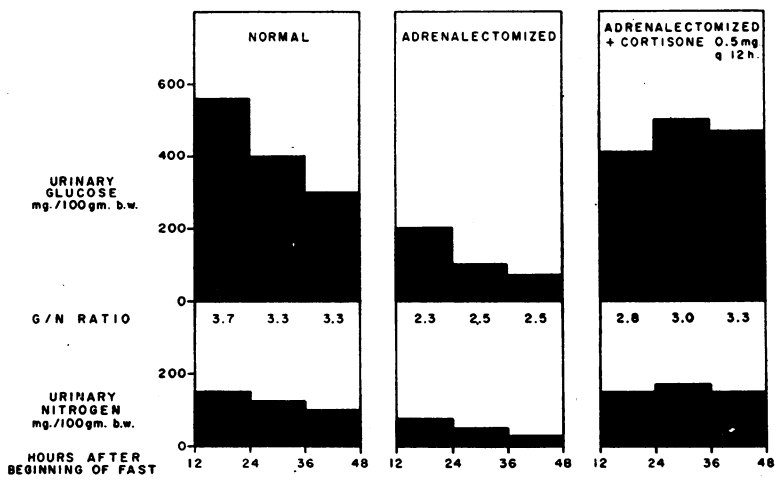


FIG. 9.—Urinary glucose and nitrogen excretion in phlorizin-treated normal, adrenalectomized, and cortisone-treated adrenalectomized rats, in the fasting state. (From Lewis *et al.*, 1940.)

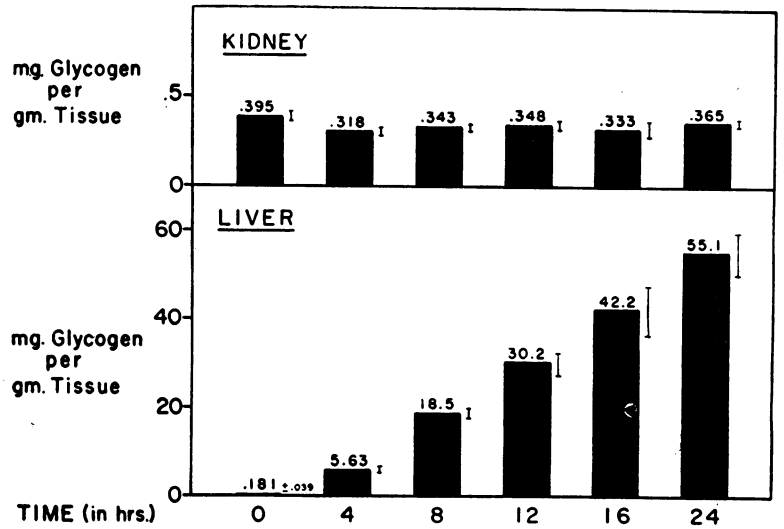


FIG. 11.—Glycogen deposition in liver and kidney of adrenalectomized rats following subcutaneous administration of cortisone (20 mg. initially, then 10 mg. four-hourly for two doses, and 5 mg. four-hourly for two doses). (From Froesch *et al.*, 1957.)

### Studies in Man

As has been previously pointed out, early studies in patients with Addison's disease suggested that in man, as in experimental animals, glucocorticoids affect the ability of the organism to produce glucose from non-glucose sources. Although isotopic techniques have not as yet been applied to the study of glucocorticoid effects in man, rather direct evidence for the gluconeogenetic effect of glucocorticoids in the intact human organism has been obtained recently from observations in two subjects with renal glucosuria on the one hand (Froesch, Winegrad, Renold, and Thorn, 1957), and in diabetic patients receiving constant infusions of fructose on the other hand (Felber, Winegrad, Renold, and Thorn, unpublished observations).

By taking advantage of the spontaneous occurrence of renal glucosuria in two patients admitted to the metabolic ward, the effects of hydrocortisone infused intravenously could be studied over a period of eight hours subsequent to a 16-hour fast. The measurements made included blood glucose as well as urinary glucose excretion; in addition inulin clearance was measured throughout and tubular glucose load and reabsorption could be calculated. The results of one of these studies are shown in Fig. 12. Blood and urine glucose was measured enzymatically. Within two hours of the beginning of intravenous hydrocortisone administration urinary glucose excretion increased, and by the end of the experimental period it reached 34 mg. a minute, a sixfold increase over base-line levels. This represents a very considerable glucose loss of approximately 1.5 g. an hour. Despite this considerably increased loss of glucose in the urine in this fasting individual, blood glucose rose by approximately 20 mg. per 100 ml. Since it may be safely assumed that, in a fasting individual given hydrocortisone, liver glycogen is increasing and not decreasing, the most reasonable conclusion which these observations allow is that of increased hepatic gluconeogenesis of the order of magnitude of at least 1.5 g. an hour as a result of hydrocortisone administration.

