doses of aldosterone which stimulate sodium transport. Both the stimulation of RNA-synthesis and sodium transport are blocked by actinomycin D, inhibitor of DNA directed RNA-synthesis.

This investigation has yielded further evidence that Karlson's theory may be applicable to the antinatriuretic action of aldosterone. The level of RNA in the renal microsomal fraction was found to be elevated in rats treated with aldosterone. If the elevation of microsomal RNA involved in initiating the antinatriuretic action of aldosterone, an increase in RNA should be detectable before or at the beginning of the antinatriuretic action. The first significant change in sodium excretion was noted in the 2-hour groups, whereas the first significant increase in the level of RNA was noted in the 1½-hour group. This would appear to indicate that aldosterone stimulated RNA-synthesis prior to affecting sodium transport. However, there is some indication that sodium transport may have been affected in the 1½-hour group (Table I). The urinary collection period here included urine from the first hour, a period in which no significant change occurred, and this may have masked a small change which occurred during the last half hour of the period. Thus while the data do not indicate clearly whether or not the increase in RNA occurred prior to or at the same time as the antinatriuretic action, they do indicate that the antinatriuretic action can not be detected prior to an increase in the level of RNA.

Summary. Using the determination of the incorporation of orotic acid-6-C^{14} into microsomal RNA-synthesis, *de novo,* it was found that aldosterone increased the microsomal level of RNA at about the time it enhanced sodium reabsorption. The lack of an effect of aldosterone on sodium reabsorption before an increase in RNA-synthesis could be detected is consistent with the hypothesis that this hormone mediates its antinatriuretic action, *in vivo, via* DNA directed synthesis of RNA.

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Effect of Blood Sugar Levels on Glycogen Content of Hypophysis and Adrenal Glands.* (31835)

PADMAKAR K. DIXIT AND ARNOLD LAZAROW
Department of Anatomy, University of Minnesota, Minneapolis

We have reported(1) increased glycogen content in hypophysis and adrenal glands of alloxan-diabetic rats. We have also reported *(loc. cit.)* the effect of experimentally induced changes in the blood sugar level on the corresponding changes in the glycogen content of these two glands.

In the present study, using microchemical methods, we have determined the effect of glucose and insulin administration on the glycogen content of adrenal cortex and medulla and also adeno- and neurohypophysis in the diabetic and normal rats.

Materials and methods. Young male rats of

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the Holtzman strain (weighing 180-200 g) were fasted overnight and injected intravenously with alloxan [40 mg per kg body weight(2)]. Diabetic rats used exhibited polydipsia, polyuria and hyperglycemia (blood sugar greater than 300 mg per 100 ml). Subdiabetic rats were produced by injecting alloxan in doses of 30 to 35 mg per kg body weight and selecting those rats which had normal fasting blood sugar level and abnormal glucose tolerance [a diabetic index (I_D) greater than 2.0(3)].

Hyperglycemia was induced in normal and subdiabetic rats by intraperitoneal glucose injections (4 g per kg body weight) every hour, while hypoglycemia was induced in the diabetic rats by injecting subcutaneously with insulin (50 units per kg body weight as a single dose). The animals were killed in a cold room by decapitation. The hypophysis and adrenal glands were rapidly removed and placed in ice-cold absolute ethanol and stored at about 5°C for 48 hours.† The component portions of the endocrine glands were separated using a dissection microscope (at 20 to 30 × magnification). The hypophyseal lobes were readily teased apart with a scalpel. The adrenal glands were cut into thin slices and after removing the capsule, the medulla and the cortex were separated.

The tissue samples were placed in microtest tubes and dried in a vacuum desiccator for 1 hour. The dried tissue samples were weighed on the Cahn electrobalance and then digested in hot 30% KOH. The glycogen was precipitated and washed with alcohol(4); after hydrolysis, glucose was determined by a microadaptation of Nelson(5) method. The glycogen values were expressed as μg of glucose equivalence per 100 mg dry weight of tissue.

In some experiments, the effect of an injection of adrenocorticotrophic hormone (ACTH) on the normal and diabetic rat adrenal cortical glycogen was studied. The hormone (purchased from the Upjohn Co., Kalamazoo) was dissolved in saline and injected intraperitoneally in a dose of 1 unit per 100 g body weight of the animal. The animals were decapitated and the adrenal glands removed for subsequent analysis.

