Epinephrine Protection Against Fatal Hyperkalemia in Adrenalectomized Rats. (18722)

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Normal humans and animals tolerate a fairly large dose of glucose solution by mouth. It is also recognized, however, that a high carbohydrate intake may precipitate Addisonian patients into a crisis(1). The factors responsible for this difference in carbohydrate tolerance are not known. At this laboratory it was observed that the administration of 2 ml of a 50% glucose solution directly into the stomachs of adrenalectomized rats was followed shortly by death. Examination of the viscera of these animals revealed only that the hearts were greatly enlarged and that death had probably resulted from cardiac standstill. The association of elevated plasma potassium concentration with the rapid onset of cardiac standstill has been demonstrated (2). It is also known that an elevated plasma potassium level is unusually toxic in animals with adrenocortical insufficiency.

It is the purpose of this report to present evidence that epinephrine protected adrenalectomized rats against the mortal effect which followed the administration of a 50% glucose solution *per os*. Epinephrine was selected to be so tested since it was recently reported(3) that this substance significantly lowered the plasma potassium concentration of normal and adrenalectomized rats.

Materials and methods. Normal intact, bilaterally adrenalectomized and bilaterally adrenal enucleated groups of male Wistar rats weighing 225-250 g were used. All rats were given a chow biscuit *ad libitum* and greens twice weekly. The adrenalectomized and enucleated rats were maintained on 1% NaCl drinking solution. Adrenal enucleation was performed by the method of Ingle and Higgins(4). A solid core of medulla and cortex was extruded from a slit in the gland and the organ was then gently milked with a forceps. A histologically normal-appearing cortex is regenerated in a short time from residual cortical cells(5). Histological study* of the regenerated adrenal glands removed from these rats showed good regeneration of all 3 cortical zones and the absence of the medulla. These rats, therefore, are termed demedullated. The adrenalectomized and demedullated rats were used in the experiments 14-16 days after their surgery. After an overnight fast the rats were lightly anesthetized with n-methylcyclo-hexenyl-methyl barbituric acid (Evipal) immediately before the administration of 2 ml of either a 50% glucose solution (c.p. Dextrose, Merck), physiological saline or mineral oil (U.S.P., light) directly into the stomach by means of a fine plastic tube. A group of adrenalectomized and demedullated rats were pre-treated with epinephrine (adrenaline tablets, Parke, Davis & Co., prepared fresh by solution in physiological saline) injected subcutaneously at a dose level of 0.04 mg/100 g of body weight (approximately 0.5 ml) 60 minutes before the administration of the glucose solution. Another group of adrenalectomized rats was pretreated with 2 ml of lipo-adrenal extract (Upjohn)† injected subcutaneously 120 minutes before the administration of the glucose *per os*. This pretreatment time-period has been shown as adequate to make available to the rat a supply of exogenous adrenal steroids(6). After the

1. Gass, H., Cherkasky, M., and Savitsky, N., *Medicine,* 1948, v27, 105.

2. Winkler, A. W., Hoff, H. E., and Smith, P. K., *Am. J. Physiol.,* 1938, v24, 478.

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

4. Ingle, D. J., and Higgins, G. M., *Am. J. Med. Sc.,* 1938, v196, 232.

5. Greep, R. O., and Deane, H. W., *Endocrinology,* 1949, v45, 42.

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† The Lipo-adrenal Extract was generously supplied by Dr. H. F. Hailman of The Upjohn Co.

