activity of the rat liver homogenates was associated mainly with the mitochondria, as had been previously demonstrated(7).

Having established that the liver cells had been separated into distinct particulate components, it was possible to determine the distribution of acetate-replacing factor activity in the cells. The results reported in Table I indicate that approximately 64% of the acetate-replacing factor activity of rat liver is associated with the mitochondria. This finding is in conformity with the apparent role of acetate-replacing factors in pyruvate oxidation and the fact that mitochondria are centers of respiratory activity in liver cells(7).

Summary. The major portion of the acetate-replacing factor activity of rat liver homogenates has been demonstrated to be associated with the mitochondria.

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Endocrine Influences on Proteinuria in the Rat: Effect of ACTH.* (18907)

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The kidney enzyme renin produces an intense though transient proteinuria when injected intraperitoneally into the normal rat (1). It has been shown that bilateral adrenalectomy abolishes this proteinuria(2), and that it is restored in the adrenalectomized rat by the injection of suitable doses of cortisone, desoxycorticosterone acetate and aqueous adrenal cortex extract(3). Hypophysectomy similarly abolishes renin proteinuria. Cortisone is effective in restoring the ability of the hypophysectomized rat to respond to renin with proteinuria while desoxycorticosterone acetate is not(4). The normal, spontaneous proteinuria of the male rat is reduced by bilateral adrenalectomy(3), and by hypophysectomy (4). Cortisone and desoxycorticosterone acetate restore this proteinuria to normal levels in the adrenalectomized rat but fail to do so in the hypophysectomized animal.

In order to define further the endocrine influences on certain types of proteinuria in the rat, the effect of various doses of pituitary adrenocorticotropic hormone (ACTH) on renin proteinuria and the spontaneous proteinuria of normal male rats has been studied.

Methods. Animals: Male albino rats of the Slonaker-Addis strain were used in these experiments.

Urine collections: All animals were taken from the colony at 4 P.M. and placed in individual urine collection cages overnight. Seventeen hours later the overnight urine collection period was terminated. The protein excreted during this overnight period is referred to as the animal's spontaneous or "base" proteinuria. During the overnight period the animals received only a 15% solution of glucose in 0.4% sodium chloride containing 0.5% of a solution of B vitamins (Betaplexin). Following the termination of the overnight urine

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TABLE I. Effect of ACTH on Base and Renin Urinary Volume and Protein Excretion.

	No. of observations	Mean body wt, g	Mean base urinary vol, ml/17 hr	Mean base protein excretion, mg/hr	Mean urinary volume after renin, ml/hr	Mean proteinuria after renin, mg/hr
			Dose schedule A			
Control	29	189	45.4 ± 8.6‡	.85 ± .31	6.14 ± 1.74	44.4 ± 22.2*
ACTH-Renin, interval 1 hr	37	209	42.4 ± 7.9	.79 ± .18	5.97 ± 1.79	70.6 ± 27.2*
ACTH-Renin, interval 3 hr	26	"	"	"	5.60 ± 1.65	41.7
Control	10	300	62	1.5	4.9 ± 1.4	76
ACTH	10	300	60	1.1	6.4 ± 1.8	77.5
			Dose schedule B			
Control	6	196	46	.82	7.7 ± .3†	63 ± 22.4*
ACTH	6	194	45	.89	5 ± 1.3†	100.1 ± 13.2*
			Dose schedule C			
Control	30	221	52.1	.71 ± .28*	5.60 ± 1.67*	57 ± 28.9*
ACTH	37	227	45.5	.91 ± .15*	2.64 ± 1.21*	91.9 ± 32.9*
			Dose schedule D			
Control	12	244			6.1	57.4
ACTH 3 mg	12	245			4.8	51.5
Control	12	220			6.5	62.2
ACTH .25 mg	12	220			6.7	56.1

* P <0.001; † P = 0.01–0.001; ‡ Figures following the means are standard deviations.

collection period, the animals had their bladders emptied by allowing them to sniff ether lightly while pressure was exerted over the bladder area. All animals then received 4 Goldblatt dog units of hog renin§ (purity 50 units per mg of nitrogen) in 4 ml of 0.85% sodium chloride solution intraperitoneally. The animals were then placed back in the metabolism cages and their urines collected during the next 60 minutes. Details of the methods for accurate rat urine collections are described elsewhere(1).

