10^{-6} in Gey and Gey buffer, complete lysis occurred in 30 minutes and no clot lysis was observed after 24 hours incubation in the lower molar concentrations mentioned above.

Discussion. This study has shown that physiological concentrations of insulin promote D-xylose penetration into intact rat diaphragm preparations *in vitro* and that the relationship between insulin concentration and D-xylose penetration is roughly linear. The response is subject to rather large day-to-day variation and efforts to eliminate such variation have not been successful.

We have demonstrated that the response is specific for insulin when compared with trypsin and chymotrypsin at low molar concentrations. Our observation of a failure of trypsin and chymotrypsin at low concentration to increase the permeability of the diaphragm to D-xylose confirms the point of view(6,7) that the insulin effect upon the penetrability of a non-utilized sugar is specific.

Our findings suggest that D-xylose penetration might be a useful technique for measurement of insulin-like activity if greater precision and reproducibility in the procedure could be obtained.

Summary. Increased D-xylose penetration of the intact rat diaphragm has been demonstrated at insulin concentrations of 50 to 500 microunits per ml, amounts that are within the range of physiological levels. Increased penetrability to D-xylose by preparations containing trypsin and chymotrypsin was observed at relatively high levels of the enzymes but not at molar concentrations equivalent to those of insulin at levels of 125 and 500 microunits per ml. The rates of penetration were approximately linear, but the day-to-day variations of this procedure were too large for a useful bioassay method.

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Stimulation *in vivo* of Renal RNA-Synthesis by Aldosterone.* (30164)

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Karlson(1) has suggested that many hormones act by stimulating the synthesis of specific proteins which in turn are responsible for the physiological actions of the hormones. He has proposed that these hormones act upon chromosomes to expose specific DNA-templates. This results in the synthesis of messenger-RNA which in turn directs the synthesis of protein by the endoplasmic reticulum. Several reports have suggested that aldosterone acts in this manner. Williamson (2) found that actinomycin D, an inhibitor of DNA-directed synthesis of RNA, blocked the antinatruretic action of aldosterone in the rat. Edelman *et al*(3) reported that actinomycin D blocked the action of aldosterone on the transport of sodium by the isolated toad bladder. Crabbé and De Weer(4) also obtained similar results with the isolated toad bladder and skin.

In this report the ability of aldosterone to increase RNA-synthesis *in vivo* has been assessed both by chemical determination of

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TABLE I. Inhibition of Antinatruretic Action of Aldosterone by Actinomycin D.

	Treatment*				Coefficient of variability (%)
	Aldosterone (7)†	Control (5)†	Actinomycin D (5)†	Actinomycin D & aldosterone (7)†	
Sodium excretion (μEq/kg body wt)	33	256	332	333	60

* Any 2 Means underscored by the same line are not significantly different. Any 2 Means not underscored by the same line are significantly different. Test of significance was made by Kramer's modification of Duncan's new multiple range test following analysis of variance using a completely randomized block design.

† Numbers in parentheses represent No. of rats in each column.

RNA as well as by incorporation of uridine 5′-monophosphate H^3 into RNA. Actinomycin D was used to inhibit the synthesis of RNA.

Methods. Male rats (Holtzman strain), weighing 95-120 g were adrenalectomized bilaterally 5 days prior to the experiment and maintained on 0.9% sodium chloride solution for drinking water and "Purina" laboratory chow. Food was removed 24 hours prior to the experiment. On the day of experiment, the rats were divided into 4 groups: 2 groups were given actinomycin D (400 μg/kg), intraperitoneally, and the other 2 groups were given the vehicle. One hour later all rats received uridine 5′-monophosphate H^3 (33 μcuries/100 g, specific activity 100 mcuries/mmole), intravenously, *via* the tail vein. A volume of approximately 0.2 ml was given so that extravascular injections could be visualized readily. Rats which received part of the injection subcutaneously were discarded. Each group was then given either aldosterone (0.34 μg/kg) or the vehicle, subcutaneously, so that the following combinations of treatments were obtained: 1) control, 2) aldosterone, 3) actinomycin D, 4) aldosterone and actinomycin D. Two hours later all rats received 0.9% sodium chloride (17 ml/kg) subcutaneously. Urine was collected during the next 2 hours using the procedure of Kagawa *et al*(5). At the end of the experiment the animals were killed and one kidney excised. Urinary sodium was measured with a Coleman flame photometer. Kidneys were homogenized in 0.25 M sucrose (0°C) with a Potter-Elvehjem tissue grinder and separated into debris (9000 × *g* pellet), microsomal (105,000 × *g* pellet) and soluble (105,000 × *g* supernatant) fractions by the method of Schneider(6) as modified by Hogeboom(7). RNA from each fraction was extracted and determined by the method of Scott *et al*(8). Incorporation of uridine 5′-monophosphate H^3 into RNA was determined with a Packard Tri-Carb liquid scintillation spectrometer using a naphthalene-dioxane system. Samples were corrected for background and quenching.

Data were analyzed by Kramer's modification of Duncan's new multiple range test(9) following analysis of variance using a completely randomized block design. $P<0.05$ was used as the criterion of significance.

Results. Table I shows the effect of actinomycin D on the antinatruretic action of aldosterone. Actinomycin D had no effect on sodium excretion by itself but completely blocked the antinatruretic action of aldosterone. These findings agree with those reported previously, when a longer urinary collection period was used(2).

The results of the spectrophotometric determination of RNA are shown in Table II. Aldosterone produced a significant elevation of RNA in all fractions. Actinomycin D by itself produced a small but significant decrease in the level of RNA in the microsomal and soluble fractions, but not in the debris fraction. When aldosterone was administered to the rats pretreated with actinomycin D, the stimulating action of aldosterone was completely blocked in all fractions.