Results. In the normal rat, the neurohypophysis contains approximately 50% more glycogen than does the adenohypophysis (439 *vs* 298 μg per 100 mg dry weight) (Fig. 1). In the diabetic rat, the glycogen content of the neurohypophysis is increased over 4-fold as compared to the normals. By contrast, the corresponding change in the adenohypophysis was about 2-fold. The ratio of glycogen content in neuro- to adenohypophysis in the normal animal was 1.48, whereas in the diabetic rat it was 2.73.

In normal rats, the glycogen content of the adrenal medulla was twice that of the cortex (808 *vs* 344 μg per 100 mg dry weight of the tissue). In diabetic rats, the glycogen content of the adrenal medulla was increased 2-fold, whereas that in the cortex was not significantly altered (Fig. 1).

When hyperglycemia was induced in normal and subdiabetic rats, the adrenal medullary glycogen was unchanged during the following 4 hours, whereas there was a dramatic decrease in the cortical glycogen; at 2 and 4 hours it was 20% of that present initially (Fig. 2).

The glycogen content of both the hypophyseal lobes of normal and subdiabetic rats was slightly elevated at 2 hours (approximately 30 to 50%, respectively) following glucose administration. At 4 hours, the values were either normal or slightly (though not significantly) elevated (Fig. 2).

The administration of a single massive dose of insulin to diabetic rats decreased the glycogen content of all the component lobes of the adrenal and hypophyseal glands (Fig. 3). In the neuro- and adenohypophyseal lobes, the decrease was gradual; at the end of 4 hours following insulin administration, the glycogen content was decreased to approximately 52 to 54% of the initial levels. The change in the adrenal cortex is, however, greater and more rapid than that in the medulla; a 35% decrease in the cortical glycogen was observed at 1 hour while at the end of 4 hours the value was decreased to 40% of the initial levels (Fig. 3).

Two hours following the injection of ACTH

† No glycogen loss could be detected during this period of shortage.

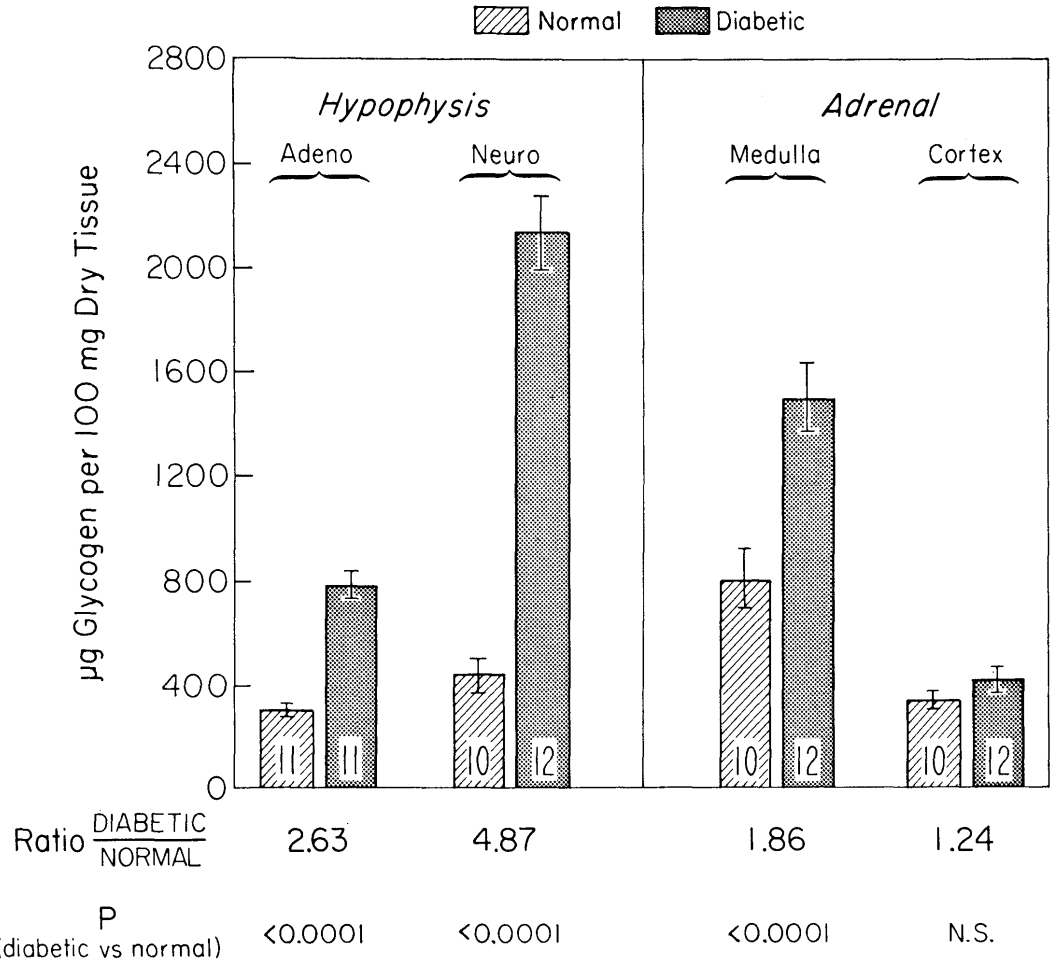
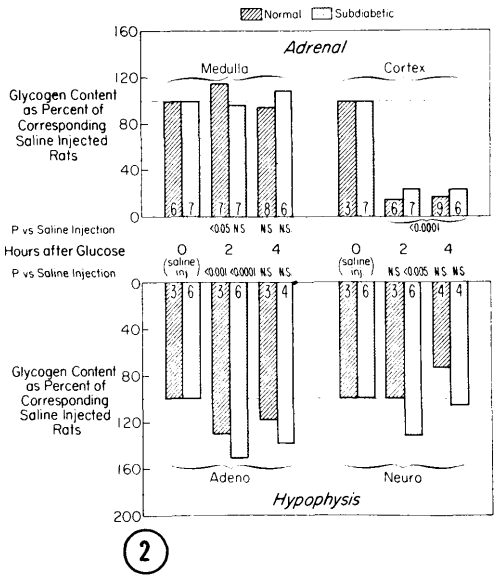