ACTH was administered subcutaneously in varying dosages and according to a variety of time schedules as described below. The dose of ACTH is expressed in terms of equivalent mg of the Armour Standard (LA-I-A). Urinary proteins were determined by a modification of Kingsley's biuret method(6).

Results. The data are expressed as means. In most cases, the standard deviation is given. P values are obtained from Fischer's(5) table of t for small numbers of observations. P values of 0.01 or less are taken to indicate a significant difference between the means.

A. *Dose schedule A.* ACTH was first administered subcutaneously in doses of 0.40 mg dissolved in 0.5 ml of 0.85% sodium chloride solution. Injections were given at 12 o'clock noon and 5 P.M. on the day before renin administration, and 10 A.M. on the day of renin administration. Control animals received 0.5 ml of 0.85% sodium chloride solution at similar times. Thirty-seven experimental animals and all of the control animals received renin at 11 A.M. Twenty-six experimental animals were injected with renin at 1 P.M. In each group the urines were carefully collected for the 60 minutes immediately following renin administration. The results are given in Table I.

ACTH when administered according to dosage schedule A, to rats weighing approximately 200 g, resulted in a 65% increase in the amount of protein excreted in the hour following renin administration over and above that shown by non-ACTH treated controls. There was no significant change from the control value in the volume or protein content of the 17-hour overnight urine or in the volume

§ Renin was generously supplied by Drs. Harry Goldblatt, Erwin Haas, and Hildegard Lamfrom.

5. Fischer, R. A., *Statistical Methods for Research Workers,* Oliver and Boyd, London, 1928.

6. Kingsley, G. R., *J. Biol. Chem.,* 1939, v131, 197.

of urine excreted in the hour following renin administration.

If too long a time was allowed to elapse between the last dose of ACTH and the administration of renin, no increase in proteinuria was noted. Thus, the proteinuria in response to renin was greatly augmented when the ACTH-renin interval was one hour, but was entirely absent if this period was extended to 3 hours. When ACTH was administered according to dosage schedule A, to rats of approximately 150 days of age and weighing 300 g there was no significant increase in renin proteinuria (Table I).

It appeared that the dose of ACTH employed above was sufficient to augment the proteinuria resulting from renin administration in rats weighing 200 g, but was too small to be effective in the 300 g animals. Consequently, ACTH was given in larger doses to a fresh group of animals.

B. *Dose schedule B.* The dose of ACTH was increased to 1.6 mg given subcutaneously at 12 o'clock noon and 5 P.M. on the day before renin injection, and 10 A.M. on the day of renin administration (Table I).

When the dose of ACTH was increased to 1.6 mg per injection, the amount of protein excreted in response to renin was increased by 63%. Thus, in spite of a 4-fold increase in the amount of ACTH administered, the increase in renin proteinuria was nearly identical to that found with the 0.40 mg dose. In this instance, however, there was a significant fall in the volume of urine excreted in the hour following the renin administration. No significant change occurred in the volume or protein concentration of the urine excreted during the 17-hour overnight period.

C. *Dose schedule C.* In order to determine whether still larger doses of ACTH given at intervals throughout the overnight collection period might augment the base proteinuria, dosage schedule C was undertaken. 4.8 mg of ACTH was given at 5 P.M. and 12 midnight of the day preceding renin administration, and at 7 A.M. on the day renin was given. The results are given in Table I. ACTH administered in this manner resulted in a 61% increase in the quantity of protein excreted during the hour following renin administration. In addition, a sharp fall in the volume of urine excreted occurred during this period, and further, the quantity of protein excreted during the 17-hour overnight period was significantly increased by ACTH administration in the time-dosage schedule indicated. The apparent fall in the 17-hour overnight urine volume in the ACTH treated animals is not a statistically significant one.

