VITAMINS & HORMONES

HARRIS R. S.

VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

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Preface

It is with pleasure that the editors present this, the twenty-third volume of *Vitamins and Hormones.*

When the first volume of *Vitamins and Hormones* was published in 1943, it was a generally held view that hormones acted by modulating specific enzyme reactions. Undoubtedly, the endocrinologists were much influenced by the remarkable success of the biochemists in establishing the mechanism of action of the vitamins (i.e., as cofactors for enzyme function). So general was the view that hormones were a species of "endogenous vitamins" elaborated by special glands that it seemed logical to have a single serial publication devoted to both hormones and vitamins. It is a source of pride that *Vitamins and Hormones* have proved sufficiently sturdy to survive what now seems a marriage of inconvenience.

The authors whose reviews are contained in the present volume have, as in the past, been encouraged by the editors to prepare selective interpretive reviews that are critical in character and that record their own outlook. The hope is that a review of this type will provide perspective in viewing the growth of a particular field and thus be of value to the scientific reader in general as well as to the specialist. The editors believe that the contributors to this volume have achieved success in the venture, and are indebted to them for their scholarly studies and their insights no less than for the time and effort they have so unselfishly devoted to the task.

October, 1965

R. S. Harris
I. G. Wool
K. A. Loraine

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Adrenal Steroids and Carbohydrate Metabolism[1]

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I. Introduction

A. Scope

During the years from 1930 to 1950 adrenal steroids were shown to produce profound alterations in carbohydrate metabolism in the mammalian organism. Among those taking part in the investigations were Britton, Cori, Engel, Hartman, Houssay, Ingle, Kendall, Levine, Long, Lukens, Russell, Thorn, and Wilhelmi. Reviews covering these investigations have appeared (see Ingle, 1942; Kendall, 1948; Long, 1942, 1953; Russell and Wilhelmi, 1954). Within the past fifteen years isotopic and enzymatic techniques have been applied in an effort to define the alterations in a more satisfactory manner and to establish the mechanism or mechanisms by which they occur (for reviews of some of those studies see Verzár, 1952; Renold *et al.*, 1956; Ashmore, 1959; Renold and Ashmore, 1960; Beck and McGarry, 1962; Long and Smith, 1962; Bush, 1962b; Rosen and Nichol, 1963). A mass of data has accumulated which is subject to varied interpretations. An attempt will be made to survey critically and to correlate a selected portion of the data. A personal estimate of future research needs will be presented. Prime emphasis will be placed upon information obtained through animal experimentation *in vivo* and *in vitro* relating to the nature of the changes in glucose metabolism. Consideration of carbohydrate metabolism will thus be restricted to the metabolism of glucose and its immediate precursors and products. However, because of the intimate relationship between the metabolism of glucose and of protein and lipid, the latter also will enter into the discussion. Only 11-oxygenated adrenal steroids and the synthetic hormones which mirror their major effects on glucose metabolism will be considered.

Important contributions to the field may inadvertently be omitted or may not be emphasized. Despite those inadequacies, it is hoped that this review will help to construct a more meaningful foundation for future studies.

B. Limitations and Definitions

The selection of a single set of experimental conditions for the study of the effects of adrenal steroids on carbohydrate metabolism would have been unfortunate. However, the almost limitless number of conditions employed makes comparisons particularly difficult. The limitations have been realized and investigators have usually carefully defined the area of their study. In particular, species selection, experimental preparation, dosage, specificity, and duration of hormone administration must be emphasized. Bush (1962a) has detailed the difficulties in assigning physiological relevance to effects of adrenal steroids observed on cells. In this review experimental conditions

cannot be enumerated in detail, but the major limitations of individual studies will be discussed.

Terms must also be clearly defined. Thus, gluconeogenesis is defined as synthesis of glucose. Glycogen is considered a storage form of glucose and therefore must be considered in measurements of glucose synthesis. An increase in glucose in a system under observation with a corresponding decrease in glycogen cannot then be considered gluconeogenesis. When isotopes are employed care in interpretation of results is necessary. Isotopes allow measurements of incorporation, but these cannot be equated with synthesis (Landau, 1960). The effects of changes in pool sizes and their rate of turnover, exchange reactions, transient and steady states, must all be considered in the evaluation of isotopic data.

