TABLE I. Effect of Insulin on Induction of Urethane Anesthesia, Dimethyl-p-Phenylenediamine Convulsions and Subcutaneous Absorption of Physiologic Saline in Rats.

Groups of 12 rats	Urethane* anesthesia (%) 10 min.	20 min.	30 min.	Glycemia,† mg %	PPD‡ convulsions (%) 10 min.	20 min.	30 min.	Glycemia,† mg %
Controls	0	13	25	112 ± 3.1	0	0	90	128 ± 4.2
Zinc-insulin (0.2 I.U. s.c.)	25	37	67	78 ± 5.9	0	90	100	80 ± 5.3

	Absorption rate of saline (%)§	Glycemia,† mg %
Controls	39	118 ± 3.7
Zinc-insulin (0.2 I.U. s.c.)	80	74 ± 6.1

* Mean readings following s.c. inj. of 100 mg/100 g of ethyl urethane.
† Mean ± S.E.
‡ Mean readings following s.c. inj. of 2.6 mg/100 g of dimethyl-p-phenylenediamine.
§ Mean percentage based on intensity of blueing 10 min. after i.v. inj. of 0.2 ml of 0.5% Evans blue.

ing effect of insulin on dextran reaction is also derived from increase in absorption. Whether insulin influences the reaction by changing permeability of cells or of ground substance remains to be shown. It has been reported that subcutaneous absorption of urethane is augmented by cortisone, in rats, and by dehydration and fasting, in mice. This was interpreted as resulting from a reduction of hyaluronic acid content in connective tissue ground substance(3). Insulin may act similarly, perhaps by influencing activity of hyaluronidase.

Fasting, especially in adrenalectomized rats, augments sensitivity to dextran(1), and similarly, activity of various drugs was more uniform in animals subjected to a short period of fasting. It is also common knowledge that the efficiency of therapeutic agents, even when given parenterally, is higher in fasting patients. That a slight decrease in glycemia, within physiologic range, activates absorption phenomena may also explain the fact that insulin given at a low dosage promotes absorption of substances injected subcutaneously.

Summary. Subcutaneous absorption of urethane and of dimethyl-p-phenylenediamine was augmented by insulin, as evidenced by acceleration of anesthesia and convulsions caused by these substances, respectively. Insulin also promoted absorption rate of intradermally injected physiologic saline.

1. Jasmin, G., Bois, P., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 656.
2. ———, *Proc. Can. Physiol. Soc.,* Meet. Toronto, June 9, 1959.
3. Hvidberg, E., Schou, J., *Nature,* 1959, v184, 646.

Received December 7, 1959. P.S.E.B.M., 1960, v103.

Potassium Metabolism in Rats with Chronic Renal Insufficiency.* (25573)

ASHTON B. MORRISON (Introduced by J. Russell Elkinton)
Dept. of Pathology, School of Medicine, University of Pennsylvania, Philadelphia

Male rats which had been subtotally (5/6) nephrectomized developed a form of chronic renal insufficiency similar in many ways to that occurring in the human patient with chronic nephritis. At death, lesions were found in the cardiovascular system and in bones of the rats, which were indistinguishable in quality from those found in some patients with chronic renal insufficiency(1). When at least one month had elapsed following operation the adrenals were enlarged(1). This enlargement in such animals suggested that during period of renal insufficiency the adrenals

* This work was supported by U.S.P.H.S. grants from Nat. Inst. of Arthritis and Metab. Dis., Bethesda, Md., and from Smith, Kline, and French Fn.

TABLE I. Effect of Low Potassium Diet on Muscle and Serum Potassium, Serum CO_2 Content and Myocardium of Subtotally Nephrectomized and Sham Control Rats.