D. *Dose schedule D.* This schedule was designed to determine whether or not ACTH administered the day preceding renin administration was necessary if an increase in renin proteinuria was to be obtained. All animals were placed in metabolism cages overnight, as described above. The following morning one-half of the experimental animals received 0.40 mg of ACTH and one hour later were injected intraperitoneally with 4 units of renin in 4 ml of 0.85% sodium chloride solution. The remaining half of the experimental animals received 4.8 mg of ACTH subcutaneously and 10 minutes later were injected with four units of renin in 4 ml of 0.85% sodium chloride solution intraperitoneally. Control animals received 0.5 ml of 0.85% sodium chloride solution subcutaneously instead of ACTH. In all instances, urines were collected for 60 minutes following renin administration (Table I).

It appears that a single dose of ACTH, whether large or small, when administered in the manner described above, will not produce an increase in protein excretion in the hour following renin injection. In rats of our colony single injections of ACTH in doses up to 25 mg have failed to produce a consistent eosinophile fall. It was not until 9.6 mg of ACTH was injected in 3 successive doses over a 17-hour period, that a satisfactory eosinopenia was produced. While the volume of urine excreted during this hour appears to be diminished in the animals given 4.8 mg of ACTH, this fall is not a statistically significant one.

Discussion. When given in the proper dose and at appropriate time intervals, ACTH is capable of markedly augmenting the intense transient proteinuria that occurs in the normal

rat following renin administration. A less spectacular, nevertheless, significant increase in the normal, spontaneous proteinuria of rats occurs following suitable doses of ACTH. If the animal is too large, or the administration of ACTH is not suitably timed, one may fail to observe any significant change from the normal in protein excretion. Though it might seem that the sharp fall in urinary volume in the hour following schedule C, renin administration was a manifestation of posterior pituitary contamination of the ACTH used, this possibility was discarded following assay of our ACTH preparations for anti-diuretic substances.¶ Further, we have recently demonstrated that a similar fall in post-renin urinary volume occurs in cortisone-treated rats. Other preliminary studies in our laboratory indicate that the fall in urine volume in ACTH and cortisone-treated animals given renin may be a result of actual renal tubular damage with plugging of the tubular lumina by protein casts. Along similar lines, Masson, Corcoran, and Page have reported renal damage in rats treated with saline, desoxycorticosterone acetate, and renin(7).

The mechanism by which ACTH augments proteinuria in the rat is not indicated by the data presented here.

Summary. 1. Normal rats respond to the intraperitoneal injection of renin with an intense, transient proteinuria. ACTH administration results in a 60 to 65% increase in proteinuria in the hour following renin administration over and above that shown by non-ACTH treated controls. 2. The normal, spontaneous proteinuria of the male rat is significantly increased by ACTH administration.

¶ The assays were performed by Dr. Irby Bunding of Armour and Co., Chicago, Ill.

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Effect of Intranasally Administered Immune Serum on Influenza Virus Present in the Mouse Lung.* (18908)

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Previous studies(1) have indicated that when mice are inoculated intranasally with small amounts of influenza virus, death occurs only in those instances in which the concentration of virus in the lungs reaches a certain level. However, when significantly larger amounts of virus are inoculated, death may occur within 2 to 4 days, but the maximum concentration of virus attained in the lungs is usually less than the critical concentration of smaller doses and, furthermore, may be less than frequently is found in the lungs of mice that have received sublethal doses. Hence, it was suggested that when the large amount of virus was inoculated the quantity introduced may, by itself, have been sufficient to kill the animal without subsequent multiplication of the agent. As part of a further investigation of this problem, a number of experiments were conducted comparing the effect of intranasally administered immune serum on the outcome of the infection, with the concentration of virus in the lungs of the mice at the time the serum was given. The purpose of this paper is to report the results of those experiments.

Materials and methods. A mouse-adapted line of the Cam strain of influenza A virus was used. The virus had been through 128 mouse passages at the beginning of the investigation and was maintained during the period of study by bi-weekly passage in mice. At each passage a number of 9- to 12-day-old

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