The present experiment was undertaken to determine the effect of activity and rest upon dogs with renal hypertension produced by placing capsules about the kidney, Page.[3] Hypertension was produced in 3 dogs with normal blood pressure by placing silk capsules about both kidneys in 2 (dogs 1 and 2, Fig. 1) and plastic capsules in one (dog 3, Fig. 1). It was produced in a 4th dog by placing a silk capsule about one kidney and removing the other. Blood pressures as determined by direct arterial puncture rose to levels between 190 and 206 mm Hg during the 25 to 36 days that elapsed before the final experiment. Cuffs were then placed about the right iliac and femoral arteries and the animals were prepared for blood pressure determination according to the technic previously described.[1]

After complete recovery from anesthesia blood pressures and pulse rates were recorded at frequent intervals for periods from 49 to 76 hours. The variation and the mean of readings of 3 dogs taken when they were feeding, moving about the cage, standing quietly, lying and resting, or sleeping are illustrated in Fig. 1. The 4th dog with one kidney removed and a silk capsule about the other exhibited unusually high pressures throughout the experiment. Base lines and cuffs were carefully checked without determining a cause. The pressure by direct arterial puncture was 200 mm Hg 9 days earlier. This animal had pressures during the experiment ranging from 275 to 305 while feeding, 265 to 315 while active, 250 to 315 while standing, 270 to 295 while resting, and from 240 to 285 while sleeping. The variations of pulse rate of all 4 dogs during activity and rest roughly paralleled the changes of blood pressure.

Under the conditions of this experiment changes of blood pressure and pulse rate in dogs with renal hypertension are quite similar to those previously reported for dogs with neurogenic hypertension.[2] The reduction of blood pressure and pulse rate that occurs in both types of hypertension does not approach the reduction of levels that occurs in normal dogs.[2]

[3] Page, I. H., *Science*, 1939, **89**, 273.

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Creatine Concentration and Weight Loss in Denervated Skeletal Muscle.

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Denervated skeletal muscle undergoes a number of degenerative changes of which atrophy is of especial interest. Hines, Thomson and Lazere[1] have pointed out that muscle strength, weight and creatine content decrease following denervation, subsequently increasing with regeneration of the nerve fibers. Since creatine, in the form of phosphocreatine, plays a vital role in muscular contraction, the present work was undertaken to compare creatine loss and weight loss in denervated skeletal muscle.

Methods. Albino rats were used. The gastrocnemius muscle of one leg of each rat was denervated by removing a section of the sciatic nerve. The opposite leg was left intact and served as a control. Two animals were sacrificed at 2-day intervals after denervation. The intact and denervated muscles were carefully removed, placed directly into tared airtight flasks, weighed and analyzed for creatine.

Of the several methods for determining creatine concentration which have been re-

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[1] Hines, H. M., Thomson, J. D., and Lazere, B., *Am. J. Physiol.*, 1942, **137**, 527.

TABLE I.

Days after denervation	Body wt	Muscle weight			Creatine concentration					Total creatine			
		Intact	Denervated	Denervated / Intact × 100	Intact	Denervated	Denervated avg	(Avg intact conc.) — (Den. conc.)	Den. creatine conc. / Intact creatine conc. × 100	Intact	Denervated	Net creatine loss	Total creatine of denervated / Total creatine of intact × 100
	g	g	g		mg%	mg%	mg%	mg%		mg	mg	mg	
4	135	0.916	0.774	84.5	411	388	373	29	94.4	3.76	3.00	0.76	79.8
4	129	0.794	0.669	84.4	390	367			91.5	2.83	2.39	0.44	84.5
6	240	1.393	1.134	81.5	393	326	338	64	83.0	5.47	3.70	1.77	67.6
6	270	1.734	1.431	82.4	394	349			88.6	6.83	4.99	1.84	73.1
8	162	1.144	0.795	69.5	421	297	312	90	70.5	4.82	2.36	2.46	49.0
8	118	0.856	0.583	68.2	394	326			82.7	3.37	1.90	1.47	56.4
10	224	1.588	1.046	66.0	402	261	298	104	64.9	6.38	2.73	3.65	42.8
10	251	1.870	1.308	69.9	411	334			81.3	7.69	4.37	2.32	56.8
12	251	1.398	0.821	58.8	406	274	292	110	67.5	5.68	2.25	3.43	39.6
12	290	1.949	1.172	60.3	395	310			78.5	7.70	3.63	4.07	47.1
14	291	1.819	0.982	54.1	404	259	246	156	62.4	7.35	2.47	4.88	33.6
14	245	1.624	0.902	55.5	397	239			60.2	6.45	2.16	4.29	33.5

ported[2-4] most investigators have employed the method of Rose, Helmer and Chanutin.[2] For rat muscles we found this method to be satisfactory. In order to simplify the manipulations the following modifications were introduced: Instead of using an autoclave for digestion of the samples, a 4-quart pressure cooker was used. This would accommodate 8 flasks, could be brought to the required pressure in 3 minutes and could be cooled in 3 minutes. Pressure was readily controlled using a laboratory burner. Approximately 1½ hours could be saved by substitution of the pressure cooker for the autoclave in each determination. Alkaline picrate solution was added to the clear protein-free filtrate and the unknown creatine concentrations were compared with standard solutions using a photoelectric colorimeter (Cenco photelometer).

Results. Our results are tabulated in Table I. Inasmuch as dry weight and creatine determination could not be made conveniently on the same muscle, the wet weights were used for comparison. In a second series of rats it was found that average dry weight ratios of denervated and intact muscle are not significantly different from average wet weight ratios on the 14th day after denervation. In this series the mean wet denervated/intact ratio was 0.585 ± 0.045 while the corresponding dry ratio was 0.552 ± 0.057. The standard error of the difference between the 2 means was ± 0.02.

