Changes in Electrolyte and Water Composition after Glucose in Unprotected and Epinephrine-Protected Rats. (19363)

ABRAHAM DURY. (With the technical assistance of Thomas N. Johnston.)
From the Dorn Laboratory for Medical Research, Bradford Hospital, Bradford, Pa.

Several investigators(1-3) have reported that the level of plasma potassium was definitely lower than the initial K level about 20 minutes after injection of epinephrine or insulin in man and other animals. Recently, Dury, *et al.*(4) reported that the pattern of change in plasma potassium concentration and the extent of fall from initial level, in the course of 2 hours after epinephrine, was different in patients with Addison's disease and with idiopathic epilepsy than the response in normal humans similarly tested. Tum Suden(5) reported that after sympathetic blockade with ergotamine, in rats, the toxic dose of potassium was diminished; and Dury and Johnston(6), showing that there was a significant decrease in plasma and skeletal muscle concentration of potassium 60 minutes after an injection of epinephrine, suggested that epinephrine may have a regulatory role in the potassium balance of the organism.

In a report from this laboratory(7) it was shown that pretreating adrenalectomized rats with a single physiological dose of epinephrine 60 minutes before admitting 2 ml of a 50% glucose solution into their stomachs protected about 80% of these animals from a fatal hyperkalemia and cardiac changes. Non-pretreated adrenalectomized rats developed a hyperkalemia and cardiac changes leading to a high percentage of mortality within a short time after glucose was given. The source of the rapidly mobilized plasma potassium in the unprotected rats, and the manner in which epinephrine-pretreatment had "blocked" the onset of hyperkalemia was not demonstrated. It is the purpose of this paper to present the changes in tissue electrolyte and water concentration in unprotected and epinephrine-protected rats after a glucose load.

Materials and methods. Male Wistar rats weighing 225-250 g were used either 6-7 days or 12-13 days after adrenalectomy. The animals were maintained on 1% NaCl drinking solution and were given a chow biscuit *ad libitum* and greens twice weekly. After an overnight fast the animals were anesthetized with n-methylcyclohexenyl-methyl barbituric acid (EVIPAL). The rats were then either given 2 ml of a 50% glucose solution (*c.p.* Dextrose, Merck) directly into the stomach by means of a fine plastic tube, or first pretreated with epinephrine 60 minutes before the glucose solution was administered. The epinephrine (Adrenaline tablets, Parke, Davis and Co.) was prepared fresh in 0.9% NaCl and injected subcutaneously at a dose level of 0.04 mg/100 g body weight (approx. 0.5 ml). After the glucose solution had been admitted into the stomach each of the rats was observed carefully for the first signs of respiratory difficulty. When this was observed blood was withdrawn from a cardiac puncture into a dry syringe containing 1 drop of heparin (Liqeamin, Organon, 10 mg/ml). On the basis of previous evidence(7) those rats which showed no signs of failure for 20 minutes after the glucose load were accepted as probably surviving the procedure and blood was obtained from these shortly thereafter. The control groups for this study were rats 6 and 12 days after adrenalectomy which had not been treated in any way, and a group of rats 6 days after adrenalectomy which were killed 60 minutes after the epinephrine injection and tissues obtained for analyses of electrolyte and water composition. The procedures followed for analyses of electrolyte and water composition of blood and tissues was the same for all animals and has been described in detail in a previous paper(6). Tissue chloride content was determined by the method of Van Slyke(8), and plasma chloride by the method of Schales and Schales(9). Plasma glucose was determined by the photometric method of Kingsley & Reinhold(10). Tissue and plasma sodium and potassium content was determined with the aid of an internal lithium standard flame photometer.

Results. The present investigation was

TABLE I. Water and Electrolyte Composition of Plasma of Non-Pretreated and Epinephrine-Pretreated Adrenalectomized Rats After a Glucose Load per os.

Animals used	Survival time after glucose, min	% plasma water	m.eq./kg plasma water Cl	K	Na	Plasma glucose, mg %
Rats 6-7 days after adrenalectomy						
a. No inj. (9)		94.1 ± .17†	112.8 ± 1.3	5.28 ± .19	147.7 ± 2.2	45
b. " " + glucose* (9)	<5 (5)	93.8 ± .17	*105.8 ± 2.6*	*7.03 ± .31*	149.6 ± 3.3	54
	10–15 (2)	94.4	110	5.88	147.6	72
	>20 (2)	93.8	106.7	4.62	152.1	118
c. Epinephrine‡ (13)		93.7 ± .11	110.8 ± 1.1	4.31 ± .4	150.8 ± 1.7	159
d. " + glucose§ (9)	10 (1)	93	115.2	5.57	×	×
	16–19 (3)	92.8	113	5.60	×	218
	>20 (5)	93.2 ± .16	115.1 ± 3.3	4.47 ± .56	152.3 ± 1.8	239
Rats 12-13 days after adrenalectomy						
e. No inj. (6)		94.2 ± .1	105.8 ± 2.5	5.79 ± .26	147.9 ± 2.1	50
f. " " + glucose (6)	<5 (5)	94 ± .19	103.1 ± 1.9	*6.68 ± .41*	147.5 ± 2.1	94
	>20 (1)	94	99.2	4.52	147.4	120
g. Epinephrine + glucose (5)	>20 (4)	93.5 ± .2	102.7 ± 1.3	*3.97 ± .08*	144.1 ± .8	239