Group	No. of rats	Treatment: Operation	Treatment: Diet	Serum: Total CO_2, mM/l	Serum: Potassium, meq/l	Muscle K as meq/100 g f.f.s.	Myocardial lesions grading
A	8	Subtotal nephrectomy	Low K	32.4 ±1.6*	2.9 ±.2	35.4 ± 1.8	2+ to 4+
B	8	*Idem*	Control	24.6 ± .7	4.6 ±.3	48.1 ± 1.5	0 to 1+
C	8	Sham operation	Low K	32.2 ± .9	3.8 ±.2	41.5 ± 1.3	0 to 2+
D	8	*Idem*	Control	26.1 ±1.1	4.6 ±.1	47.1 ± 1.2	0 to 1+
				p†	A & C <.01	A & C <.05	

f.f.s. = fat-free solids.

* Means ± stand. errors. Stand. errors calculated as $\sqrt{\Sigma (x-\bar{x})^2 \Big/ \frac{n-1}{n}} = \sigma$. $\bar{x}$, mean of x.

† p determined from ± test of differences, D, between means of 2 groups where $\sigma D = \sqrt{\sigma x_1^2 + \sigma x_2^2}$, $t = \frac{D}{\sigma D}$.

were more active than in the normal animal. Adrenal overactivity may lead to potassium depletion(2), and if adrenals of subtotally (5/6) nephrectomized rats were overactive, it might be expected that such animals would show a tendency to become potassium depleted more readily than normal rats. To test this, it was decided to feed subtotally (5/6) nephrectomized rats a low potassium diet and discover whether they became more potassium depleted than sham operated control rats fed the same diet. The criteria used to assess potassium depletion in the rats were, fall in serum potassium levels, rise in total CO_2 of serum (resulting from metabolic alkalosis of potassium depletion), extent of depletion of muscle potassium, and degree of focal myocardial necrosis.

Materials and methods. Male Wistar rats weighing 250 g were subtotally (5/6) nephrectomized. Sham operations were carried out on another group. Six weeks after operation, half of the sham control group (Group C) and half of subtotally nephrectomized group (Group A) were fed a diet low in potassium† but containing 30 meq of magnesium/kg of diet and adequate in all other nutrients. Remaining animals (Groups B and D) were fed the same diet to which potassium had been added (0.85 g KH_2PO_4 and 0.58 g KCl to 100 g diet). This diet was continued 70 days, when blood was taken under oil from the aorta in each animal and the animal killed. The muscle was analyzed for potassium by the method of Darrow(3) using a Baird flame photometer. Serum CO_2 content was measured manometrically by the method of Van Slyke and Neill(4). The heart was fixed, bisected and after embedding was sectioned so that the myocardium of each ventricle and auricle might be examined. The extent of myocardial lesion was graded 0, 1+, 2+, 3+, or 4+ depending on number of lesions seen on low-power examination of a couple of complete sections through middle of heart, by an observer who had no knowledge of the grouping of the animals.

Results are presented in Table I. There was clearly a greater depletion of muscle potassium in subtotally (5/6) nephrectomized animals (Group A) than in sham operated controls (Group C), when both were on a low potassium diet. Serum potassium levels were also significantly lower in the subtotally

† Low Potassium Diet bought from Nutritional Biochemicals Corp., Cleveland. Composition: cornstarch, 64%; casein, 30%; butterfat, 3.5%; calcium carbonate, 1.3%; sodium chloride, 1%; and an adequate supplement of all known vitamins. The diet on analysis contained 8 meq potassium/kg, 340 meq sodium/kg, and 7.0 meq magnesium/kg. A mixture of magnesium chloride and magnesium sulphate was added to bring level of magnesium in stock diet to 30 meq of magnesium/kg of diet.

nephrectomized group, than in the sham operated control group on a low potassium diet. In studies by others(5), degree of elevation of serum CO_2 in potassium deficient rats is correlated closely with the fall in muscle potassium, but in animals reported here, where there was clearly a greater degree of muscle potassium depletion in sub-totally nephrectomized animals (Group A) than in sham operated controls (Group C), there was no significant difference in serum CO_2 content. The most severe myocardial focal necrosis and fibrosis was observed in subtotally nephrectomized animals made potassium deficient (Group A). These lesions were very much more severe in subtotally nephrectomized potassium depleted animals than in sham animals subjected to potassium deficiency. Degree of necrosis and scarring naturally varied from animal to animal, but in none of the sham controls of Group C was the lesion as severe as in the least affected of subtotally nephrectomized group (Group A).