II. Steroids and Glucose Balance in the Intact Animal

In most of the mammalian species examined, the administration of adrenal glucocorticoids leads to an increase in the blood glucose concentration (the dog is an exception; see de Bodo and Altszuler, 1958). Sprague (1951), Conn (1953), Thorn *et al.* (1957), and Fajans (1961) have reviewed the results obtained in man. Increases in the glycogen content of rat and mouse liver and to a much smaller extent, when at all, of rat and mouse muscle glycogen have also been observed (Long *et al.*, 1940). Administered glucocorticoid increases blood glucose concentration and liver carbohydrate

TABLE I

Glucose and Glycogen Content in Fasted Adrenalectomized Rats Injected with Cortisol[a,b]

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

[a] From Long *et al.* (1960b).

[b] All values are means in milligrams per 100 gm body weight. Dose was 10 mg subcutaneously. Glucose is calculated from the blood glucose concentration assuming a glucose space of 25%; muscle glycogen assuming a muscle mass of 50% of body weight.

[c] Not significantly different from controls.

content in the guinea pig, but the responses are smaller and occur more slowly than in the rat (Azuma and Eisenstein, 1964).

It has generally been concluded that adrenal steroids increase carbohydrate content when administered to an animal. In the strictest sense carbohydrate content of the entire carcass of the animal would have to be determined to justify this conclusion. In some tissues, content has been estimated as glucose, following preliminary treatment of homogenates to hydrolyze polysaccharides and phosphorylated sugars. Usually carbohydrate content has meant glucose and glycogen content as measured by more or less specific analytical procedures. The data of Long *et al.* (1960b) illustrate the changes in glucose and glycogen content, as a function of time, following the administration of cortisol to adrenalectomized rats (Table I). The most dramatic increase is in liver glycogen, although a significant increlase in blood glucose concentration occurs prior to that in glycogen (simiar data have been obtained by Munck and Koritz, 1962) (see Fig. 6).

Increased carbohydrate content after steroid administration to intact or adrenalectomized animals appears to be well established, but the mechanism by which it occurs remains in doubt. Increased content can be consequent to either increased glucose synthesis, i.e., increased gluconeogenesis, or to decreased utilization, or both. Evidence considered to support all three of these possibilities has accumulated.

A. Glucose Production

The paucity of measurements of hepatic glucose production in animals receiving steroids is surprising in view of the several techniques that have been developed for its measurement (see Steele *et al.*, 1956; Dunn *et al.*, 1957; Madison and Unger, 1958; Shoemaker *et al.*, 1959). The techniques have been briefly reviewed by Randle and Morgan (1962). The assumptions inherent in the isotopic techniques have been summarized by Wrenshall and Hetenyi (1962). The term production rather than gluconeogenesis is used here, since none of the techniques actually measure the rate of formation of new glucose, as defined, unless changes in liver glycogen content are assessed. In the techniques employing nonisotopic glucose, even when changes in glycogen content are encompassed, net balance across the liver rather than the synthesis of new glucose is measured (Madison *et al.*, 1963).

When the effect of steroids on glucose production is discussed, reference is frequently made to the ingenious studies of Welt *et al.* (1952). Rats previously maintained on a medium carbohydrate diet were fasted 24 hours and anesthetized; then a continuous infusion of C^{14}-labeled glucose was begun in a quantity sufficient to produce glycosuria. From the sixth to the twelfth hour of infusion, a plateau in the specific activity of urinary