Continuous infusions of fructose have also been used in order to further analyse the activity of hepatic gluconeogenesis. It is well recognized that fructose is metabolized mainly in the liver and its major pathway of metabolism involves the splitting of fructose-1-phosphate into two three-carbon fragments. The hepatic production of glucose from fructose then involves the resynthesis of six-carbon compounds from three-carbon fragments (Fig. 13). Since fructose is metabolized by liver at a very nearly constant rate under almost all conditions tested, the fraction of fructose which is metabolized to glucose is likely to parallel its gluconeogenetic activity at that time. Measurement of the conversion of fructose to glucose is therefore likely to provide an estimation of hepatic gluconeogenetic activity.

Patient B. B., a 67-year-old woman with mild diabetes mellitus and not receiving insulin, was given a prolonged infusion of fructose with a constant infusion pump for a period of eight hours at the rate of 0.5 g./kg./hour (Fig. 14). Blood fructose rapidly increased to levels around 50 mg. per 100 ml., then remained constant. Blood glucose steadily declined from levels around 170 to levels around 130 mg. per 100 ml. Since this patient's blood glucose behaved similarly if the overnight fast was prolonged by eight hours, this observation was interpreted as suggesting that most of the fructose administered (approximately 200 g.) had been deposited as liver glycogen or metabolized by pathways other than the production of glucose—for example, produc-

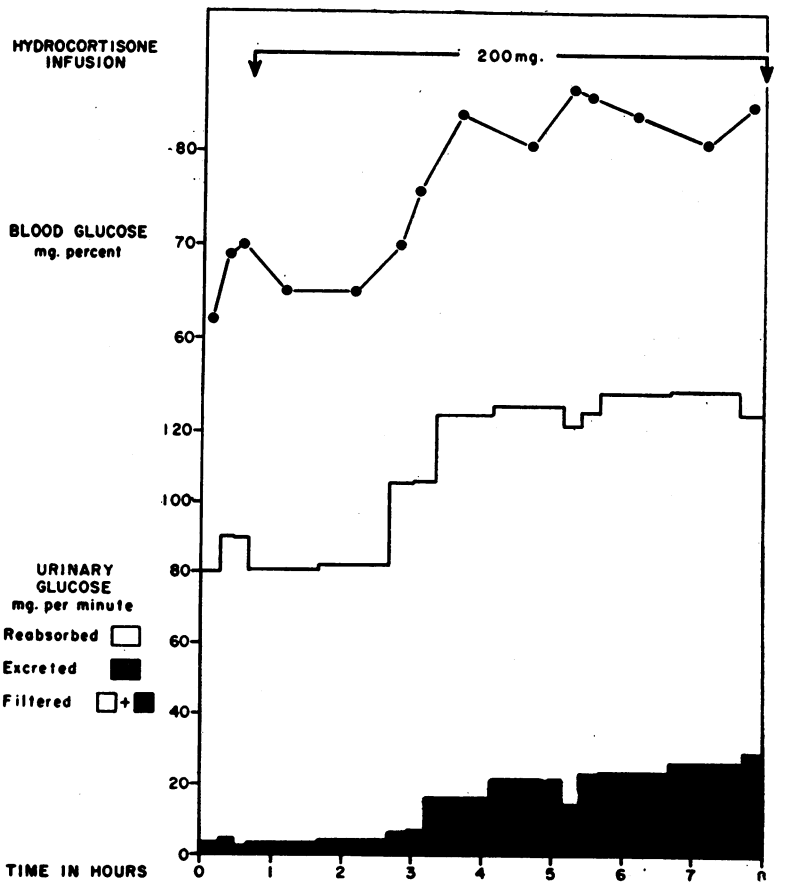


FIG. 12.—Effect of hydrocortisone on blood glucose and renal glucose clearance in a fasted patient (a man (F. N.) aged 22, weight 73 kg., height 168 cm.) with renal glucosuria: II. (From Froesch, Winegrad, Renold, and Thorn, 1957.)

