one of our animals, Dog 3, which received 1,000 mg/kg of Tibione, a maximum blood concentration of 0.33 mg per 100 ml (3.3 γ/ml) was found. This level was even less than that achieved by 1/100 the dose of nicotinaldehyde thiosemicarbazone, *i.e.*, 10 mg/kg. At the end of eight hours after the latter dosage, the level still remained above the maximum level obtained with Tibione. (Fig. 2).

Our results are substantially in agreement with those of a recent work by Spinks(12), who found blood levels of Tibione of the order of 3 γ/ml in the first one to two hours in therapeutic applications. His value (.4 γ/ml) in dogs using a dose of 5 mg/kg is the same as our value for nicotinaldehyde thiosemicarbazone suggesting that at this dosage level the low solubility of Tibione does not yet limit absorption.

The photostability of the compounds in n-hexanol in diffuse daylight can be expected from Spinks' data and appears confirmed by our results. No erratic readings were observed with our standards and all spectrophotometric readings, which were routinely checked back after the absorption curves were recorded, were stable within .005 of the density scale of the Beckmann spectrophotometer. Our recovery factor for Tibione using n-hexanol is .91 as compared to about .80 using chloroform reported by Spinks.

In rats having received comparable amounts of nicotinaldehyde thiosemicarbazone hydrochloride and Tibione, *i.e.*, 0.05% in their diets for 13 weeks, the concentration in the blood of the former agent (7.5 γ/ml) was roughly 10 ten times that of the latter agent (0.77 γ/ml). It is thus apparent from these studies in rats as well as those in dogs that nicotinaldehyde thiosemicarbazone hydrochloride is absorbed to a very great degree as compared with Tibione.

Summary. (1) A spectrophotometric method has been developed for the determination of nicotinaldehyde thiosemicarbazone in blood. (2) Following single oral doses of 200 mg/kg in dogs, the maximum blood concentration achieved by nicotinaldehyde thiosemicarbazone was ten to forty times greater than that of p-acetylaminobenzaldehyde thiosemicarbazone. (3) Following the chronic daily oral ingestion of 0.05% of these drugs in the diet of rats, the average blood concentration attained by nicotinaldehyde thiosemicarbazone was approximately 10 times that attained by p-acetylaminobenzaldehyde thiosemicarbazone.

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12. Spinks, A., *Brit. J. Pharmacol.*, 1951, v6, 35.

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Changes in Plasma and Tissue Water and Electrolytes After Epinephrine Administration in Rats. (19092)

ABRAHAM DURY AND THOMAS N. JOHNSTON.
From the Dorn Laboratory for Medical Research, Bradford Hospital, Bradford, Pa.

The great difference in the concentrations of potassium in the extracellular and intracellular fluid compartments has often raised the question of hormonal regulation of the "balance" between these two compartments. The adrenal cortex has been particularly considered as the secretory source of hormonal regulation of potassium balance in the organism because of the changes that occur in adrenocortical deficiency and when desoxycorticosterone is used. However, this view has not been generally acceptable(1). The effect of desoxycorticosterone on the renal excretion of potassium has been shown by Harrison and Darrow(2) to be sufficient to explain the change in potassium balance. It is of interest

1. Gaunt, R., Birnie, J. H., and Eversole, W. J., *Physiol. Revs.*, 1949, v27, 4.

2. Harrison, H. E., and Darrow, D. C., *Am. J. Physiol.*, 1939, v125, 631.

that the relevant literature provides evidence that other known hormonal agents than adrenocortical steroids affected the level of plasma potassium. D'Silva(3), Brewer *et al.* (4), Keys(5) and others have shown that the injection of adrenaline and insulin will cause a fall in the level of plasma potassium in man and animals. Dury *et al.*(6) have reported that there was a distinctive pattern of change in the plasma potassium level in normal humans after the injection of epinephrine which was different from that obtained in epileptic patients and those with Addison's disease. The significance of epinephrine as a hormonal regulator of potassium balance has been raised by the demonstration of Tum Suden(7) that after sympathetic blockade by ergotamine in rats the toxic dose of potassium is diminished, and by the recent report of Dury(8) that adrenalectomized rats were protected from fatal hypergalemic levels when pretreated with epinephrine. Furthermore, Dury(9) has presented evidence that epinephrine decreased the level of plasma potassium without the mediation of the anterior pituitary-adrenocortical axis.