glucose was observed; and from this and the specific activity of the infused glucose, the extent of endogenous dilution, and hence glucose production, was estimated. In a series of normal rats production was 6.8 mg/100 gm body weight per hour and in a series of rats injected with 5 mg of cortisone acetate, twice daily for 6–7 days, the figure was 46.8 mg/100 gm/hr. In the normal rat 132 mg/100 gm/hr of glucose-C^{14} was infused to produce glycosuria, and in the cortisone-treated rat 35 mg/100 gm/hr. The rats receiving cortisone, on the day prior to fasting, excreted an average of 6.3 gm of glucose and were therefore presumably hyperglycemic. The theoretical basis for these experiments and the procedures employed appear sound, although some limitations in experimental design exist (see the discussion of Boutwell and Chiang, 1954). The effect of administering the same glucose load to the cortisone-treated as the normal rats, was examined. Glucose production was then estimated at 61 mg/100 gm body weight per hour. Since only production from nonlabeled carbon is measured by this technique, recycling, i.e., glucose formation from C^{14}-labeled products formed from glucose-C^{14}, could not be estimated. The authors concluded, as has frequently been stated, that cortisone administration resulted in an approximately sevenfold, i.e., 46.8/6.8, increase above normal in the rate of gluconeogenesis. Actually, these experiments provide no information as to what change in glucose production from normal occurs in the rat on administration of cortisone. Production in the normal, as measured in these experiments, represents production in a rat given glucose to the point of glycosuria. In the dog, elevation of glucose concentration to the point of glycosuria would probably result in a cessation of hepatic glucose production; and indeed, in the dog fed a high carbohydrate diet, hepatic production of glucose ceases with only a very small increase in blood glucose concentration (Combes *et al.*, 1961; Landau *et al.*, 1961). In the cortisone-treated rat, since glycosuria was present prior to glucose administration, production would presumably be less dependent upon the glucose load. While quantitative comparisons are difficult, there is other evidence to indicate that glucose production in the normal rat is greater than 6.8 mg/100 gm/hr. Thus, Russell (1942) found that the eviscerated rat, maintained on a normal diet prior to evisceration, required 13.5 mg/100 gm/hr of glucose to maintain a normal blood glucose concentration. This may be a minimum value in view of the possible decrease in glucose tolerance that accompanies surgery, the suggestive evidence of the need of the liver to prevent impaired glucose tolerance (Coen *et al.*, 1961), and the possibility that a basal secretion of insulin must be present to achieve a measure of basal glucose utilization. In isotope studies as those of Welt *et al.* (1952), production of glucose by the whole body is measured and the kidney as well as the liver can participate in glucose production (Krebs, 1963, 1964)

In the dog as much as 13% of glucose production may be attributable to the kidney (McCann and Jude, 1958).

The rate of decline in specific activity of glucose after a single injection of glucose-C^{14} can be employed to measure hepatic glucose production (Dunn *et al.*, 1957). Figure 1 presents the graph of Ashmore *et al.* (1961) illustrating the data for rats fasted 18 hours and receiving control or cortisol injections 3 hours prior to the administration of glucose-C^{14}. As noted by Ashmore *et al.* (1961), if glucose production from unlabeled carbon precursors were increased by cortisol administration, the rate of decline of

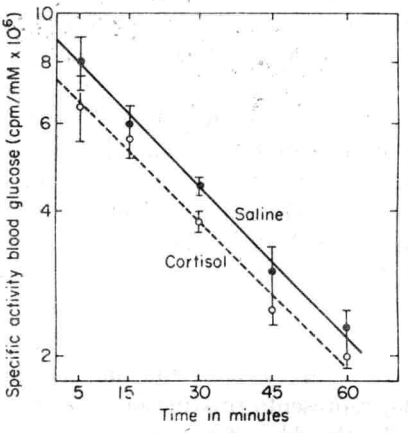

FIG. 1. Rate of decline of specific activity of blood glucose-C^{14} in cortisol-treated and control rats. Log specific activity of blood glucose has been plotted as a function of time after intravenous injection of 5 μc of glucose-6-C^{14} into cortisol- and saline-injected rats. Each curve represents the mean of three experiments, and total spread of points is indicated. From Ashmore *et al.* (1961).

blood glucose specific activity in the cortisol-treated rats should have been increased. The slopes are remarkably similar. Assuming that the blood glucose concentration in both groups of animals was about 100 mg/100 ml and did not change during the third to fourth hour, and assuming a glucose space 30% of body weight, then glucose turnover in both normal and cortisol-treated rats as estimated by the method of Dunn *et al.* (1957), would be about 42 mg/100 gm body weight per hour.[2] The change in blood glucose concentration upon cortisol administration observed by Moriwaki and Landau (1963), under conditions similar to those of Ashmore *et al.* (1961), would not significantly alter the calculation. Von Holt *et al.* (1961) estimated by another method, from the decline in blood glucose specific

[2] Rate of turnover, $R = (2.3 \times 100)/1 \times \log 8/2 = 139$ mg/100 ml/hr; $139 \times 0.3 = 42$ mg/100 gm/hr.

activity in the normal rat fasted 15 hours, a glucose turnover of 62 mg/100 gm/hr.