* 2 ml-50% glucose sol. into stomachs.
† Mean ± S. E.
‡ Cardiac blood and tissues taken 60 min after epinephrine pretreatment at .04 mg/100 g wt.
§ 2 ml-50% glucose sol. into stomach 60 min after epinephrine pretreatment at .04 mg/100 g wt.
Figures in italics are significantly different from controls.

designed to detect in the non-pretreated and epinephrine-pretreated groups: 1) the changes, if any, from the respective controls of the composition of water and electrolytes in the plasma, skeletal muscle and liver of rats which showed impending mortal failure after the glucose load was placed in their stomachs; and 2) the changes, if any, in the plasma, muscle and liver composition of rats which obviously were surviving the administration of the glucose load.

Survival. The number of rats in the non-pretreated and epinephrine-pretreated groups which showed signs of failure at several time-periods after glucose *per os* and those surviving the procedure are shown in the second column of Table I. It is apparent that epinephrine-pretreatment had protected a large percent of the rats from failure after the administration of glucose. Similar survival and mortality results have been reported from this laboratory(7) for rats 16 days after adrenalectomy and similarly treated.

Plasma changes. The data presented in Table I show that impending mortal failure in rats 6 and 12 days after adrenalectomy was related to an increased plasma potassium concentration following the admission of a glucose load into their stomachs. In the non-pretreated series (b,f) there was a marked increase in the potassium concentration in the groups which showed signs of failure within 5 minutes after glucose. In the three rats of this series which showed no signs of failure throughout the designated survival period the plasma K concentration was found to be in the range of normal intact controls and somewhat lower than the mean value of the adrenalectomized controls. The relationship between increased plasma potassium concentration and low survival rate demonstrated in the non-pretreated series (b,f) is supported by the data of the epinephrine-pretreated series (d,g) in which a large percentage of the rats survived the glucose load. It is evident that a rapidly developed hyperkalemia was the single plasma change which followed the administration of the glucose load in a large number of rats in the non-pretreated series. Epinephrine-pretreatment, however, had somehow altered conditions so that after the glucose load the plasma potassium concentration had been maintained within normal range in this series of rats.

Muscle and liver changes. Non-pretreated series. The muscle electrolyte and water composition of the groups of rats in series b and f after the glucose load was found to be not

TABLE II. Tissue Water and Electrolyte Composition of Non-Pretreated and Epinephrine-Pretreated Adrenalectomized Rats After Glucose.

Series	Survival time, min*	Muscle water, g/kg wet tissue			Muscle electrolytes, m.eq./kg wet tissue			Total water, g/kg wet liver	Liver electrolytes, g/kg wet tissue	
		Total	ECW	ICW	Cl	K	Na		K	Na
				Rats 6-7 days after adrenalectomy						
a.†	(controls)	770 ± 1‡	117 ± 4	653 ± 5	13.7 ± .6	90.1 ± 1.6	36.8 ± 1.9	719 ± 4	77.3 ± 3.3	40.5 ± 3
b.	<5 (5)	770 ± 4	107 ± 3	663 ± 5	12 ± .4	86.3 ± .7	33 ± 3	722 ± 5	*68.9* ± *.6*	42.8 ± 2
	10–15 (2)	772	125	656	13.4	93.6	30.1	731	79.3	37.3
	>20 (2)	766	115	651	12.9	91.7	34.9	721	74.7	42.5
c.	(controls)	775 ± 1	129 ± 4	646 ± 3	14.9 ± .4	85 ± 1.1	40.8 ± .8	720 ± 2	83.9 ± 2.2	37.1 ± 2
d.	10 (1)	764	105	659	12.7	92	38.2	709	82.4	43.1
	16–19 (3)	759	114	645	13.2	91.2	35.4	724	84.6	35.4
	>20 (5)	764 ± 1	127 ± 2	637 ± 1	14.6 ± .5	*93.5* ± *3.1*	*33* ± *3.2*	714 ± 4	81.7 ± .8	38.6 ± 2
				Rats 12-13 days after adrenalectomy						
e.	(controls)	768 ± 7	112 ± 2	656 ± 8	12.5 ± .4	85.1 ± 1.2	38.4 ± 1.8	721 ± 2	81.6 ± 2.5	32.7 ± 2
f.	<5 (5)	775 ± 3	114 ± 5	661 ± 5	12.5 ± .7	87.5 ± .9	38.9 ± 1.8	729 ± 3	*71.9* ± *2.2*	*40.7* ± *2*
	>20 (1)	775	111	664	11.6	97.8	23.4	733	81.6	34.9
g.	>20 (4)	777 ± 2	131 ± 8	646 ± 6	14.1 ± .7	88.3 ± 2.7	35.3 ± 3.3	723 ± 3	80.2 ± 4.6	37.6 ± 4