tion of pyruvate and lactate. When the procedure was repeated, together with the concurrent administration of hydrocortisone at the rate of 25 mg. an hour from the third hour onwards, blood fructose levels were not affected but blood glucose rose sharply, beginning two hours after initiation of hydrocortisone administration. The maximum level reached was 280 mg. per 100 ml. Whereas urinary glucose excretion during the last four hours of fructose

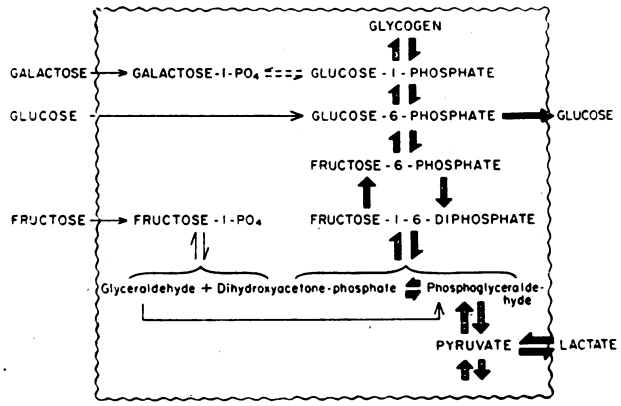


FIG. 13.—Major pathways of hexose metabolism in liver.

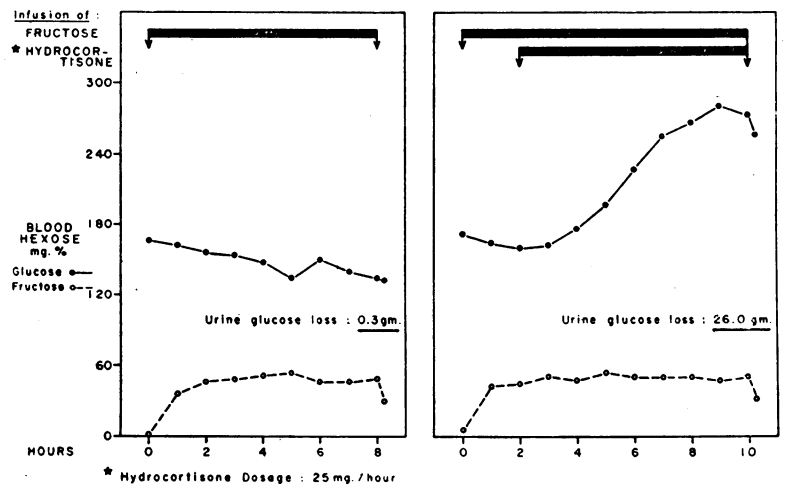


FIG. 14.—Changes in blood glucose levels in a woman (B. B.) aged 67 with diabetes mellitus during the continuous intravenous infusion of fructose (0.5 g./kg./hr. for 8 hours). (From Felber, Winegrad, Renold, and Thorn, unpublished observations.)

infusion averaged less than 50 mg. an hour on the control day, it averaged 5 g. an hour on the day of hydrocortisone administration.

The strikingly increased blood glucose levels and urinary glucose excretion during the hydrocortisone–fructose infusion periods is again interpreted as strong evidence in favour of accelerated hepatic synthesis of glucose from intermediary metabolites as a result of glucocorticoid action in man.

## Cushing's Syndrome and Diabetes Mellitus

The metabolic abnormalities in patients with Cushing's syndrome provide the opportunity to compare a disorder associated with primary glucocorticoid excess with one associated with primary insulin deficiency (diabetes mellitus). The incidence of abnormalities of carbohydrate metabolism in 36 patients with Cushing's syndrome is summarized in Table IV. The following criteria have been observed: an elevated fasting blood glucose was considered to be one above 110 mg. per 100 ml., using the Somogyi–Nelson method. An abnormal glucose-tolerance test represented the failure to observe a return to normal glucose levels within 60–90 minutes after the intravenous administration of

TABLE IV.—*Abnormalities of Carbohydrate Metabolism Occurring in 36 Patients with Cushing's Syndrome*

| | Patients | % |
|---|---|---|
| Abnormal glucose-tolerance curve | 29 | 81 |
| Glucosuria | 14 | 38 |
| Fasting hyperglycaemia | 12 | 33 |
| Overt diabetes mellitus | 10 | 28 |

a glucose load of 0.5 g. per kg. body weight administered over a period of 30 minutes. Glucosuria was considered positive when present constantly or on more than one occasion. Overt diabetes was diagnosed in the presence of persistently elevated fasting blood glucose levels and glucosuria, usually requiring insulin therapy.