Ashmore *et al.* (1961) administered glucose-6-C^{14} in an attempt to obtain a measure of recycling. In recycling, the glucose-6-C^{14} metabolized in muscle yields lactate-3-C^{14} (Fig. 2) and resynthesis of glucose from the lactate by the liver[3] yields glucose-1,6-C^{14}. Actually, because of random-

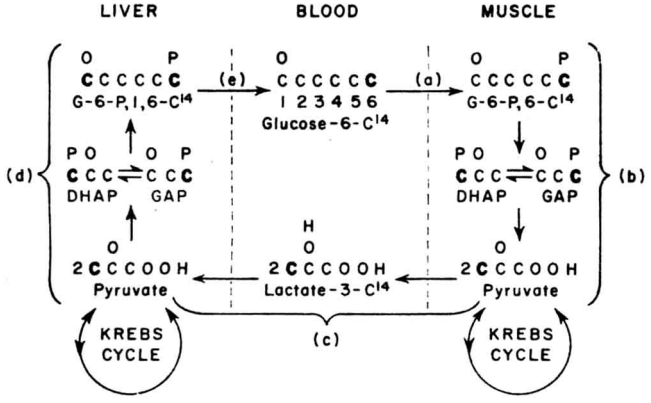

FIG. 2. Recycling of glucose carbon: (a) glucose-6-C^{14} is phosphorylated to glucose-6-P,6-C^{14} by muscle; (b) glycolysis of a molecule of the phosphate yields 2 molecules of pyruvate-3-C^{14} with dihydroxyacetone-P (DHAP) and glyceraldehyde-3-P (GAP) as intermediates; (c) lactate-3-C^{14} formed in the muscle from pyruvate enters the liver (or kidney) from the blood and is oxidized to pyruvate; (d) the carbons of the pyruvate then form glucose-6-P,1,6-C^{14} via the condensation of DHAP with GAP; (e) and on hydrolysis glucose-1,6-C^{14} is released into the blood. Note that in the formation and breakdown of glucose-6-P, DHAP contains carbons 1, 2, and 3 and GAP contains carbons 4, 5, and 6. The carbons of pyruvate in both liver and muscle are randomized in the Krebs cycle, but if equilibration of DHAP and GAP is complete one-half the C^{14} from the pyruvate, and hence the glucose-6-C^{14}, will appear in carbons 4, 5, and 6. The activity in carbon 1 will equal that in carbon 6, 2 equal that in 5, and 3 equal that in 4. If equilibration is incomplete, more C^{14} appears in carbons 4, 5, and 6 than 1, 2, and 3.

ization of C^{14} in the Krebs cycle, C^{14} would be incorporated into all three carbons of lactate, but nevertheless the C^{14} in carbons 1, 2, and 3 relative to carbons 4, 5, and 6 of glucose isolated from the blood, would be a measure of the extent of recycling. Formation of glucose-1,6-C^{14} in the liver by cleavage of the glucose-6-C^{14} to dihydroxyacetone-P and glyceraldehyde-3-P and then resynthesis is considered to be negligible. Isotopic equilibration of the triose phosphates is assumed complete. If equilibration did not occur, incorporation into carbons 4, 5, and 6 relative to carbons 1, 2, and 3

[3] Metabolism to lactate and resynthesis to glucose of glycogen is the well-known "Cori cycle."

of the glucose would be favored. Under the experimental conditions, equilibration is probably sufficient to justify the assumption that it is complete (Landau *et al.*, 1955). C^{14} in carbons 1, 2, and 3 of the blood glucose of the normal and cortisol-treated rats 1 hour after injection of glucose-6-C^{14} in the experiment of Ashmore *et al.* (1961) was less than 5% of the total activity. Since one-half of the C^{14} incorporated into glucose during recycling is in carbons 4, 5, and 6, Ashmore (1960) concluded that less than 10% of the C^{14} in the blood glucose could be attributed to the Cori cycle. However, this is not a measure of the glucose formation by recycling since a steady state probably was not achieved. The lactate-C^{14} would be diluted by the pools it traversed so that incorporation to a small extent could indicate considerable formation from lactate. This reservation is emphasized by an experiment in glucagon-treated, fasted normal rats (Friedmann *et al.*, 1963; see their Table I), where the proportion of radioactivity in carbon 6 relative to carbon 1 through 5 of blood glucose reached a constant percentage of specific activity of about 15% within 2 hours after administration of glucose-1-C^{14}, but was only 100 $(6.2 \pm 0.6)/(151 \pm 5) = 4.2\%$ after 1 hour. Von Holt *et al.* (1961) examining the randomization of C^{14} of glucose-6-C^{14}, estimate that 12% of blood glucose originates from resynthesis of degradation products of glucose in fed rats and 50% in rats fasted 15 hours. Von Holt and Fister (1964), from the randomization of C^{14} of glucose-6-C^{14} during its incorporation into glycogen of fasting cortisol-treated rats, have concluded that the incorporation occurs to a large extent after degradation of the glucose to three carbon compounds and resynthesis.

