treated animals. In animals eating *ad lib.*, estrogen treatment for 3 weeks or more resulted in a depression of body weight accompanied by a voluntary restriction in food intake; body weight was more greatly depressed than food intake. Restriction of food intake in the controls to amounts eaten *ad lib.* by the treated animals duplicated the effects of the estrogen on depression of body weight, since the growth curves of pair-fed control and treated groups became parallel.

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Received June 25, 1957. P.S.E.B.M., 1957, v96.

Free Amino Acid Patterns of Certain Tissues from Potassium-Deficient Dogs.* (23394)

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Rats placed on potassium-deficient diets, with or without simultaneous administration of desoxycorticosterone acetate (DCA), exhibit typical changes of the electrolyte compositions of plasma and skeletal muscle(1,2). Thus, there is an increased plasma bicarbonate content and a lowered plasma chloride concentration in association with a loss of skeletal muscle potassium and an unequivalent gain of muscle sodium. Cooke *et al.*(3) developed the thesis that the unequivalent distribution of potassium and sodium is accompanied by a passage of hydrogen ions into the muscle cell, thus accounting for the alkalosis of the extracellular fluid. Dogs fed a potassium-deficient diet, with or without simultaneous administration of DCA, also exhibit a loss of skeletal muscle potassium and an unequivalent gain of sodium. However, the increased plasma bicarbonate content and fall of the plasma chloride concentration become conspicuous only with simultaneous dietary restriction of chloride(2,4).

Evidence was recently presented(5), on the basis of paper chromatography, that the amino acids lysine and arginine were increased in the skeletal muscle, diaphragm and kidneys from potassium-deficient rats, while simultaneously there appeared to be decreased concentration of the amino acids aspartic acid and glutamic acid in these same tissues. The suggestion was made that possibly the changes in the concentrations of basic and acidic amino acids in the skeletal muscle of the rat may compensate in part the discrepancy between the potassium loss and sodium gain. This view was supported by the observations of Eckel, Pope and Norris(6) that from 8-40% of the metallic cation deficit present in the potassium-depleted rat skeletal muscle could be accounted for by the amino acid lysine.

The results with rats prompted the present study of the free amino acid patterns of skeletal muscle, left ventricle and kidneys from potassium-deficient dogs. Special attention was given to potassium and sodium concen-

* This investigation was supported by a research grant from the Nat. Inst. of Health.

trations and free amino acid patterns of skeletal and heart muscle in relation to changes of plasma chloride and bicarbonate concentrations.

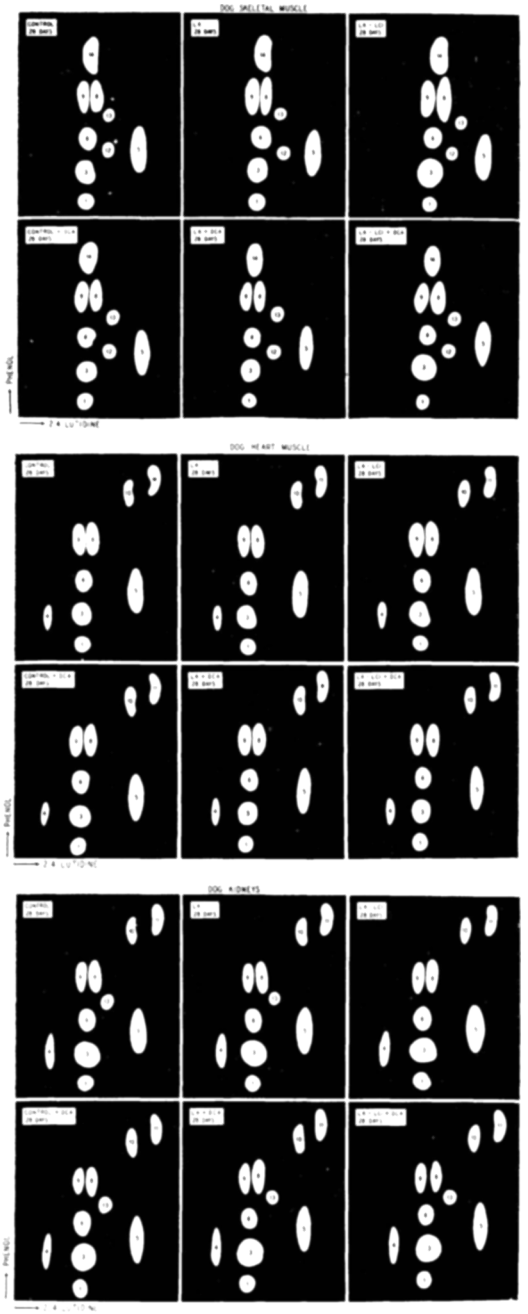

FIG. 1, 2 and 3. Free amino acid patterns of skeletal muscle, left ventricle and kidney. Numbers within the tracings indicate the following substances: 1, aspartic acid; 3, glutamic acid; 4, lysine; 5, taurine; 6, glycine; 8, glutamine; 9, alpha-alanine; 10, valine; 11, leucine; 12, serine; 13, threonine; 14, carnosine.