6. Gershberg, H., Fry, E. G., Brobeck, J. R., and Long, C. N. H., *Yale J. Biol. and Med.,* 1950, v23, 32.

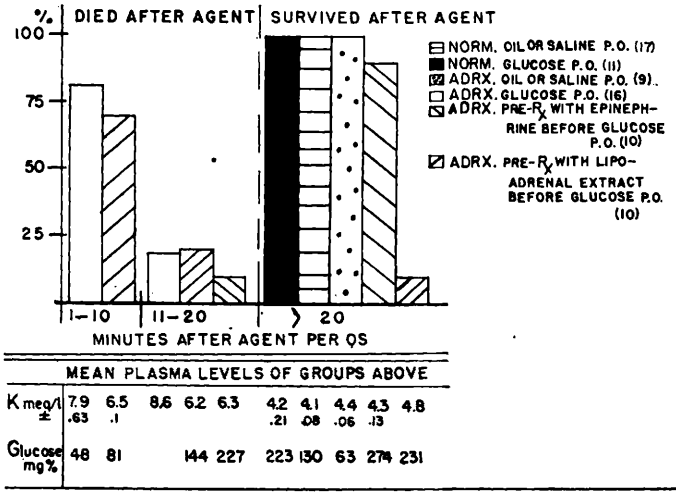


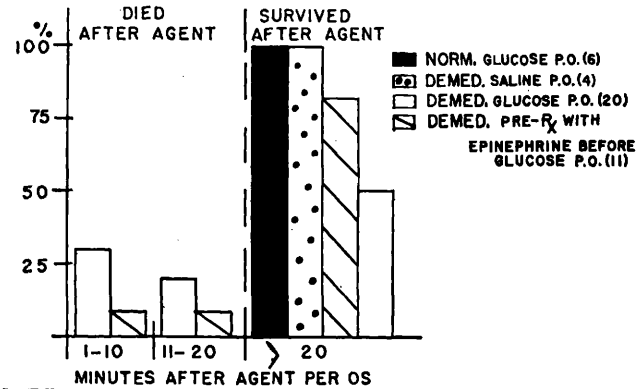
FIG. 1. Protection of adrenalectomized rats with epinephrine pretreatment against mortality and hyperkalemia following 2 ml of a 50% glucose solution *per os*. No. of rats in each group is in the parentheses. ± is the std error of the mean.

agent had been admitted into the stomach, each of the rats was observed carefully for the first signs of failure, respiratory difficulty or anoxia. At this time a cardiac puncture was made and blood withdrawn into a dry syringe containing 1 drop of heparin (Liqueamin, Lilly, 10 mg/ml). The blood was immediately centrifuged. Aliquots of plasma, if free from hemolysis, were taken for the determination of plasma potassium and glucose concentration. The potassium concentration (m.eq./L) was determined with a lithium internal standard flame photometer. Plasma glucose (mg%) was determined photometrically by the method of Kingsley and Reinhold(7). Since all the adrenalectomized rats showed signs of failure in less than 20 minutes after the glucose solution had been admitted into the stomach, those rats which had no signs of failure for 20 minutes were accepted as probably surviving the procedure and sacrificed 25-30 minutes after the agent had been given.

Results. Adrenalectomized groups. Inspection of Fig. 1 shows that 100% of a group of adrenalectomized rats were mortally affected less than 20 minutes after 2 ml of a 50% glucose solution was admitted directly into their stomachs. Pretreatment of another group of adrenalectomized rats with 2 ml of lipoadrenal extract had not altered the results. However, pretreatment with a single injection of epinephrine 60 minutes before giving the glucose solution *per os* protected another group of adrenalectomized rats. It is apparent from the results obtained in the control groups (the normal rats given glucose solution, and the normal and adrenalectomized groups given either physiological saline or mineral oil *per os* that the procedure, *per se,* was innocuous. Inspection of the plasma potassium concentration of the groups in Fig. 1 shows that the potassium levels were significantly higher in those which did not survive after the glucose solution than the plasma potassium levels of the controls or the adrenalectomized group protected with epinephrine. These results suggest that the rapid rise in plasma potassium concentration probably was responsible for the mortality of the unprotected adrenalectomized groups of rats.

Demedullated groups. Greep and Deane(5) presented histochemical evidence (presence of lipid droplets, and ascorbic acid granules) of the functional status of the regenerated adrenal cortex of demedullated rats. This was confirmed by us in the present study. A significant fall in the number of circulating eosinophiles 180 minutes after an injection of

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



MEAN PLASMA LEVELS OF GROUPS ABOVE								
K meq/l ±	5.4 (.26)	4.9	5.2 (.97)	4.6	4.2 (.19)	3.3 (.15)	3.4 (.13)	3.5 (.09)
Glucose mg%	130	240	157	297	230	73	321	143

FIG. 2. Protection of demedullated rats with epinephrine pretreatment against mortality and hyperkalemia following 2 ml of a 50% glucose solution *per os*. No. of rats in each group is in the parentheses. ± is the std error of the mean.

epinephrine in demedullated rats has been reported by Dury(8). This is further evidence of the functional secretory status of the regenerated adrenal cortex. The plasma potassium concentration, however, in demedullated rats is significantly lower than in normal or adrenalectomized rats(3). These points must be considered in the interpretation of the results obtained with the demedullated groups.