As noted previously, increases in blood glucose concentration are produced less readily by glucocorticoids in dogs than other species (de Bodo and Altszuler, 1958). In the hypophysectomized dog, given a constant infusion of glucose-C^{14}, a decrease in glucose output was found and production returned to normal after several days of cortisol administration. Glucose output was reduced somewhat below normal in adrenalectomized dogs [$(3.46 \pm 0.11$ gm/m^2/hr compared to 4.01 ± 0.20 gm/m^2/hr (Steele *et al.*, 1959)]. Again recycling is not included in these estimates.

Lecocq *et al.* (1964) measured the effect of acute cortisol administration on hepatic glucose output in dogs with portacaval shunts. This preparation, because of hemodynamic changes, may not be representative of normal metabolism. After 30–90 minutes of cortisol infusion, following a priming dose, a 33% decrease in hepatic glucose output was observed. Since outputs were measured directly, recycling was included in the estimate. A similar estimate was obtained from the difference in the blood glucose specific activity across the liver during the infusion of glucose-C^{14} and the total amount of glucose released from the liver, calculated as the product of the hepatic vein glucose concentration and blood flow. The specific activity of the

hepatic artery glucose, though unfortunately not reported, would be expected to have increased during the glucose-C^{14} infusion, if the release of unlabeled glucose decreased. Ninomiya *et al.* (1964) also report a decrease in glucose production on infusion of cortisol into dogs as measured by a method using successive tracer injections. Daily injections of methylprednisolone are estimated to have increased the rate of glucose production by 37% in 1 week and 83% in 2 weeks with an increase in the glucose pool of 36% and in the glucose space of 30%. The kinetic analysis employed for these estimates may not give an accurate measure of endogenous glucose production since it involves a number of assumptions as yet unproved to hold for the intact animal. Perkoff *et al.* (1963) reported an increase in glucose tolerance in the first few hours following cortisol administration to man and, in preliminary experiments, described a decrease in splanchnic glucose output. Those observations would accord with an acute decrease in hepatic glucose output following cortisol administration.

Before completion of this section, we should consider the changes in hepatic glucose output that would be necessary to account for the incresaes in carbohydrate content that have been observed. For example, if glucose output were known in the adrenalectomized rat, from the experiments of Long *et al.* (1960b) (Table I) one could calculate how much the output would have had to increase to provide during a 4-hour period the difference between 212 and 239 mg of glucose per 100 gm body weight. In these and the experiments of Ashmore *et al.* (1961), where liver glycogen content increased, assuming an output of 13–62 mg/100 gm/hr, only a small, and barely detectable, increase in hepatic glucose output could account for the additional carbohydrate.

B. Glucose Utilization

In the strict sense disappearance of glucose from the blood should not be called utilization, although under most experimental conditions the assumption that uptake is an adequate measure of utilization is probably justified.

Ingle *et al.* (1953) found that, on infusion of glucose and insulin into eviscerated rats given adrenal cortical extract for 24 hours, terminal blood glucose concentrations were higher than in control rats not given extract. Cortisone and cortisol were relatively ineffective. Bondy *et al.* (1954) observed no difference in glucose concentration at the end of periods up to 6 hours between eviscerated rats given adrenal extract and saline-injected controls. When insulin was also injected, glucose tolerance was greater in the group receiving the extract. In an adrenalectomized group maintained in an environment at 26°–27°C, cortisone given for 24 hours depressed glucose tolerance. The dose of cortisone was larger than that employed by