Discussion. These findings indicate that rats with chronic renal insufficiency induced by subtotal (5/6) nephrectomy become more potassium deficient on a low potassium diet than sham-operated control animals on the same diet. While this finding is consistent with adrenal cortical overactivity in subtotally nephrectomized rats, there remains the possibility that the increased loss of potassium resulted directly from impaired renal function. When such sub-totally nephrectotomized animals are on a normal diet, excretion of potassium is no greater after than before subtotal nephrectomy(6). Preliminary experiments have shown that a sub-totally nephrectomized rat can reduce its urinary loss of potassium in much the same fashion as a normal rat, when placed on a low potassium intake. These facts suggest that nothing like a form of potassium losing nephropathy is induced by the operation. Whatever may be the initiating factor in the overall increased loss of potassium, when subtotally nephrectomized animals are placed on a low potassium diet, it seems likely that increased secretion of adrenocortical hormones is involved in the process. Increased secretion of adrenocortical hormones might also explain the greater incidence and severity of myocardial lesions in rats with a subtotal nephrectomy on a low potassium diet (Group A), than in sham controls on the same diet (Group C), since administration of adrenocortical hormones increases susceptibility of the myocardium to damage in potassium deficiency(7,8). In previous experiments(9,10) subtotally (5/6) nephrectomized rats formed and excreted more urea and lost more potassium than sham operated controls, when both were starved for 4 days. Such findings were again possibly the result of greater adrenal activity in subtotally (5/6) nephrectomized animals. These observations concerning potassium metabolism of rats with chronic renal insufficiency may have a bearing on metabolic derangements in chronic nephritis in the human, and are to be reported elsewhere in detail.

Summary. Subtotally 5/6 nephrectomized rats and sham operated controls were fed a diet deficient in potassium. Subtotally nephrectomized animals had significantly less potassium in their muscles, and a lower serum potassium than control animals; there appeared to be no difference in serum CO_2 content in the 2 groups; subtotally nephrectomized rats showed more focal necrosis and fibrosis of the myocardium than control animals on a low potassium diet.

1. Morrison, A. B., Robbins, T., Gordon, J., *Am. J. Path. (Proc.)*, 1958, v34, 563.

2. Kepler, E. J., Sprague, R. G., Mason, H. L., Power, M. H., *Rec. Prog. Hormone Res.*, 1948, v2, 345.

3. Darrow, D. C., Harrison, H. E., Taffel, M., *J. Biol. Chem.*, 1939, v130, 487.

4. Peters, J. P., Van Slyke, D. C., *Quantitative Clinical Chemistry*, v1, Williams & Wilkins Co., Baltimore, 1932, p. 285.

5. Hollander, W., Winters, R. W., Williams, T. F., Bradley, J., Oliver, J., Welt, L. G., *Am. J. Physiol.*, 1957, v189, 557.

6. Platt, R., Roscoe, M. H., Smith, F. W., *Clin. Sci.*, 1952, v11, 217.

7. Selye, H., Bajusz, E., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 580.

8. Darrow, D. C., Miller, H. C., *J. Clin. Invest.*, 1942, v21, 601.

9. McCance, R. A., Morrison, A. B., *Quart. J. Exp. Physiol.*, 1956, v41, 365.

10. Morrison, A. B., Gordon, J., *Fed. Proc.*, 1957, v16, 366.

Received December 7, 1959. P.S.E.B.M., 1960, v103.