Effect of Adrenalectomy on Spontaneous and Induced Proteinuria in the Rat.* (17803)

T. Addis,† J. Marmorston, H. C. Goodman,‡ Alvin L. Sellers, and Margaret Smith.

From the Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, Calif.

Addis, Barrett, Boyd, and Ureen(1) have demonstrated that an intense transient proteinuria follows the intraperitoneal injection of the kidney enzyme renin into the normal rat. Addis and Boyd(2) state that the proteinuria following renin injection is abolished in the rat by bilateral adrenalectomy. In the course of our studies on the mechanism of proteinuria, we have confirmed and extended these observations and have demonstrated that treatment not only with aqueous adrenal cortex extract (ACE), but also with synthetic desoxycorticosterone acetate (DOCA), and 11-dehydro-17-hydroxy-corticosterone acetate (cortisone) will restore the ability of the adrenalectomized rat to respond to renin with a marked proteinuria. The effect of adrenalectomy on proteinuria produced by the intraperitoneal injection of human serum albumin, as well as on the spontaneous, slight proteinuria present in the normal rat have also been investigated.

Methods. Two hundred seventeen male and female albino rats of the Slonaker strain were used in these experiments. Proteinuria in response to renin injection was determined on the fifth and tenth postoperative days in the adrenalectomized and control animals. Four dog units(3) of hog renin diluted in 4 cc of physiological saline solution were given intraperitoneally. The animals were then placed in individual metabolism cages, and the urine excreted over the ensuing 60 minutes was carefully collected. The methods for accurate rat urine collections are described in detail by Addis, *et al.*(1). Animals given hormone replacement therapy received daily injections from the day of operation until the termination of the experiment. The dosage schedule was as follows: desoxycorticosterone acetate, 0.5 mg subcutaneously; adrenal cortex extract, 2 cc intraperitoneally; and cortisone, 1.0 mg subcutaneously daily. Serum albumin proteinuria was produced by injecting rats intraperitoneally with 16 cc of 6% human serum albumin§ in normal saline solution, according to 2 dosage schedules. Group A, consisting of 6 adrenalectomized and 4 control animals, received 16 cc of 6% serum albumin in normal saline solution intraperitoneally at 9:00 a.m. and 4:00 p.m. on the fourth postoperative day, and at 10:00 a.m. on the fifth postoperative day. (A total of 3 g of albumin.) They were then placed in individual metabolism cages, and urine was collected over the ensuing 18-hour period.

Group B, consisting of 8 adrenalectomized and 4 control animals, received this dose at 9:00 a.m. and 4:00 p.m. on the fifth postoperative day (a total of 2 g of albumin). They were then placed in individual metabolism cages, and their urine was collected during the following 18-hour, overnight period. Adrenalectomies were performed through bilateral flank incisions uring ether anesthesia. All adrenalectomized rats were maintained on normal saline drinking solution. Urinary

* This work was made possible by grants from the United States Public Health Service and The American Foundation for High Blood Pressure.

Desoxycorticosterone acetate was supplied by Dr. G. Babcock, Jr. of Schering Corp., Bloomfield, N.J.

Adrenal cortex extract was supplied by Dr. David Klein of The Wilson Laboratories, Chicago, Ill.

† Dr. Addis' untimely death occurred before this paper was completed.

‡ Columbia Foundation Research Fellow.

1. Addis, T., Barrett, E., Boyd, R. L., and Ureen, H. J., *J. Exp. Med.*, 1949, v89, 131.

2. Addis, T., and Boyd, R. L., *Fed. Proc.*, 1949, v8, 443.

3. Goldblatt, H., Katz, Y. J., Lewis, H. A., and Richardson, E., *J. Exp. Med.*, 1943, v77, 309.

§ Obtained through the courtesy of the American Red Cross.

TABLE I.
Abolition of Renin Proteinuria by Adrenalectomy and Its Restoration by Adrenal Hormone Therapy.

	No. of observations	Renin proteinuria, mg/hr
Normal controls	96	31.7
Sham operated controls	75	37.7
Adrenalectomy	50	1.0
" + DOCA	12	33.1
" + ACE	14	16.5
" + Compound E	23	33.5

proteins were determined by a modification of Kingsley's biuret method(1).

Results. (A) *Effect of Adrenalectomy on Renin Induced Proteinuria.*

All rats excrete protein in their urine. In the normal male rat this proteinuria may amount to 1.0 mg per hour. In the hour following the intraperitoneal injection of 4 dog units of renin, the protein excretion increases markedly. While there is a great deal of individual variation in the amount of protein excreted during this hour, the average in 96 animals shown in Table I is 31.7 mg. In view of the variability in protein excretion, the value of 37.7 mg/hr for the sham operated control is not significantly greater than the normal control value. The data shown in Table I indicate that bilateral adrenalectomy renders the saline-maintained rat incapable of responding to renin injection with significant proteinuria.