However, no data have been presented in the experiments referred to above on what changes actually occurred in blood and tissue electrolytes after the administration of epinephrine other than its effect upon plasma potassium level. In this report data will be presented on the water and electrolyte content in the blood and tissues of intact and adrenalectomized groups of rats which were untreated compared with those 60 minutes after the administration of epinephrine.

Materials and methods. Normal intact and adrenalectomized (6-7 days) male Wistar rats weighing 225-250 g were used. The rats were given a chow biscuit *ad libitum* and greens twice weekly before they were used in the experiments. The adrenalectomized rats were maintained on 1% NaCl drinking solution. After an overnight fast the animals were anesthetized with n-methylcyclo-hexenyl-methyl barbituric acid (Evipal) and either used immediately without further procedure or were pretreated with a subcutaneous injection of epinephrine and then killed 60 minutes later. The epinephrine (adrenaline tablets; Parke, Davis & Co.) was prepared fresh by solution in physiological saline and injected at a dose level of 0.04 mg/100 g body weight (approximately 0.5 ml). Cardiac blood was obtained in a dry syringe containing 1 drop of heparin (Organon, 10 mg/ml) and immediately centrifuged. Aliquots of plasma were taken for the determination of potassium, sodium, chloride, glucose and the per cent of plasma water. Immediately after the cardiac blood had been obtained the abdomen and thorax were opened; the heart and hepatic vein incised and bled; and a specimen of liver weighing approximately 1 g was removed. The specimen was gently blotted free of blood and weighed immediately. The gastrocnemius muscle of each hind leg was then exposed, freed of nerves lying on its surface or entering the body of the muscle and weighed immediately. One muscle specimen was used for the determination of chloride content by the method of Van Slyke(10). The other muscle and the specimen of liver were used for the analyses of sodium, potassium and water content after weighing, drying and ashing. The specimens were dried overnight at 105°C; and ashed overnight at 425°C. Sodium and potassium determinations of plasma and tissues were done after proper dilutions with the aid of an internal lithium standard flame photometer. Plasma chloride was determined by the method of Schales and Schales(11). Plasma glucose was determined by the photometric method of Kingsley and Reinhold(12). The concentration of plasma electrolytes as

3. D'Silva, J. L., *J. Physiol.*, 1937, v90, 303.

4. Brewer, G., Larson, P. S., and Schroeder, A. R., *Am. J. Physiol.*, 1939, v126, 708.

5. Keys, A., *Am. J. Physiol.*, 1938, v121, 325.

6. Dury, A., Holler, J. W., and Smith, C., *Fed. Proc.*, 1950, v9, 35.

7. Tum Suden, C., *Am. J. Physiol.*, 1947, v149, 589.

8. Dury, A., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 199.

9. Dury A., *Endocrinology,* in press.

10. Van Slyke, D. D., *J. Biol. Chem.*, 1941, v140, 879.

11. Schales, O., and Schales, S. S., *J. Biol. Chem.*, 1941, v140, 879.

TABLE I. The Reduction in Plasma Potassium 60 Min After Injection of Epinephrine in Intact and Adrenalectomized Rats.

Animals used	% plasma water	Plasma electrolytes m. eq./kg plasma water			Plasma glucose, mg %
		Cl	K	Na	
Normal					
No inj (12)	92.6 ± .06*	108.5 ± .6	4.76 ± .1	158.1 ± .8	102
After epinephrine† (10)	92.4 ± .08	107.6 ± .8	4.41 ± .08 (7) .025‡	156.7 ± .8	301
Adrenx					
No inj (9)	94.1 ± .17	112.8 ± 1.3	5.28 ± .19 (8)	147.7 ± 2.2	45
After epinephrine (13)	93.7 ± .11 (12)	110.8 ± 1.1	4.31 ± .14 (9) .0009	150.8 ± 1.7 (11)	159

* Mean ± S.E. † Epinephrine injected subcutaneously at level of .04 mg/100 g body wt. ‡ P value of the difference between the means occurring by chance alone; when not given P was not significant. No. in parentheses is the No. of rats.

m.eq./kg plasma water and the concentration of muscle and liver electrolytes as m.eq./kg wet tissue were derived from the data. The methods for the calculation of other derived data which are presented in the results were:

$$[Cl]_e = \frac{[Cl]_p}{0.95}; \frac{(Cl)_t}{[Cl]_e} \times 1000 = (H_2O)_e$$

g/kg wet tissue; $(H_2O)_t - (H_2O)_e = (H_2O)_i$ g/kg wet tissue. The subscripts p, t, e and i represent plasma, whole tissue, extracellular and intracellular phase, respectively. The difference between the means of the plasma and tissue water and electrolytes in the groups of untreated and epinephrine pretreated intact and adrenalectomized rats were tested for statistical significance.