FIG. 1. Glycogen content of pituitary (adenohypophysis and neurohypophysis) and adrenal (cortex and medulla) in normal and diabetic rats. The number at bottom of each bar denotes number of observations, while vertical line at top of each bar represents standard error of mean. Average blood sugar of normal rats was 118 mg per 100 ml; that of diabetic rats was 400 mg per 100 ml.

there was a decrease in glycogen content of normal rat adrenal cortex to about 73% of the initial levels, and similar decrease was observed at 4 hours following the hormone injection. Due to the small number of samples analyzed, this decrease was not found to be statistically significant, however. Noble and Papageorge(6) and Greenberg and Glick(7) have earlier reported a decrease in the normal rat adrenal cortical glycogen following ACTH injection. The glycogen content of the diabetic rat adrenal cortex was not altered at 2 hours although a very slight (*i.e.*, 13%) decrease was observed at 4 hours, which was not statistically significant.

Discussion. The present studies indicate that whereas the glycogen content of both the adeno- and neurohypophysis increased in alloxan-diabetes, the change is much greater in the neurohypophysis.

The adrenal medulla contains proportionately greater amounts of glycogen than the cortex, thus our microchemical studies confirmed the histochemical observations of Hunt and Hunt(8) who reported that glycogen is more concentrated in the rat adrenal medulla than in the cortex. Of the two component



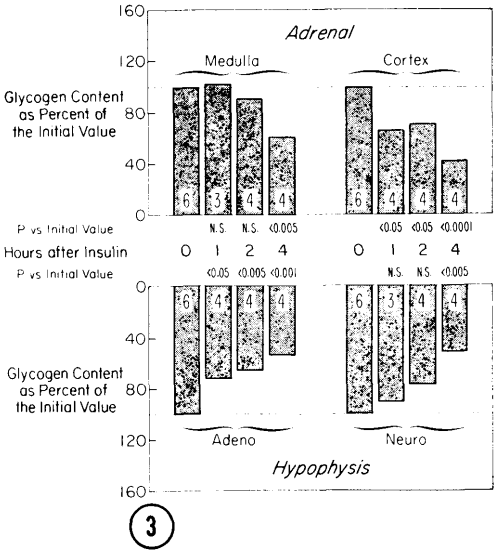


FIG. 2. Effect of glucose injection (in normal and subdiabetic rats): glycogen content of adrenal cortex, medulla, adeno and neurohypophysis. Blood sugar levels of normal rats were 128, 664 and >846 mg% at 0, 2 and 4 hours, respectively, after glucose injections; corresponding values in the subdiabetic rats were 137, 798 and >846 mg%.