(B) *Renin Proteinuria in Adrenalectomized Rat Receiving Adrenal Cortex Steroids.* Both desoxycorticosterone acetate and cortisone restore the ability of the adrenalectomized rat to respond to renin injection with massive proteinuria (Table I). Adrenal cortex extract does not seem to restore this function as completely as the other compounds in the dosage used in these experiments.

(C) *Human Serum Albumin Proteinuria.*

The normal rat excretes about 510 mg of protein in the 18 hours following the last intraperitoneal injection of human serum albumin. After bilateral adrenalectomy, 50-100 mg of protein are excreted in the 18

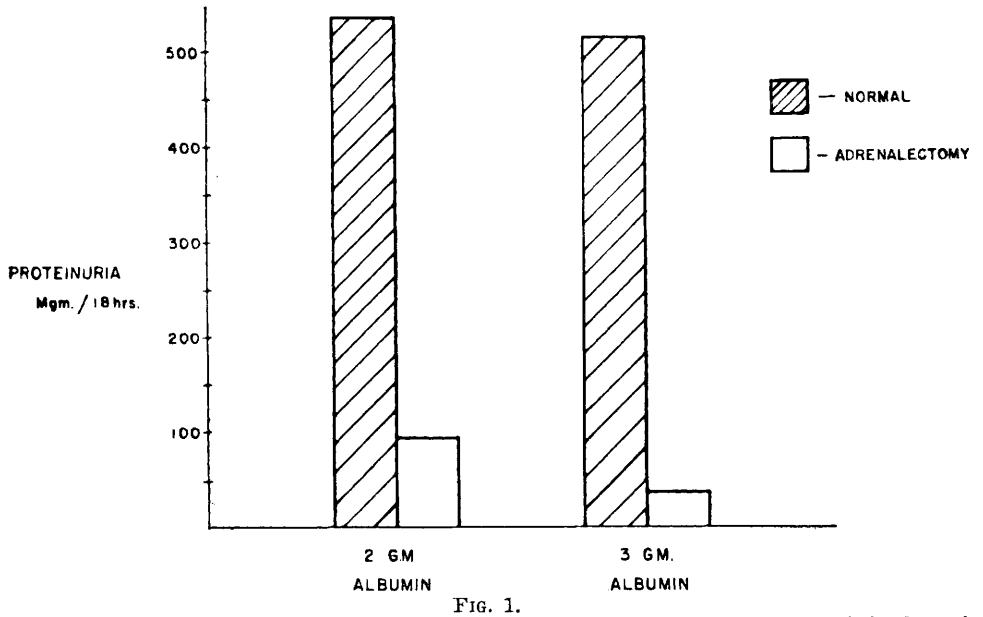

FIG. 1.
Effect of adrenalectomy on the proteinuria produced by the intraperitoneal injection of human serum albumin.

TABLE II.
Effect of Adrenalectomy and Adrenal Steroid Therapy on Spontaneous Proteinuria of Male and Female Rats.

	Male			Female	
	No. of observations	Mean proteinuria mg/hr/100 cm² body surface	Stand. dev.	No. of observations	Mean proteinuria mg/hr/100 cm² body surface
Normal controls	51	.19*	.052	75	.10
Adrenalectomy	25	.11*	.052	44	.08
" + Cortisone	23	.19*	.071	18	.10
" + DOCA	12	.16*	.035	18	.07
" + ACE	12	.12	.035	24	.06

* The difference between the means of the normal controls, adrenalectomy plus cortisone, and adrenalectomy plus DOCA and the mean of the untreated adrenalectomized animals exceeds three times the standard error of the difference between these means.

hours following the albumin injections. As with renin, there is great variability in the amount of protein individual animals excrete following albumin injection. This variability is not indicated by the averages shown in Fig. 1. Nevertheless, the effect of adrenalectomy in decreasing this proteinuria is so clear cut and striking as to be beyond doubt.

Normal male rats have a higher rate of spontaneous protein excretion in their urine than normal female rats(4). (Table II). The data in Table II indicate that bilateral adrenalectomy decreases the rate of spontaneous proteinuria in the normal male rat. The results have been expressed in mg/hr 100 sq. cm. of body suface in order to allow comparison of animals with different body weights and kidney mass. A statistical analysis of these results shows that the decrease in proteinuria in the male rat following adrenalectomy is a significant one.

The administration of cortisone in dosage of 1 mg subcutaneously daily restored the spontaneous protein excretion of adrenalectomized male rats to normal values within 5 days. Desoxycorticosterone acetate (0.5 mg daily) proved less adequate in this regard, while adrenal cortex extract (2 cc intraperitoneally daily) did not increase the protein excretion over that found in the untreated controls. Bilateral adrenalectomy apparently does not alter the level of normal spontaneous proteinuria in the female rat. (Table II).