Incorporation of uridine 5′-monophosphate H^3 into RNA following aldosterone and/or actinomycin D treatment is shown in Table III. In all fractions, aldosterone was found to significantly increase uridine 5′-monophos-

TABLE II. Stimulation of Renal Synthesis of RNA by Aldosterone and Inhibition of this Effect by Actinomycin D.

Fraction (μg of RNA/fraction/ 100 mg kidney, wet wt)	Treatment*				Coefficient of variability (%)
	Aldosterone (7)†	Control (5)†	Actinomycin D & aldosterone (7)†	Actinomycin D (5)†	
Microsomal	165	98	82	63	16
Soluble	48	34	24	26	14
Debris	99	43	40	35	33

* † See footnotes to Table I.

TABLE III. Stimulation of Incorporation of Uridine 5'-monophosphate H^3 into RNA by Aldosterone and Inhibition of this Effect by Actinomycin D.

Fraction (cpm/ fraction/g kidney, wet wt)	Treatment*				Coefficient of variability (%)
	Aldosterone (7)†	Control (5)†	Actinomycin D & aldosterone (7)†	Actinomycin D (5)†	
Microsomal	276	143	16	20	48
Soluble	417	155	78	100	62
Debris	546	203	234	243	47

* † See footnotes to Table I.

phate H^3 incorporation. When the actinomycin D group was compared with control group, incorporation was found to be significantly decreased only in the microsomal fraction. There was a small decrease in incorporation by the soluble fraction; however, it was not found to be significant. Pretreatment with actinomycin D was found to completely block the aldosterone-induced incorporation in all fractions.

Discussion. The data presented here show that aldosterone stimulated, *in vivo,* the synthesis of renal RNA in the rat and that this effect occurs during the same period as the antinatruretic action of this hormone. Both the antinatruretic action of aldosterone and its stimulation of RNA-synthesis were blocked by actinomycin D, an inhibitor of RNA-synthesis. Thus, these data are consistent with the hypothesis that aldosterone mediates its antinatruretic action by stimulation of RNA-synthesis and subsequent protein-synthesis. Similar findings have been reported by Porter *et al* with the isolated toad bladder(10). These workers found that aldosterone stimulated incorporation of uridine H^3 into RNA and that this occurred prior to its effect on sodium transport. Thus the ability of aldosterone to affect sodium transport *via* stimulation of RNA-synthesis would appear to be the same in both mammalians and amphibians.

Levels of RNA were increased in the microsomal, soluble and debris fractions of the kidney. The debris fraction in these experiments included the nuclei as well as such structures as cell walls and mitochondria. Increased levels of RNA in all these fractions are consistent with the above hypothesis. Initially aldosterone stimulates DNA directed synthesis of RNA in nuclei. This is reflected by the increased levels of RNA in the debris fraction in the experiments here. Subsequent migration of messenger-RNA into the cytoplasm and then attachment to the ribosomes of the endoplasmic reticulum would account for the increased levels of RNA found here in the soluble and microsomal fractions, respectively.

The levels of RNA of some fractions of the kidney of the actinomycin D treated group were less than levels of the control group. This is not unexpected inasmuch as this agent in a non-specific inhibitor of RNA-synthesis. Consequently, all RNA-synthesis is affected and such decreases in levels as were found indicate the amount of synthesis stimulated by substances other than aldosterone.

Summary. Spectrophotometric determination of RNA and isotopic determination of the incorporation of uridine 5'-monophosphate H^3 into RNA have shown that aldosterone stimulates RNA-synthesis *in vivo*. This occurs during the time aldosterone exerts its antinatruretic action. Actinomycin D, an inhibitor of RNA-synthesis, blocked both of these effects. These observations are consistent with the hypothesis that the antinatruretic action of aldosterone is mediated *via* DNA directed synthesis of messenger-RNA which in turn stimulates the synthesis of a protein involved in the transport of sodium.

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Effect of Bovine Gamma Globulin on Subsequent Immunization with Dinitrophenylated Bovine Gamma Globulin.* (30165)

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It has been shown that when rabbits are immunized with a hapten-protein conjugate, an anamnestic response can be obtained only when the same hapten-carrier protein conjugate is used for reinjection. The same hapten on a different, unrelated carrier protein does not provoke an anamnestic response(1).

The present study reports results obtained when the carrier protein is injected prior to injection of the hapten-protein conjugate.

Materials and methods. Animals. Random-bred, albino rabbits weighing approximately 3-4 kg were used for immunization. Hartley-strain, albino guinea pigs 250 ± 50 g, were used for passive cutaneous anaphylaxis (PCA) reactions.

Antigens. (a) Bovine gamma globulin (BGG) Armour Pharmaceuticals, Lot T 30203. (b) 2,4-dinitrophenylsulphonic acid (DNPS) (Eastman Kodak, Rochester, N. Y.) twice recrystallized from water and used for coupling to 2 different carrier proteins, *i.e.*, BGG and crystalline bovine serum albumin (BSA) (Armour Pharmaceuticals, Lot W 69312).

Coupling. Coupling was effected as described previously(1). Four different preparations of dinitrophenylated BGG (DNP-BGG) were used for immunization. These preparations differed only in the numbers of dinitrophenyl (DNP) groups present per molecule of BGG. The numbers of DNP groups per molecule (G/M) were 15, 33, 47, and 55.

The dinitrophenylated bovine serum albumin (DNP-BSA) had 28 G/M and was used for eliciting PCA reactions only.

Immunization. Two experiments were performed, with 20 rabbits in each. Ten rabbits were pre-immunized with BGG, and 10 were used as controls in each experiment. The pre-immunized rabbits received 3 mg of BGG in saline for 3 consecutive days. Sixteen days

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