TABLE I. Renal Arteriovenous Differences in Hemoglobin Concentration Measured as Cyanmethemoglobin. A negative value indicates an arteriovenous increase.

Animal No.	Before diuresis (g %)	During diuresis (g %)
1	.73	.08
2	.73	.23
3	.19	.10
4	.00	.10
5	−.05	−.12
6	.26	.08
7	.94	−.06
Mean	.40	.073
S.E.	±.15	±.035
P from the distribution of t	.05–.02	.10–.05

stab wounds. A polyethylene catheter was also implanted in an external jugular vein. A first pair of renal arterial and venous samples was taken some 10-15 minutes after the laparotomy had been closed and the subject had been heparinized. An infusion of 500 ml of a 10% aqueous solution of mannitol was then given through the catheter in the jugular vein. This required a considerable part of an hour. A second pair of renal arterial and venous samples was taken 20-30 minutes after this infusion had been completed.

Results and discussion. At the time the first pair of blood samples was taken, the flow of urine from the ureteral catheters amounted to only an occasional drop; when the second pair was taken, it had increased to several drops per second. The renal arteriovenous differences in the apparent hemoglobin concentration are given in Table I. The arteriovenous reduction that preceded diuresis diminished during diuresis 0.33 ± 0.15 g% (mean and standard error), for which the value of P from the distribution of t is 0.05-0.02. This would appear to indicate that diuresis tends to eliminate the observed renal arteriovenous reduction in the concentration of whatever is measured as cyanmethemoglobin in accord with the expectation mentioned in the introduction.

Summary. Diuresis tends to eliminate an arteriovenous reduction in the apparent concentration of hemoglobin measured as cyanmethemoglobin.

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Mediation of Aldosterone Induced Anti-Natriuresis *via* RNA-Synthesis *de novo.** (31834)

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Numerous reports have indicated that the effect of aldosterone on sodium transport, both *in vitro* and *in vivo,* is mediated *via* DNA directed synthesis of RNA(1-5). The effect of aldosterone on sodium transport in the isolated toad bladder(1) and the rat kidney(5) was found to be accompanied by elevated levels of intracellular RNA. In both preparations the stimulation of sodium transport and the increase in levels of RNA were blocked by prior administration of actinomycin D(1,5). When the isolated toad bladder was employed to examine these effects chronologically, RNA-synthesis was found to be stimulated 30 minutes prior to any change in sodium transport(2). In this report the effects of aldosterone on RNA-synthesis and renal sodium transport have been investigated, *in vivo,* in the rat as a function of time.

Methods. Orotic acid-6-C^{14} incorporation into RNA. Male rats (Holtzman strain) weighing 95-120 g were adrenalectomized

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TABLE I. A Chronological Comparison of Orotic Acid-6-C^{14} Incorporation into Renal Microsomal RNA and the Onset of Anti-Natriuresis Following Aldosterone Administration.*

	Treatment		Probability level
	Control	Aldosterone	
Sodium excretion (μEq/kg)			
1 hr	740 ± 216 (6)	823 ± 296 (6)	$P > .05$
1.5 "	704 ± 363 (7)	486 ± 109 (5)	$P > .05$
2 "	1891 ± 649 (6)	621 ± 214 (6)	$P < .05$
Microsomal RNA (cpm/mg RNA)			
1 hr	428 ± 120 (6)	396 ± 43 (6)	$P > .05$
1.5 "	497 ± 73 (7)	672 ± 96 (5)	$P < .05$
2 "	818 ± 56 (6)	963 ± 84 (6)	$P < .05$

* Each value is mean ± standard deviation of the number of animals designated in parentheses. Data were analyzed by the Student "t" test.

bilaterally 5 days prior to the experiment and maintained on 0.9% sodium chloride for drinking water and "Purina" laboratory chow. Food was removed on the fifth day. On the sixth day, orotic acid-6-C^{14} (3 μcuries/100 g body weight, specific activity 4.8 mcuries/mmole) was injected intravenously *via* the tail vein. The radioactive tracer was injected in a volume of approximately 0.2 ml to permit visualization of extra-vascular injection. Next, 0.9% sodium chloride solution (17 ml/kg) was administered, subcutaneously. Then the rats were divided into 2 groups and either aldosterone (0.34 μg/kg) or its vehicle was injected subcutaneously. The administration of the 3 injections to each rat took about 2-3 minutes. The urinary collection period was begun after the last injection and urine was then collected for 1, 1½ or 2 hours using the procedure of Kagawa *et al*(6). The injections and times of collection were staggered in order to keep the collection period constant. Because of the number of animals employed, the experiment was performed in 3 parts, the 1-hour study on one day, the 1½-hour study on a second day and the 2-hour study on a third day. Urinary sodium was analyzed with a Coleman flame photometer. At the end of the collection period, kidneys were excised and the microsomal fraction was obtained by the method of Schneider(7) as modified by Hogeboom(8). RNA was extracted and counted as described previously(5).

Data were analyzed using the Student "t" test(9). $P<0.05$ was used as the criterion of significance.

Results. Orotic acid-6-C^{14} incorporation into RNA. Table I shows the effect of aldosterone at various times after administration on the incorporation of orotic acid-6-C^{14} into renal microsomal RNA and on the excretion of sodium. When the aldosterone group was compared to the control group at 60 minutes after treatment, no significant changes were observed in incorporation of orotic acid or excretion of sodium. Ninety minutes after administration of aldosterone the incorporation of the C^{14}-precursor was found to be increased in the microsomal fraction. No difference was observed at this time between groups in regard to sodium excretion. After 120 minutes, a significant anti-natriuresis was observed in addition to a significant increase in incorporation of orotic acid-6-C^{14}.

Discussion. Karlson(10) has postulated that many hormones exert their physiological actions by initiating the formation of new proteins (enzymes). He derived this theory from observations of changes in insect chromosomes. During development (molting and metamorphosis) of these insects, puffs of DNA appear at certain sites along their giant chromosomes. It was found that these puffs are induced by ecdysone, a hormone from this insect's prothoracic gland. Once these puffs were formed, RNA could also be identified in this region. Thus, he proposed that the action of some hormones may be to induce DNA puffing on chromosomes to expose a specific DNA template for RNA-synthesis and that this RNA (messenger RNA) would then migrate to ribosomes located along the endoplasmic reticulum and thus relay information necessary for synthesis of specific proteins.

The evidence accumulated in the past few years suggests that the mechanism proposed by Karlson may be applicable to aldosterone. It has been reported that aldosterone is bound intracellularly and concentrates near or in the nucleus(1). RNA-synthesis, *in vitro* and *in vivo,* has been found to be stimulated by

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)

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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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