Discussion. Our data establish a relationship between the adrenal cortex and protein excretion by the kidney, but do not indicate the mechanism of this interaction. It is possible that adrenalectomy decreases protein excretion by altering the renal blood flow, or decreasing the rate of glomerular filtration of protein. Lotspeich(6) and Friedman, Mackenzie, and Friedman(7) have reported essentially normal rates of glomerular filtration in the adrenalectomized rat maintained on saline solution. The later workers have demonstrated that renal blood flow is unaltered in the saline treated, adrenalectomized rat when studied 4-6 days postoperatively. While the urinary output of adrenalectomized rats is less than that of normal controls, Lotspeich(6) maintains that this is due to an increased tubular reabsorption of water. Small changes in these renal functions could be responsible for the fall in spontaneous protein excretion found in the adrenalectomized male rats, but could hardly account for the gross decreases in renin and human albumin proteinuria following bilateral adrenalectomy. The effect of adrenalectomy

4. Addis, T., *Proc. Calif. Acad. Med.*, 1931-32, pp. 38.

5. Addis, T., Final Report, Contract 338, Office Of Scientific Research and Development, Committee on Medical Research, 1946.

6. Lotspeich, W. D., *Endocrinology*, 1949, v44, 314.

7. Friedman, S. M., Mackenzie, K. R., and Friedman, C. L., *Endocrinology*, 1948, v43, 123.

on the glomerular permeability to protein, as well as on the tubular reabsorption of protein is now under investigation.

Summary. 1. Bilateral adrenalectomy abolishes or greatly reduces the types of experimentally produced proteinuria studied in the saline maintained rat.

2. The normal, spontaneous proteinuria of the male rat is significantly reduced by bilateral adrenalectomy.

3. Cortisone administration to bilaterally adrenalectomized male rats increases their spontaneous proteinuria to normal control levels.

4. Adrenal cortex extract, desoxycorticosterone acetate, and cortisone restore the ability of the salt-maintained, adrenalectomized rat to respond to renin injection with massive proteinuria.

We are indebted to Drs. Harry Goldblatt, Erwin Haas, and Hildegarde Lamfrom for supplying us with the renin used in this study.

We wish to thank Mr. J. Manders and Mr. A. Mallinger for their technical aid.

Received March 12, 1950. P.S.E.B.M., 1950, v74.

The Dermal Loss of Iron.* (17804)

WILLIAM S. ADAMS, ALAN LESLIE, AND MELVIN H. LEVIN.
(With the technical assistance of Mrs. Howard W. Luchsinger.)
(Introduced by William G. Clark.)

From the Medical Service, Wadsworth Hospital, Veterans Administration Center, and the Department of Medicine, University of California, Los Angeles, Calif.

It has been generally accepted that the human being has a limited capacity to lose iron except by hemorrhage. Voluminous data obtained from careful iron balance studies have indicated that: (a) very little dietary iron will keep a normal individual in iron balance; (b) the loss of iron in the urine and stool amounts to no more than 1 mg per day; (c) only a small amount of iron is absorbed from the gastro-intestinal tract even when iron is given in large amounts; and (d) the body supply of iron, once across the intestinal barrier, is closely conserved(1-5). The findings of Mitchell and Hamilton(6) who report as much as 6.5 mg of iron lost in sweat per day indicate a hitherto unsuspected avenue of iron loss.

This paper presents the results of 118 determinations of iron in sweat obtained from 25 normal subjects.

Method. 1. The method of Leslie and Levin(7), used in obtaining the sweat sample, consists essentially of carefully washing one forearm with soap and water, rinsing with copious amounts of distilled water, and drying carefully with specially cleaned towels. Finger cots are applied to preclude fingernail contamination and a full-length rubber glove is then drawn over the hand and forearm to catch the sweat. The other arm is placed in a water bath at 110° F for one hour. In most instances a sufficient volume of sweat is obtained. The sweat which accumulates in the glove is measured in a graduated centri-

* Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions of the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

1. Hahn, P. F., *Fed Proc.*, 1948, v7, 493.

2. Hahn, P. F., *Advances in Biol. and Med. Physics*, 1948, v1, 287.

3. Widdowson, E. M. and McCance, R. A., *Biochem. J.*, 1937, v31, 2029.

4. McCance, R. A., and Widdowson, E. M., *J. Physiol.*, 1938, v94, 148.

5. Hahn, P. F., Bale, W. F., Hettig, R. A., Kamen, M. D., and Whipple, G. H., *J. Exp. Med.*, 1939, v70, 443.

6. Mitchell, H. H., and Hamilton, T. S., *J. Biol. Chem.*, 1949, v178, 345.

7. Leslie, A., Levin, M., *Am. J. Med.*, in press.