FIG. 3. Effect of insulin injection (50 U/kg body weight) injected as a single dose: glycogen content of adrenal cortex, medulla, adeno and neurohypophysis. Average blood sugar levels of diabetic rats at 0, 1, 2 and 4 hours after insulin injection were 450, 316, 261 and 109 mg%, respectively.

parts of the hypophysis, the neurohypophysis contained somewhat greater amounts of glycogen.

The glycogen content of the adrenal cortex is unaltered in diabetes, although that of the adrenal medulla is significantly increased and may account for the previously observed increase in the glycogen content of the whole gland(1).

Hyperglycemia (glucose injection) and lowering of blood sugar level (following insulin administration) produced a corresponding increase and decrease respectively in the glycogen content of the adeno- and neurohypophysis. Secretion of growth hormone by adenohypophyseal acidophilic cells is markedly stimulated by a rapid fall in blood sugar level and suppressed by glucose administration(9). It is possible that the decrease in the glycogen content of the adenohypophysis following insulin injection may be related to the secretion of growth hormone.

A rapid decrease in the glycogen content of the adenohypophysis following insulin administration may also perhaps be associated with an increased synthesis of adrenocorticotrophic hormone (ACTH) by the adenohypophysis in response to "stress" as suggested by Jacobovitz and Marks(10). The release of ACTH by the adenohypophysis is stated to be dependent upon the ability of this gland to synthesize this hormone *de novo*(11), hence it would be interesting to determine the changes in the plasma ACTH levels following hyper- and particularly hypoglycemia.

In contrast to the adeno- and neurohypophysis, the glycogen content of the adrenal cortex is decreased markedly by both hyperglycemia (glucose injection) and a decrease in blood sugar levels (insulin administration). Perhaps these treatments stimulate ACTH and epinephrine secretion respectively. Noble and Papageorge(6) have reported that ACTH and epinephrine administration caused a decrease in glycogen content of the normal rat adrenal cortex to approximately 65% of that of the untreated controls. ACTH, in fact, produced significant decreases in the glycogen content of fasciculata and

