7. Ong, E. B., Shaw, E., Schoellmann, G., J. Biol. Chem., 1965, v240, 694.

8. Schwert, G. W., Takenaka, Y., Biochim. Biophys. Acta, 1955, v16, 570.

9. Schaffer, N. K., May, C. S., Jr., Summerson, W. H., J. Biol. Chem., 1953, v202, 67.

10. Dixon, G. H., Kauffman, D. L., Neurath, H., ibid., 1958, v233, 1373.

11. Oosterbaan, R. A., Van Adrichem. M. E., Biochim. Biophys. Acta, 1958, v27, 423.

12. Walsh, K. A., Neurath, H., Proc. Nat. Acad. Sci., U. S., 1964, v52, 884.

13. Avakian, S., Clin. Pharmacol. Therap., 1964, v5, 712.

14. Krahl, M., The Action of Insulin on Cells, Academic Press, New York, 1961.

15. Hartley, B. S., Nature, 1964, v201, 1284.

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Na and K Bound to an Ether Soluble Compound in Myocardium of Renal Hypertensive Dogs.* (30230)

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Sodium metabolism has been suspected of being deranged in the disease state hypertension. The evidence for this is the effectiveness of a low sodium diet in clinical hypertension (1) and the use of desoxycorticosterone and high NaCl intake to produce experimental hypertension(2).

Such a derangement of sodium metabolism has not been readily shown by methods of tissue analysis. In our laboratory we have been interested in the fact that sodium is contained in an ether extract of tissues, presumably bound to an ether soluble compound. This study is to determine whether the amounts of this compound might be altered in the myocardium of hypertensive compared with normotensive dogs.

Methods. Animals. Adult dogs, 11 male and 1 female, 18 to 28 kg, were maintained on a standard commercial diet. The mean blood pressure was measured 2 to 3 times a week by femoral artery puncture with a 20-gauge needle, until a constant control pressure was obtained. The control mean blood pressures were from 95 to 130 mm Hg. The dogs were then subjected to the Grollman procedure to produce renal hypertension. In the Grollman procedure a figure-of-eight ligature is placed around one kidney; 2 weeks later the contralateral kidney is removed. All surgery was carried out under pentobarbital anesthesia (30 mg/kg).

After recovery from surgery the blood pressure was measured weekly or biweekly until hypertension became established or until it was apparent that hypertension would not develop. Hypertension was considered present when the mean blood pressure was maintained at 40 mm Hg above preoperative levels. The mean blood pressure in dogs with hypertension ranged from 160 to 175 mm Hg. The dogs were sacrificed by anesthetizing with pentobarbital and rapidly opening the chest and removing the heart for samples. Triplicate samples were taken from each ventricle.

Of the 11 dogs subjected to the Grollman procedure 5 developed hypertension. Six dogs showed no rise in blood pressure with blood pressures between 90 to 130 mm Hg. These and one sham operated animal were considered controls.

Analytical technique. The samples of myocardium (1 to 3 g) were frozen. Later they were thawed and subjected to an extraction procedure for ether soluble compounds containing sodium and for ether soluble compounds containing potassium (ESC-Na and ESC-K)(3). Previous studies in the laboratory revealed no changes in these components due to storage in the frozen state. Tissues from a control dog were analyzed at the same time as tissues from a hypertensive

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TABLE I. Na and K in an Ether Extract of Myocardium.

	Hypertension		Control		
	No.	meq/kg	No.	meq/kg	P
		Left ventricle			
Na	5	9.98 ± 1.45*	7	5.77 ± 1.18	<.02
K	5	7.58 ± .83	7	5.47 ± .54	>.05
		Right ventricle			
Na	5	7.41 ± 1.02	7	5.56 ± .45	>.05
K	5	5.25 ± .53	7	4.78 ± .42	>.05

* Avg ± standard error.

animal to control the effect of minor variations in technique on the results.

Statistics. The values obtained from the triplicate analysis were averaged for each tissue of each dog. The data on the control and experimental groups of animals were then analyzed using Student's "t" test as described by Fisher(4).

Results. The results of analyses of the right and left ventricles are shown in Table I. The values are reported in milliequivalents of Na or K bound by the compounds in the extract. There was an increase in the ESC-Na and also in ESC-K in the left ventricle in hypertension. However, the increase of ESC-K was not significant, nor was there a correlation between the values of ESC-Na and ESC-K in the left ventricle.

Discussion. Except for changes in electrolyte content of the aorta in hypertensive rats, workers have generally failed to find consistent changes in the electrolyte content of tissues of hypertensive compared to control animals(5). Evidence implicating a disturbed sodium metabolism in hypertension has been cited above. The existence of a compound binding sodium has been hypothecated to fulfill one theory of a sodium transport mechanism(6). The discovery of ESC-Na is then compatible with this theory. It seems possible that the amount of this compound might be affected by the disease process hypertension. This compound contains phosphate and is presumed to be a phospholipid.

We have found changes in the ESC-Na content of the myocardium of the left ventricle. The significance of this finding is yet to be evaluated. The finding of a chemical disturbance in the ventricle in hypertension is compatible with the morphological evidence reported by Muirhead that the myocardium is involved in the disease process (7). Unfortunately there is insufficient material obtainable from the arterioles where classical lesions of hypertension occur for analysis for ESC-Na.

Summary. The sodium and potassium contained in a phospholipid extract of tissue were determined on tissues of renal hypertensive and operated normotensive control dogs. Hypertension produced an increase in the sodium contained in a phospholipid extract of left ventricle. There was a slight but not statistically significant increase in the sodium bound in the phospholipid extract of the right ventricle. There was potassium bound by these phospholipids but the amount did not change significantly in either ventricle in hypertension.

1. Grollman, A., Harrison, T. R., Mason, M. F., Baxter, J., Crampton, J., Reichsman, F., J. Am. Med. Assn., 1945, v129, 533.

2. Knowlton, A. I., Loeb, E. N., Stoerk, H. C., Seegal, B. C., J. Exp. Med., 1947, v85, 187.

3. Susat, R., Vanatta, J. C., Proc. Soc. Exp. Biol. and Med., 1962, v109, 317.

4. Fisher, R. A., Statistical Methods for Research Workers, Oliver & Boyd, Edinburgh, 1936, 128.

5. Tobian, L., Physiol. Rev., 1960, v40, 280.

6. Ussing, H. H., Handbuch der Experimentellen Pharmakologie, Supplement, Springer-Verlag, Berlin, 1960, v13, 1.

7. Muirhead, E. E., Vanatta, J. C., Grollman, A., Arch. Path., 1949, v48, 234.

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