Methods. Young adult mongrel female dogs were treated in exactly the same manner as described previously(4). The animals given DCA received 0.5 mg DCA (Percorten, Ciba Pharmaceutical Products, Inc.) per kilo body weight per day by subcutaneous injection. At the close of the given dietary period, the animals were placed under sodium pentobarbital anesthesia for collection of blood and tissues. The sample of skeletal muscle (lumbar portion, sacrospinalis), the kidneys and the heart were quickly excised, blotted free of adhering blood, and cleaned of capsulae or connective tissue. An aliquot of the tissues was quickly weighed and immediately placed in cold (0°C) tungstic acid (1 part tissue plus 9 parts tungstic acid (prepared in the ratio of 1 part 10% sodium tungstate: 1 part ⅔ NH_2SO_4:7parts H_2O)), homogenized, and filtered in the cold (0°C). One-tenth ml aliquots of these tungstic acid filtrates were used directly for paper chromatography, utilizing the same technic as in previous experiments with rat tissues(5), employing as developing solvents phenol (80%) and 2,4 lutidine (65%). The methods for chemical analyses of plasma, skeletal muscle and left ventricle were the same as previously described(4).

Results. Fig. 1, 2, and 3 show the average tracings of the chromatograms obtained with tungstic acid filtrates of skeletal muscle, left ventricle and kidney from normal and potassium-deficient dogs. In Table I are presented the electrolyte data for plasma, skeletal muscle and left ventricle.

For a given tissue (skeletal muscle, left ventricle and kidney) the substances identified qualitatively with the volume of tungstic acid filtrate used in spotting the chromatograms were the same in the control and potassium-deficient dogs (Fig. 1,2,3). From previous experience with rat tissues(5), the absence of a difference between the chromatograms in the control and experimental dogs, particularly with respect to skeletal muscle, came as a surprise. In the case of rats, the conspicuous changes in skeletal muscle with

TABLE I. Average Electrolyte Composition of Plasma, Skeletal Muscle, and Left Ventricle.

Diet*	Plasma†		Skeletal muscle		Left ventricle	
	HCO_3	Cl	Na	K	Na	K
	mEq					
Control	24.8	116.8	8.6‡	37.8‡	15.6	41.1
" + DCA	25.7	113.2			15.2	39.0
Low K	20.3	122.8	10.3‡	33.8‡	15.1	40.6
" + DCA	31.7	108.5	14.3‡	26.5‡	14.7	37.6
" & low Cl	36.5	101.8	13.9‡	34.2‡	18.9	37.5
Idem + DCA	43.8	96.6	30.5	30.8		

* Dietary period of 28 days. Two dogs in each group.

† Plasma values are given in terms of 1000 g water, and muscle values in terms of 100 g fat-free solids.

‡ Skeletal muscle data from comparably treated dogs reported previously(4).

a cellular potassium loss was a gain of the amino acids lysine and arginine and a tendency toward a loss of the amino acids glutamic and aspartic. In the dog similar changes were anticipated, particularly in the animals fed the low-potassium or low-potassium and low-chloride diets, with or without simultaneous administration of DCA, since the skeletal muscle exhibited a lowered potassium content under these circumstances (Table I).

Discussion. No explanation can be given for the difference between the free amino acid patterns of skeletal muscle from potassium-deficient dogs and those previously reported (5) for skeletal muscle from potassium-deficient rats. The present findings do not exclude the possibility that the conditions under which the extraction of amino acids was effected in rat tissues are unsuitable for dog tissues. However, the fact that the pattern of identifiable amino acids in the skeletal muscle from the 2 species is identical argues against the observed difference being attributable to the method of amino acid extraction. The difference also appears unexplainable on the basis of smaller electrolyte changes in dog plasma or skeletal muscle. For example, the dogs fed the low-potassium and low chloride diet and given DCA exhibited marked alterations in the electrolyte compositions of both the plasma and skeletal muscle (Table I), yet the free amino acid pattern was identical with that of the control.

The significance of the alterations of apparent concentrations of basic and acidic amino acids with respect to the mineral cation deficit observed in skeletal muscle from potassium-deficient rats requires further study. Additional experiments involving quantitative measurement of free amino acids are required to evaluate this point, and, such data may help to explain the differences observed between rats and dogs.

Summary. Tracings of the amino acid patterns obtained chromatographically on the tungstic acid acid filtrates of skeletal muscle, left ventricle and kidney from normal and potassium-deficient dogs have been presented. The amino acid patterns of the tissues from control and potassium-deficient dogs were found to be identical, even in the presence of marked changes in the electrolyte compositions of plasma and skeletal muscle. Differences between amino acid patterns of the skeletal muscle from potassium-deficient dogs and of the skeletal muscle from potassium-deficient rats previously reported were pointed out.

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Received June 25, 1957. P.S.E.B.M., 1957, v96.