Results. Inspection of the data in Table I reveals that aside from the known hyperglycemic effect of epinephrine the level of plasma potassium was significantly decreased in the normal and adrenalectomized groups of rats 60 minutes subsequent to the epinephrine injection. The results indicate that the action of the agent was limited in its effect to the plasma potassium and did not change the concentration of the chloride or sodium nor the per cent of plasma water. The change in the level of plasma potassium was more marked in the adrenalectomized than the intact groups of rats subsequent to the epinephrine injection. A difference in the extent of absolute decrease of the plasma potassium level after epinephrine had been noted in other animal studies(9) of intact and adrenalectomized rats. A similar difference in the extent of plasma potassium decrease after epinephrine has been reported in humans(6). It has been observed that the level of potassium was decreased to a greater extent and over a longer period of time in subjects with Addison's disease than in normal humans after the administration of epinephrine.

The results of the analyses of water and electrolyte content of the gastrocnemius muscle of intact and adrenalectomized groups of rats untreated and after the administration of epinephrine are given in Table II. Inspection of these data reveals that the adrenalectomized group 60 minutes subsequent to the epinephrine had a significant decrease in its potassium content as well as potassium concentration per kg of tissue water in comparison with the control group. There also were significant changes in the distribution of the tissue water with a significant decrease in the intracellular phase concomitant with an increase in the extracellular phase. The change in the muscle water balance is in accord with the change in the potassium content of the tissue. It is noteworthy that the sodium and chloride content of the muscle in the adrenalectomized-epinephrine treated group were not changed when compared with the control group.

In the intact groups of rats epinephrine administration resulted in changes in muscle potassium content and the balance between extracellular and intracellular water similar

12. Kingsley, G. R., and Reinhold, J. G., *J. Lab. and Clin. Med.*, 1949, v34, 713.

TABLE II. The Effect of Epinephrine on Muscle Water and Electrolytes in Intact and Adrenalectomized Rats.

Animals used	Muscle water, g/kg wet tissue			Muscle electrolytes					
				m. eq./kg wet tissue			m. eq./kg tissue water		
	Total	ECW	ICW	Cl	K	Na	K	Na
Normal									
No inj (12)	757 ± 1.2*	92 ± 1.2	664 ± 1.6	10.6 ± .14	99.3 ± 1.3	30.9 ± 1.3	131.1 ± 2.2	40.8 ± 1.7
After epinephrine† (10)	763 ± 1.6	108 ± 3.2	655 ± 3.2	12.2 ± .3	93 ± 1.8	37.2 ± 1.5	121.9 ± 2.5	48.1 ± 1.9
	.006‡	.0001	.01	.0001	.02	.003	.01	.009
Adrenx									
No inj (9)	770 ± 1.4	117 ± 4	653 ± 4.7	13.7 ± .6	90.1 ± 1.6	36.8 ± 1.9	117 ± 2.2	56.4 ± 4.9
After epinephrine (13)	775 ± 1.1	129 ± 3.6	646 ± 3.4	14.9 ± .4	85 ± 1.1	40.8 ± .8	109 ± 1.5	51.5 ± 2.3
	.01	.0001	.0001		.01		.01	

* Mean ± S.E. † Epinephrine injected subcutaneously at level of .04 mg/100 g body wt. ‡ P value of the difference between the means occurring by chance alone; when not given P was not significant.
No. in parentheses is the No. of rats.

to those described in the adrenalectomized group. However, in addition to the above changes there was also a significant increase in both the sodium and chloride content of the muscle. In conjunction with the latter changes it will be noted that the epinephrine-treated intact group experienced a much greater increase in the total and extracellular water content than the adrenalectomized group subjected to the same procedures.