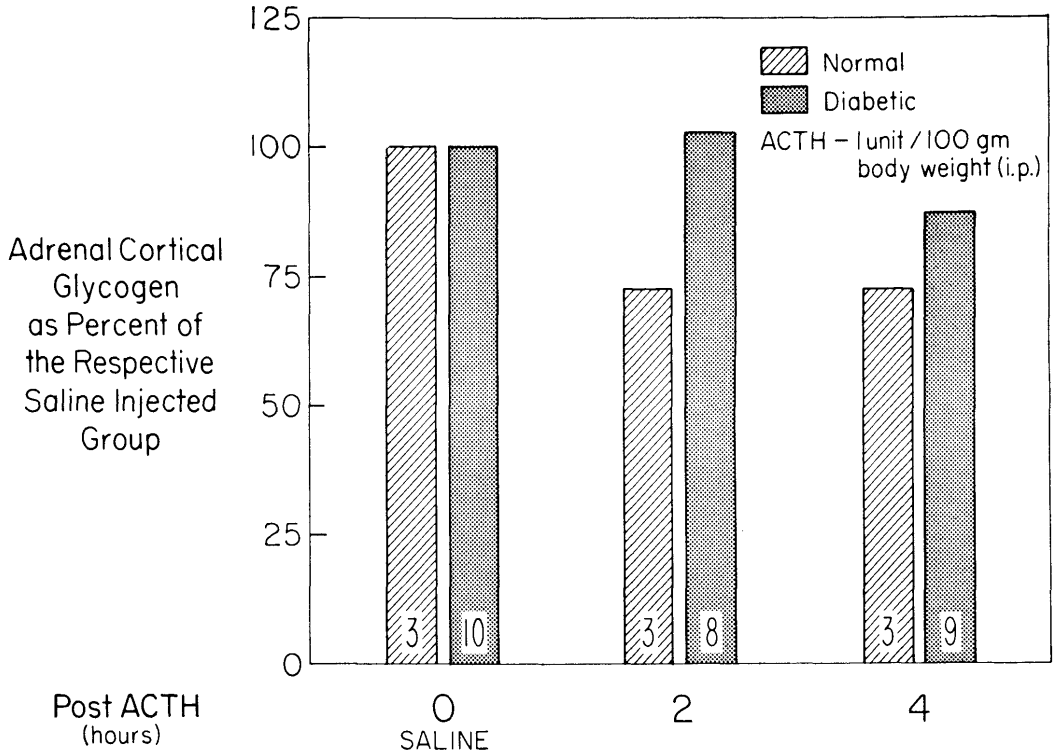


FIG. 4. Effect of ACTH (Adrenocorticotrophic hormone) injection on adrenal cortical glycogen of normal and diabetic rats. There was no significant change in blood sugar levels of either normal or diabetic rats following ACTH injection. The 27% decrease in glycogen content of the normal rat adrenal cortex at 2 and 4 hours after ACTH injection was not found to be statistically significant due to the small number of samples analyzed. The slight decrease (13%) in glycogen content of the diabetic rat adrenal cortex at 4 hours following ACTH administration was not statistically significant.

glomerulosa of the cortex, the decreases in the reticularis and medulla were slight(7). We have also observed a decrease (to 73% of the initial value) in glycogen content of the normal rat adrenal cortex (Fig. 4) following ACTH administration, although no such effect was observed in the diabetic animals. The decreased adrenal cortical glycogen in the normal rat may perhaps be due to the accumulation of 3′5′ adenosine monophosphate (3′, 5′, AMP), resulting in the activation of the enzyme phosphorylase, thus accelerating the breakdown of glycogen(12). The reason for the decreased response of the diabetic rat adrenal cortex to ACTH administration is not clear and needs further investigation.

The glycogen content of adrenal medulla is less susceptible to changes in blood sugar levels. Hyperglycemia (glucose injection) caused no appreciable alteration in medullary glycogen of normal and subdiabetic rats at 2 and 4 hours. A decrease in blood sugar to normal levels at about 4 hours (following insulin administration to diabetic rat) caused a 40% decrease in medullary glycogen content. Insulin-induced hypoglycemia is reported to cause an increased urinary excretion(13) and also an elevation of plasma epinephrine in patients(14). It is, therefore, tempting to speculate that decrease in glycogen may be related to a hormonal secretion by adrenal medulla.

Summary. Glycogen content of the components of hypophysis and adrenal glands is determined. In the normal rat, neurohypophysis contains more glycogen than does the adenohypophysis (439 *vs* 298 μg per 100 mg dry weight of tissue). In the alloxan-diabetic animals, the medullary glycogen

content was greatly elevated. Hyperglycemia produced by glucose injection caused little change in the glycogen content of neuro- and adenohypophysis and also in the adrenal medulla, but significantly decreased that of the adrenal cortex. Fall in blood sugar levels (insulin injection) decreases the glycogen content of all the components of the hypophysis and adrenal glands. Perhaps the changes in glycogen content are related to hormonal synthesis and secretion. Although injection of ACTH causes a 27% decrease in the glycogen content of the normal rat adrenal cortex at 2 and 4 hours, no significant decrease was found in the diabetic animals at these time periods.

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Urea Excretion in the Potassium-Deficient Rat.* (31836)

A. L. FINN† AND L. G. WELT‡

Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, North Carolina

The pathogenesis of the renal concentrating defect accompanying potassium deficiency in mammals(1,2) remains obscure. Although some evidence suggests that there may be a defect in the absorption of sodium from the ascending limb of the loop of Henle(3), it is clear that the countercurrent mechanism is operative in potassium depletion, since early distal tubular fluid is similarly hypotonic in potassium depleted and control animals(4,5). The impairment of urinary concentrating ability could also be due to a decrease in the permeability of the distal tubule and/or collecting duct system, from which, in the presence of antidiuretic hormone, water moves into the interstitium along its osmotic gradient.

Distal tubular permeability has been indirectly evaluated in potassium depletion and appears to be impaired in the dog(6) and in man(7,8), but direct micropuncture studies have effectively ruled out this possibility in the rat under ordinary solute loads(4,5). With regard to the role of the collecting duct, direct micropuncture data are unvailable in the rat but there appears to be no osmotic disequilibrium across the collecting duct of the potassium-depleted hamster(9).

Current concepts of renal physiology indicate that urea plays an important role in the concentrating mechanism(10,11,12,13). Most available evidence suggests that urea is

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† Work done during tenure of a Postdoctoral Fellowship from Nat. Heart Inst. Present address: Depts. of Physiology and Medicine, Yale University School of Med., New Haven, Conn.

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