Body weights of the animals varied widely among themselves and consequently the actual weight differences in intact and denervated muscles were not directly comparable. The percentage ratio of denervated to intact muscle weights is tabulated. This ratio can be used as an index of the relative weight loss of the denervated muscle. In the present instance this procedure is valid since the intact limb was unaffected by any treatment, as discussed in a previous paper.[5]

The creatine concentration of the intact muscles was found to lie between 390 and 425 mg % with an average value of 401.5 mg %. The 35 mg % variation is beyond the experimental error of the method and is due largely to the variation between animals. Denervation of the muscle of one limb caused little or no progressive effect on the creatine concentration of the opposite intact muscle. For comparison with the creatine concentration of the denervated muscle the average creatine concentration of the intact muscles is probably a better value than any one individual determination since the intact values were relatively constant. The time course of the difference between either the average or individual creatine concentration of the intact muscles and the individual creatine concentration of the denervated muscles parallels the denervated muscle weight loss. The fact that small denervated muscle weight values are associated with small creatine *concentrations* is evident from a correlation coefficient of 0.96 which is highly significant with a probability of much less than 0.01. The significance of this finding is in the fact that while one would expect the smaller denervated muscles to have a smaller total creatine content, there is actually a smaller creatine *concentration* in the denervated muscles which means that total creatine loss is greater than can be accounted for simply on the basis of muscle weight. This implies that if creatine is intimately related to the contractile mechanism, then the impairment of the contractile mechanism with denervation occurs on a steeper gradient, that is, more rapidly and to a greater extent than is reflected by muscle weight loss.

Conclusions. Average dry weight ratios of denervated and intact muscles are not significantly different from average wet weight ratios on the 14th day after denervation. Creatine concentration parallels weight loss following denervation during the experimental period used. Although one determination appears to be as valid as the other

[2] Rose, William C., Helmer, Oscar M., and Chanutin, Alfred, *J. Biol. Chem.*, 1927, **75**, 543.

[3] Horvath, S. M., *J. Cell. and Comp. Physiol.*, 1941, **17**, 315.

[4] Seecof, David P., Linegar, Charles R., and Myers, Victor C., *Arch. Int. Med.*, 1934, **53**, 574.

[5] Solandt, D. Y., DeLury, D. B., and Hunter, John, *Am. J. Physiol.*, 1943, **140**, 247.

in estimating the extent of atrophy it is evident that there is a greater net loss of creatine than can be explained simply on the basis of weight loss. Both weight loss and creatine loss are progressive with time up to 14 days after denervation.

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Potassium vs. Biotin in the Treatment of Progressive Paralysis in Dogs.

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In earlier reports from this laboratory[1,2] a progressive paralysis was described in dogs subsisting on a diet lacking some members of the B complex and very low in potassium, 0.006%. This paralysis was invariably fatal if untreated and ran its course in 12-24 hours after the progressive stage was established. The mineral lack was suspected as a contributing cause, but since the first dog treated with potassium chloride, 700 mg total dose (55 mg/kg), failed to improve and later responded to 1 mg total dose (80 μg/kg) of synthetic biotin, we then centered our attention on the significance of biotin.

While this work was in progress, Elvehjem and his colleagues[3] succeeded in curing with much larger doses of potassium several dogs that had become paralyzed while subsisting on the same deficient diet. Consequently, the next 3 dogs to become paralyzed in this laboratory were treated orally with potassium chloride in much larger doses, 2-5 g total dose (200-500 mg/kg) as compared with the 0.7 g (55 mg/kg) used in the first unsuccessful treatment. This dose level proved entirely adequate, resulting in prompt and complete reversal of the paralytic process.

There is a marked difference in the therapeutic effects of potassium and biotin on the paralysis even in the same animal (Fig. 1). In making therapeutic tests, only animals that had reached the progressive stage of the paralysis were used. In our experience, animals with neck symptoms only are subject to spontaneous recovery, and, therefore, are not reliable for such tests. The paralyzed animal is usually somewhat emaciated, markedly dehydrated, anuric and suffers from complete anorexia during the attack. After treatment with either biotin or potassium chloride and about the time there is evidence of clinical improvement, copious urination occurs.

In the potassium-treated dogs the reversal of the paralysis was very rapid after it was once initiated. There was usually a delay of 3-6 hours before any clinical improvement occurred in our potassium-treated dogs with a dosing schedule of 1 g of potassium chloride in water solution given orally at 30-60 minute intervals. It was rather dramatic to see a dog sprawled on the floor, apparently functionless in neck and all 4 extremities, suddenly stagger to his feet and in a matter of a few minutes regain function to the extent of being able to walk in an almost normal manner. Without further treatment there is a prompt return of appetite, usually some gain in weight and return to normal function while still subsisting on the deficient diet. A much smaller total dose of potassium is required to overcome anorexia than to overcome the paralysis (Fig. 1). A single adequate potassium treatment affords protection from paralysis for 6-10 weeks. The smallest dose curative of paralysis was 200 mg of potassium chloride per kg of body weight. One hundred mg per kg failed to cure, and intermediate amounts have not yet been tried.

In contrast to this, the biotin-treated dogs

1 Smith, S. G., *Science*, 1944, **100**, 389.

2 Smith, S. G., *Am. J. Physiol.*, 1945, **144**, 175.

3 Elvehjem, C. A., personal communication, December, 1945.