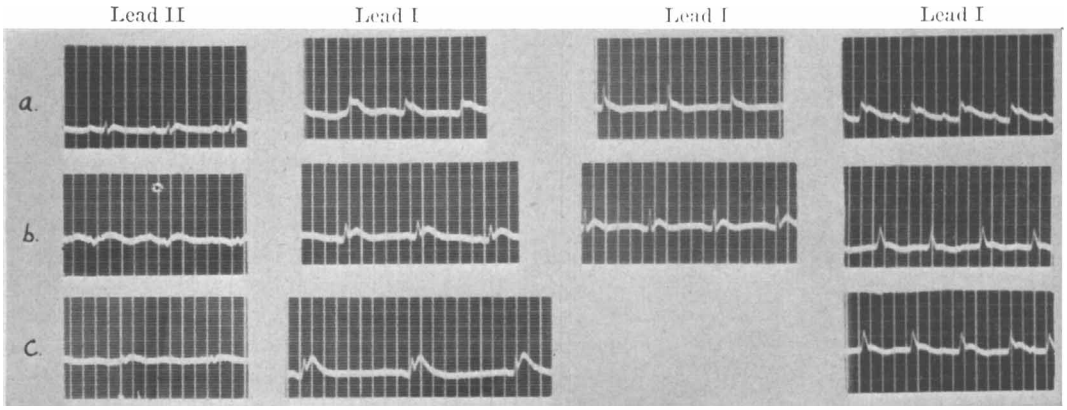
* Tissues taken at indicated survival times for analyses. † See Table I for legends. ‡ Mean ± S. E.
No. of animals in group in parentheses. Figures in italics are significantly different from controls.

different from the mean control values (Table II). However, the results of analyses of liver composition show a marked decrease in the liver potassium concentration in the groups of this series which failed within 5 minutes after the glucose load. This change, however, was not found in the few rats of this series which had survived the glucose administration for 10 minutes or more. Evidently a rapid "unloading" of liver potassium had taken place in a large number of the rats not pretreated with epinephrine.

Muscle and liver changes. Epinephrine-pretreated series. The results of analyses of muscle electrolyte and water composition of the groups in series d and g show that only the potassium concentration was significantly increased after the glucose load *per os* (Table II). This change occurred not only in the rats which had shown no signs of failure, but was found in the few rats of this series which failed 10-19 minutes after the glucose load. The results of analyses of liver composition in this series revealed that neither the concentration of liver potassium nor the other constituents had been altered after the glucose load *per os*. The finding is in marked contrast to the large decrease in liver potassium concentration found in the non-surviving groups of the non-pretreated series.

In summary, the results of the analyses of muscle and liver composition in these series of rats showed that in the non-pretreated series the essential finding in those groups of rats presenting signs of mortal failure within 5 minutes after glucose *per os* was a marked decrease in liver potassium concentration whereas muscle K concentration remained unchanged. These results were different from those found in the epinephrine-pretreated series of rats in which liver K concentration was found unchanged after the glucose load from the mean control value and the muscle potassium concentration was found to be increased.

Discussion. This investigation was undertaken in an attempt to gather evidence which would elucidate the basis for the difference in the effect of a glucose load *per os* in non-pretreated and epinephrine-pretreated adrenalectomized rats. The data in Table I showed



Rat #2G. Non-pretreated. a. Before glucose *per os*. b. 1½ min after glucose. c. 3 min after glucose; signs of failure; before blood was drawn.

Rat #12G. Non-pretreated. a. Before glucose *per os*. b. 3 min after glucose. c. 5 min after glucose; signs of failure; before blood was drawn.

Rat # 3G. Non-pretreated. a. Before glucose *per os*. b. 17 min after glucose; signs of failure; just before cardiac blood was drawn.