TABLE V.—*Steroid Diabetes Mellitus Versus Classical Diabetes Mellitus*

1. *Depletion of body protein out of proportion to other symptoms of deranged intermediary metabolism.*
2. Relative insulin resistance.
3. No tendency to severe ketoacidosis.
4. Evidence suggestive of maintained peripheral utilization of glucose (i.e., normal changes in serum $PO_4$, blood lactate, and pyruvate during glucose-tolerance test).
5. Exaggerated fructose conversion to glucose, even when glucose-tolerance test normal.

"Glucocorticoid diabetes" has several characteristics which differentiate it from non-steroid diabetes mellitus (Table V). In the first place, one is impressed by the *marked overall protein depletion exhibited by patients with Cushing's syndrome.* This may be true even in cases where the diabetes is relatively mild or non-existent. Osteoporosis, thinning of the skin with striae, "bruisability," and loss of muscle mass are much more striking in this disorder than one ordinarily sees even in long-standing, severe, and poorly treated classical diabetes mellitus. These observations, of course, suggest that steroid diabetes inherently reflects excessive glucose formation from endogenous protein sources with consequent severe depletion of the supporting body tissues. Secondly, the hyperglycaemia and glucosuria of Cushing's syndrome may be relatively resistant to insulin therapy, although this is not uniformly true. Thirdly, when glucose-tolerance tests are carried out in conjunction with the measurement of indices of peripheral glucose utilization, such as changes in serum inorganic phosphate, serum potassium, or blood pyruvate and lactate, it is commonly observed that these indices show a normal response to the glucose load even in the presence of an abnormal glucose profile. Indeed, it has been suggested that blood pyruvate and lactate levels are elevated in the fasting state in patients with hyperadrenocorticism and respond by an abnormally *brisk* increase to the administration of a glucose load (Frawley, 1955; Henneman and Bunker, 1957).

The most likely interpretation of these findings is that of persistent adequate peripheral glucose utilization—that is, of normal, or perhaps better than normal, insulin secretion, albeit frequently inadequate to achieve completely normal blood glucose levels in the presence of excessive gluconeogenetic activity. Fig. 15 finally illustrates that in certain patients with Cushing's syndrome increased gluconeogenesis may be detected *in the presence of a normal intravenous glucose-tolerance test* by means of the blood glucose response to an acute intravenous fructose load. After the intravenous administration of 0.5 g. of fructose per kg. over 10 minutes this patient's blood glucose increased by 24 mg. per 100 ml., whereas metabolically normal individuals rarely show any significant blood glucose rise under these conditions.

The intimate dependence of the hyperglycaemia of Cushing's syndrome on steroidogenesis can be well demonstrated by temporarily blocking the secretion of glucocorticoids by amphenone administration. This can be achieved

quite readily in patients with adrenal cortical carcinoma (Thorn, Renold, Goldfien, Nelson, Reddy, and Hertz, 1956; Hertz, Renold, Reddy, Pittman, Graff, and Thorn, 1956), as illustrated in Fig. 16. In patient S. D. the administration of amphenone resulted in a sudden decrease in plasma and urine steroid levels and concomitantly in a dramatic improvement of her diabetic state. Fasting blood glucose levels approached normal values, glucosuria markedly decreased, and insulin could be withheld. The severity of the diabetic state was restored to pre-amphenone levels within hours of amphenone withdrawal.

Studies with amphenone, as well as the comparison of the glucosuric effect of corticotrophin with that of known quantities of cortisone or hydrocortisone, give no support to the concept that patients with Cushing's syndrome and diabetes secrete a glucocorticoid of much greater potency than the major known glucocorticoids.

### Preliminary Studies of Insulin Reserve in Man

The availability of corticotrophin and adrenal steroids has provided the clinical investigator with an important means of stressing the insulin-secreting mechanism in normal individuals as well as in patients with potentially decreased insulin reserve. Fajans and Conn (1954) have reported that glucose-tolerance tests carried out shortly after the administration of glucocorticoids may unmask a group of potential diabetic patients, as yet compensated but perhaps approaching decompensation. We have explored the possibility that the glucosuric response to a standard glucocorticoid stress might be applicable to the evaluation of insulin reserve in a manner sufficiently practical to be suitable for large-scale application. These studies were begun when a purified glucose-oxidase preparation became available, thus providing for the first time an adequate and specific method for the determination of small amounts of glucose in urine. With this procedure the influence of non-specific reducing substances, which may greatly exceed the normal quantity of glucose present in the urine, can be readily eliminated.