The mortality of demedullated rats after the administration *per os* of 2 ml of a 50% glucose solution and the protection which epinephrine pretreatment gave to these rats is shown in Fig. 2. Fifty percent of a group of demedullated rats survived the administration of the glucose *per os*. Pretreatment with a single injection of epinephrine 60 minutes before the administration of the glucose solution protected the demedullated rats as it had protected the adrenalectomized rats. The difference in the percent survival between adrenalectomized and the demedullated groups after the glucose solution was given may be explained on the basis of the points mentioned above. Although the plasma potassium concentration of the demedullated rats increased after the administration of the glucose solution, it did not reach a mortal level since these rats had initially a low potassium concentration. Secondly, since the regenerated adrenal cortices have been shown to be functional, demedullated rats would not be unduly sensitive to a rise in plasma potassium concentration a little above the "normal" level.

Discussion. The experiments presented here showed that adrenalectomized rats were extremely sensitive to a dose of glucose administered directly into their stomachs. The factor apparently responsible was the rapid rise in the plasma potassium concentration which followed this procedure. In previous experiments it had been shown that the adrenal cortex and extracts of the adrenal gland protected animals from potassium toxicity. In these experiments it was shown that epinephrine protected the adrenalectomized rats, whereas pretreatment with lipo-adrenal extract had not protected these animals. Additional evidence that a functional adrenal cortex in the absence of the adrenal medulla was inadequate protection against the rapid mobilization of plasma potassium is indicated by the results obtained from the experiments with the demedullated groups of rats.

Recently, Dury(3) showed that epinephrine must be considered a homeostatic agent in the regulation of the plasma potassium level. The

8. Dury, A., *Endocrinology*, 1950, v47, 387.

experiments reported here provide further evidence of the physiological function of epinephrine in the adjustment of the organism to a situation of "stress" which involved a rapid change in the concentration of a blood electrolyte. The source of the rapidly mobilized plasma potassium after the administration of glucose *per os* and the "blocking" of this phenomenon with epinephrine is under investigation.

Summary. A hyperkalemia and cardiac standstill resulting in 100% mortality was induced within 20 minutes after the administration of 2 ml of a 50% glucose solution into the stomachs of adrenalectomized rats. Pretreatment with epinephrine 60 minutes before the administration of the glucose solution protected the adrenalectomized rats. Pretreatment with lipo-adrenal extract 120 minutes before giving the glucose solution did not protect the adrenalectomized rats. The admission of the glucose solution to demedullated rats with functional adrenal cortices resulted in a 50% mortality. The difference in mortality of the adrenalectomized and demedullated rats was ascribed to the initial relatively "low" plasma potassium concentration of the latter group. Epinephrine pretreatment protected the demedullated group too. These data are discussed as indicating that epinephrine protected the experimental groups of rats by inducing a lower than "normal" plasma potassium concentration and "blocking" a change to supranormal levels after the glucose *per os*.

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Effect of Iodide, Thiouracil and Thyroxine on the Disappearance of Thyroidal I^{131}. (18723)

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A direct method for determining *in vivo* the thyroidal secretion rate in rats has been described(1). The method consists of injecting a tracer dose of I^{131} and making daily observations over the thyroid area in terms of counts per second for a period of about 2 half-lives of I^{131} (16 days). These observations, when plotted on semilog paper, can be fitted to a straight line, and from this line the proportional rate of diminution of thyroidal I^{131} can be determined and expressed as per cent per day. This proportional secretion rate, when calculated in terms of μg of thyroxine secreted per day, agrees with calculations made by other methods. Furthermore, it has been shown that hypophysectomy, which is known to depress thyroidal activity, reduced the secretion rate to one-twelfth that of normal animals(2). Further proof that this method measures hormonal secretion was obtained by determining the effect of thiouracil, thyroxine and sodium iodide on the disappearance of thyroidal I^{131}.

Material and methods. Weanling male rats (Sprague-Dawley) were used under conditions as described previously(2). After 2 weeks on an iodine deficient diet, the animals were injected subcutaneously with a tracer dose of 1 μc of radio-iodine, and observations were made daily as described. After the 48-hour count was obtained, the animals were separated into four groups: (1) controls; (2) 1% thiouracil in the drinking water; (3) 1% sodium iodide in the drinking water; (4) 10 mg d-l-thyroxine injected daily by subcutaneous route. Observations were continued for 16 days, and the proportional rates of disappearance of thyroidal I^{131} were determined. Three experiments, performed at different times of the years, are shown in Table I.

Results. As shown in Table I, the normal rate of hormonal secretion was between 8 and 9% per day with close agreement in the 3

1. Albert, A., *Endocrinology,* 1951, v48, 334.

2. Randall, R. V., Lorenz, N., and Albert, A., *Endocrinology,* 1951, v48, 339.