Rat #8G. Epinephrine pretreated. a. 60 min after epinephrine and just before glucose. b. 5 min after glucose. c. 21 min after glucose; no signs of failure; just before cardiac blood drawn.

FIG. 1. Electrocardiographic records of non-pretreated and epinephrine-pretreated adrenalectomized rats before and after glucose *per os*.

that epinephrine-pretreatment protected rats 6 and 12 days after adrenalectomy from a high mortality rate which otherwise ensued shortly after a glucose load was placed into the stomachs of the non-pretreated adrenalectomized rats. The results indicated that rapid increase in plasma potassium concentration, the marked decrease in liver potassium composition, and the low survival rate found in the non-pretreated rats shortly after a glucose load were apparently related. It is suggested that the sequence of events after the glucose load was probably as follows: a rapid release of liver potassium into the blood stream followed by a marked increase in the level of blood potassium. This rapid change affected the electrical activity of the heart which was evidenced by cardiac failure and standstill found in this series of rats. Reproductions of the electrocardiographs of some of the non-pretreated and epinephrine-pretreated rats before and after the glucose load are shown in Fig. 1. The altered cardiac activity in the former group are clearly consonant with the findings of increased potassium concentration and support the explanation for the rapid failure of a large percentage of rats in this series.*

Insofar as the possible manner in which epinephrine-pretreatment protected the adrenalectomized rats after a glucose load, the available data does not warrant more than speculation of the mechanism. However, two facts are clear from the results presented: 1) The level of plasma potassium was maintained in this series after the glucose *per os* at approximately the control level in a large percentage of the animals. Even in the few rats of this series which failed during the allotted survival period there was not the sharp increase in potassium level found in the rats of the non-pretreated series. 2) The results of analyses of the liver composition in this series suggest that a "release" of liver potassium did not occur after the glucose load. It had been shown in a previous study(6) that the concentration of potassium in plasma and muscle was significantly decreased from its initial level 60 minutes after an epinephrine

* EKG changes will be reported later. Rats were attached to a Cambridge Electrocardiograph with German Silver electrodes, designed by Drs. Hundley and Pecora of the National Institutes of Health, and made by Surveyor Service Co., Silver Springs, Md. Electrocardiographic paper was fed through machine at a speed of 100 mm/sec.

injection. This status before the administration of the glucose load could explain the evident maintenance of the plasma level in these rats. Consideration, also, should be given to the possibility that the epinephrine-pretreatment had resulted in metabolic adjustments related to increased liver glycogenolysis and peripheral utilization of glucose in these animals so that the sudden imposition of a glucose load *per os* could be taken into the metabolic pool without seriously impairing the functions of the animal.

Summary. 1. The survival rate, and the results of tissue composition are presented for rats showing signs of failure and those surviving the administration of a glucose load *per os* (2 ml– 50% solution) 60 minutes after epinephrine-pretreatment (0.04 mg/100 g wt) and those which were not pretreated. 2. Signs of mortal failure in a large percentage of the non-pretreated series of rats shortly after glucose *per os* was associated with a significant increase in the plasma potassium concentration and a significant decrease in the liver potassium composition only. 3. In the epinephrine-pretreated series a large percentage of the rats survived the glucose load *per os*. It was shown that the level of plasma potassium and the liver potassium concentration were unchanged from the control values. The muscle potassium concentration was found significantly increased after glucose in this series. 4. Representative EKG records of non-protected and epinephrine-protected rats are shown. The EKG changes in the former group are consonant with the analytical results indicating that hyperkalemia was responsible for failure of these animals. 5. The results were discussed as suggestive of: a) the sequence of changes in the non-pretreated series which led to mortal failure and low survival rate shortly after the glucose *per os*; and b) the possible manner in which epinephrine-pretreatment had altered the metabolic status of these rats before the glucose *per os* was given and thereby "protected" them from the changes in tissue composition found in the former group.

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Specific Inhibition of Precipitation as an Aid in Antigen Analysis with Gel Diffusion Method.* (19364)

BERTIL BJÖRKLUND. (Introduced by J. L. Melnick.)

From the State Bacteriological Laboratory and the Department of Virus Research, Karolinska Institutet, Stockholm, Sweden.

Antigenic material derived from bacteria, animal tissues, and similar sources is usually immunologically extremely heterogenous. Even chemically pure synthetic antigens almost always exhibit a complex immunological pattern giving rise to a number of distinct antibodies. The heterogeneity of antigens is not always demonstrable in the ordinary flocculation, agglutination or complement fixation procedures. More information can be gained by the use of the quantitative precipitation reactions performed according to the princi-

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