Careful studies in well over 100 normal subjects have shown that the 24-hour glucose excretion of normal adults averages 115 mg. and rarely exceeds 200 mg. daily, despite rather wide variation in the dietary carbohydrate intake. With steroid administration (dosage selected for standard test: 50 mg. daily of prednisone) there occurs almost invariably an immediate increase in true glucose excretion on the first day. This may amount to between 200 mg. and 10 g. a day. With continued steroid administration in the non-diabetic subject, the glucose level returns to normal by the second or third day (Fig. 17), whereas the diabetic patient is unable to compensate for the added metabolic stress and continues to excrete increasing quantities of glucose throughout the period of steroid therapy (Fig. 18). It would seem reasonable to assume, therefore, that increased insulin secretion contributes to the metabolic readjustment leading to a compensation of the initial glucosuria in subjects with normal islet-cell reserve. Preliminary results in a group of students, with and without diabetic family history, suggest that standardized intensive steroid administration for two or more days, combined with measurement of the urinary excretion of true glucose, may unmask latent diabetes, which otherwise escapes detection, and may further provide some measure of the functional reserve of the pancreatic islets in as yet well-compensated states.

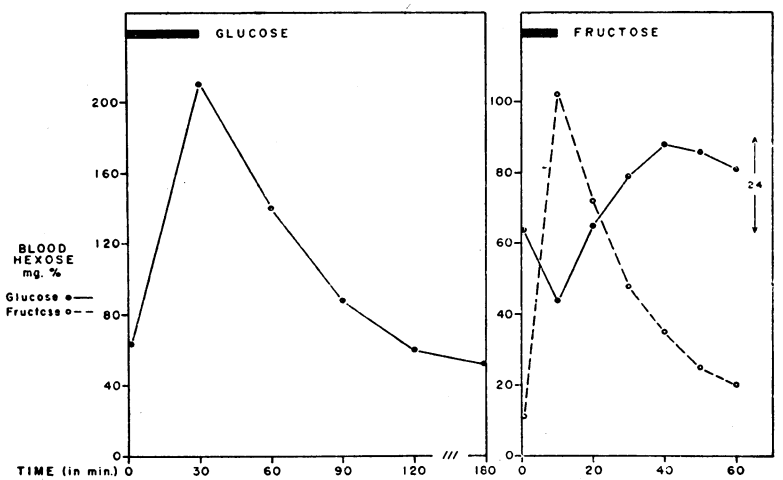


FIG. 15.—Intravenous glucose- and fructose-tolerance tests in a woman (F. B.) with Cushing's syndrome (glucose: 0.5 g./kg./30 minutes; fructose: 0.5 g./kg./10 minutes).

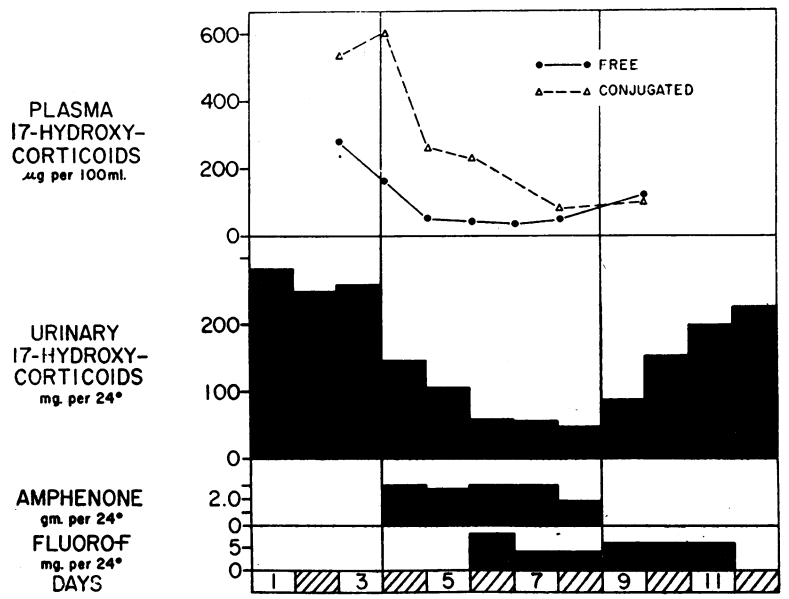


FIG. 16.—17-Hydroxycorticoids in urine and plasma during amphenone Rx in a woman (S. D.) aged 41 with adrenal cortical carcinoma. (From Thorn, Renold, Goldfien, Nelson, Reddy, and Hertz, 1956.)