The results of the analyses of liver sodium, potassium and total water content in intact and adrenalectomized groups of rats are shown in Table III. Comparison of the respective epinephrine-treated groups with their controls shows that the total water, sodium and potassium content were not statistically different 60 minutes after epinephrine. It is well known that the hyperglycemia which follows the administration of epinephrine is the result of a rapid hepatic glycogenolysis. It is also known that there is a relationship between liver carbohydrate and potassium metabolism(13,14). Theoretically, therefore, livers which are metabolically in the status of high glycogenolytic activity should be lower than "normally" in potassium content. The results presented here show that this was not the case at the time after epinephrine injection which was chosen for liver analyses. Further investigation of this is certainly indicated.

Discussion. The present results illustrate that epinephrine administered to rats resulted 60 minutes later in a significant decrease in the level of plasma potassium and the potassium content of muscle tissue. There also was a significant decrease in the muscle content of intracellular water together with a significant increase in the extracellular water content. The physiological importance of this mechanism was recently shown(8) by demonstrating that epinephrine protected adrenalectomized rats from an experimentally induced fatal hyperkalemia.

Inspection of Table II showed that the muscle potassium content was decreased 60 minutes after epinephrine administration in

13. Fenn, W. O., *Physiol. Revs.*, 1940, v20, 377.
14. Fenn, W. O., *J. Biol. Chem.*, 1939, v128, 297.

TABLE III. The Lack of an Effect of Epinephrine on Liver Water and Electrolytes in Intact and Adrenalectomized Rats.

Animals used	Total water, g/kg tissue	Electrolytes: m. eq./kg wet tissue K	Na	m. eq./kg tissue H_2O K	Na
Normal					
No inj (12)	686 ± 2.8*	83.7 ± 2 (11)	34.5 ± 2 (11)	122.1 ± 3	50.3 ± 2.8
After epinephrine† (10)	689 ± 2.8	87.7 ± 2.3	33.2 ± 1.3	127.3 ± 3.4	48.1 ± 1.9
Adrenx					
No inj (9)	719 ± 4.1	77.3 ± 3.3	40.5 ± 3.1	107 ± 4.8	56.4 ± 4.9
After epinephrine (13)	720 ± 1.9	83.9 ± 2.2	37.1 ± 1.6	116 ± 3	51.5 ± 2.3

* Mean ± S.E. † Epinephrine injected subcutaneously at level of .04 mg/100 g body wt. No. in parentheses is the No. of rats.

both the adrenalectomized and intact groups when compared with their respective controls. However, the sodium content of the muscle of adrenalectomized rats was not changed after epinephrine, but both the sodium and chloride content was increased in the muscle of the intact-treated group. It is apparent that some factor(s) other than epinephrine, *per se,* was related to this difference in results between the intact and adrenalectomized groups. There is sufficient evidence in the literature that adrenocortical steroids induce sodium retention in the blood and tissues. It has also been demonstrated that epinephrine will induce the release of adrenal steroids shortly after its injection in an animal. It is therefore suggested that the change in the muscle content of sodium in the intact epinephrine-treated group as distinguished from the absence of any sodium change in the adrenalectomized group was the resultant of the mechanism outlined above. However, the decrease in the potassium content of the muscle tissue of both intact and adrenalectomized epinephrine-treated groups, as well as the significant decreases in the level of plasma potassium, suggest that epinephrine probably has a significant role in the physiological regulation of potassium balance in the fluid compartments of the body.

Summary. Analyses of plasma and tissue water and electrolytes content were made in intact and adrenalectomized groups of rats untreated and 60 minutes after epinephrine injection. Evidence was presented that the plasma potassium concentration was significantly decreased in intact and adrenalectomized rats after epinephrine compared to their respective controls. The potassium content of muscle tissue was significantly decreased in the epinephrine treated intact and adrenalectomized groups. There was no change in the water, sodium or chloride concentration of plasma after epinephrine in either intact or adrenalectomized groups. However, there was a significant decrease in intracellular water content and increase in extracellular water content which was briefly discussed as linked to the change in potassium content. None of these changes were determined in liver tissue of the intact or adrenalectomized groups 60 minutes subsequent to epinephrine injection. In view of the above results and the evidence that the sodium and chloride content of muscle of the intact group was significantly increased after epinephrine but not so in the adrenalectomized group, the results were briefly discussed as evidence of an effect of epinephrine on the potassium "balance" in the compartments of the organism.

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