### Conclusion

The adrenal cortex influences to a major degree the metabolism of carbohydrate, protein, and fat by the mammalian organism. These effects are mediated by adrenal cortical steroids which have, in common with other biologically active steroids secreted by the adrenal cortex, a 21-carbon chain, the unsaturation in ring A, and oxygen substitutions in position 3, 20, and 21. In addition, the most specific structural requirements relating to intermediary metabolic activity of naturally occurring corticoids appear to be the oxygen substitutions in positions 11 and 17. According to our present knowledge, the main carrier of this activity in man is hydrocortisone (cortisol).

The effects of adrenal cortical steroids on intermediary metabolism have been studied in isolated tissues, in intact experimental animals, and in man. In man these studies have included observations made after the administration of exogenous steroids as well as the analysis of

the syndrome resulting from excess or absence of endogenous glucocorticoids—that is, Cushing's syndrome and Addison's disease. *Increased hepatic gluconeogenesis* is the most readily demonstrated metabolic effect of these steroids, and has been established in isolated liver as well as in the intact animal and human organism. The time lapse between the administration of the hormones and the first appearance of evidence suggesting increased gluconeogenesis is approximately one to three hours, and is the same in the intact human organism and in tissues isolated from treated animals. In experimental animals, corticosteroids also *inhibit the synthesis of long-chain fatty acids* by liver and, to a less degree, by adipose tissue. In addition a direct effect upon the *catabolism of body protein* can be demonstrated in the liverless organism. The occurrence of marked protein depletion in patients with Cushing's syndrome at a time when glucose tolerance is still normal or affected only to a minor degree suggests that the latter effect is independent of the effect on hepatic gluconeogenesis and that it may be closer to the primary site of corticosteroid action.

Although our understanding of the effects of adrenal-cortical steroids on intermediary metabolism has been improved we are as yet unable to assign discrete metabolic sites to any of these effects, let alone suggest a primary site of corticosteroid action which could account for all their known metabolic effects.

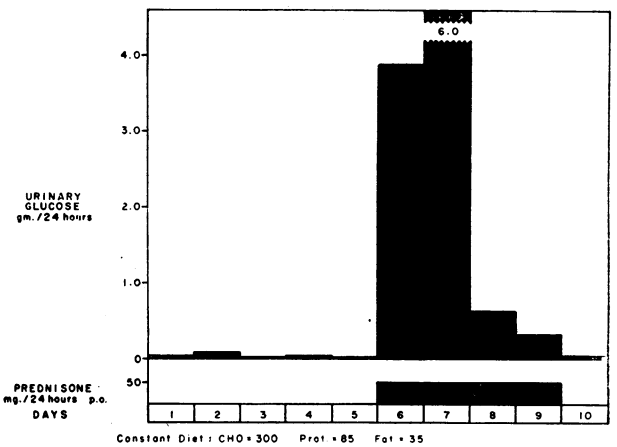


FIG. 17.—Effect of prednisone administration on urinary excretion of true glucose by a non-diabetic woman (H. W.) aged 22.

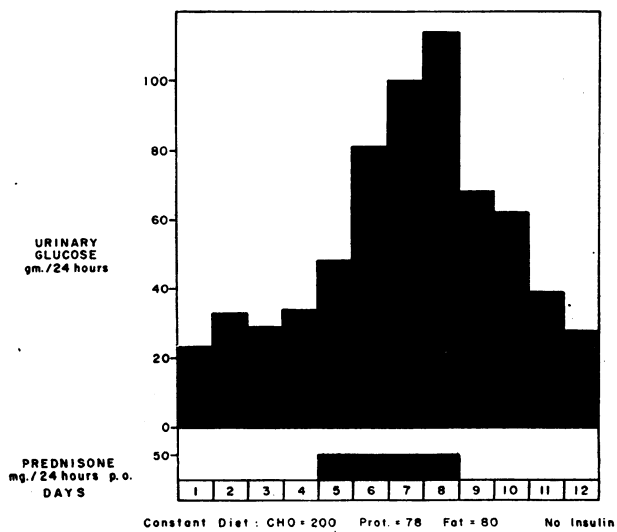


FIG. 18.—Effect of prednisone administration on urinary excretion of true glucose in a woman (E. L.) aged 65 with diabetes mellitus.

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