A Comparative Study of Various Hyperglycemic Agents in Potassium Deficient Rats (32734)

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Conn (1) has drawn recent attention to the alterations in carbohydrate metabolism produced by potassium deficiency. Such changes have been investigated in both experimental animals (2–5) and in man (6). It has been reported that potassium deficient animals have an increased hyperglycemic response to diazoxide (7), an experimental nondiuretic thiazide whose hyperglycemic effects have received much attention. These reports, coupled with the knowledge that potassium has an important role in normal carbohydrate metabolism (8,9) have led us to this comparative study of the responses of various hyperglycemic agents (diazoxide, hydrocortisone, mannoheptulose, glucagon, and epinephrine) in potassium deficient animals.

Methods and Materials. Sprague-Dawley male rats[3] (145–155 gm) were divided into two groups. The low potassium group was fed a special low potassium diet[4] and deionized water *ad libitum*. The control group was fed the low potassium diet with added potassium salts and deionized water *ad libitum* in order to restore the potassium content of the diet to a normal level. (For contents of each diet see Table I.) Both groups remained on this regimen for 25 days.

On the day of each experiment a random group of 10 or 12 animals was selected for confirmation of potassium deficiency. Animals were housed 2–3 per cage, urine samples were collected under toluene for 24 hours, and levels of sodium, potassium, and chloride were determined. While in metabolic cages, the animals received deionized water so that leakage could not alter total electrolyte levels. After the urine collections, blood samples were obtained by decapitation for determination of serum sodium, potassium chlorides, and carbon dioxide content.

TABLE I. Contents of Diets.[a]

Special low potassium diet, fortified with vitamins	%
d(+)-Dextrose	62.7
Casein	30.0
Butterfat	3.5
$CaCO_3$	1.3
NaCl	1.0
$MgCl_2$	1.5
Potassium content (analysis)	.001

[a] *Special control diet,* same as low potassium diet but with 200 gm KCl and 375 gm K_2HPO_4 added per 100 lb. of diet.

Immediately following this, a muscle sample was obtained from the left quadriceps muscle. The samples (wet wt. 100–200 mg) were prepared for determination of potassium by homogenizing with 5 ml of deionized water using a glass grinder. Sodium, potassium, chloride, and carbon dioxide levels in blood and muscle were determined by standard Technicon AutoAnalyzer methods.

A number of rats in the baseline group had additional samples of muscle and liver taken for glycogen estimation. Liver samples were taken from 3 different lobes to insure a reliable average value in each animal. The samples were quick frozen with liquid nitrogen, and tissue homogenates were assayed for glycogen by potassium hydroxide digestion and orcinol reaction of the ethanol–sodium sulfate precipitated glycogen by a modification of the method of Bruckner (10).

The rats receiving the low potassium diet were then divided in two groups, one group

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[3] Supplied by Dublin Farms, Dublin, Virginia.

[4] Supplied by Nutritional Biochemicals Co., Cleveland, Ohio.

TABLE II. Weights of Rats Receiving Low Potassium or Control Diets for 25 Days.

Each value represents the average of 12 animals.

Group	Diet	Av wt. (gm)		
		Initial	Final	Gain
Diazoxide	Low potassium	150	175	25
	Control	150	250	100
Epinephrine	Low potassium	150	180	30
	Control	150	220	70
Mannoheptulose	Low potassium	150	180	30
	Control	150	240	90
Hydrocortisone	Low potassium	150	175	25
	Control	150	250	100
Glucagon	Low potassium	150	160	10
	Control	150	250	100

received the drug and the other group received the vehicle as control. This was done also in the animals receiving the control diet, thus producing a total of four groups in each experiment.

All samples for blood sugar levels were obtained from the tail vein and estimated by the standard autoanalyzer method of Hoffman (11). Animals were fed until the time of experiment. Rats receiving mannoheptulose were fasted for 18 hours prior to the experiment. All animals were allowed free access to deionized water between blood samplings.

Diazoxide[5] suspended in methyl cellulose was administered in a dose of 100 mg/kg (i.p.). Blood samples were taken at 0, 90, 180, and 300 min.

Epinephrine dissolved in a normal saline (1:10,000) was administered in a dose of 450 μg/kg (i.p.). Blood samples were taken at 0, 90, 180, and 270 min.

Glucagon dissolved in normal saline was administered in a dose of 600 μg/kg (i.p.) and blood samples were obtained at 0, 15, 35, 55, and 75 min.

Mannoheptulose[6] dissolved in distilled water was administered in a dose of 750 mg/kg (i.p.). Blood samples were taken at 0, 90, and 180 min. Samples were diluted 1/20 with distilled water, and blood sugar was determined by glucose oxidase method (12) in order to measure true glucose values as mannuheptulose interferes with the Hoffman (11) determination.

Hydrocortisone was administered in a dose of 240 mg/kg i.p. while controls were given an equivalent volume of normal saline. Blood samples were collected at 0, 60, 150, and 240 min.

Statistical analyses were performed by standard error estimation and by the Student *t* test.

Results. Twenty-five days on the low potassium diet decreased the rate of growth while the animals receiving the same diet with added potassium grew normally (Table II). There were very few deaths with the low potassium diet.

Pilot experiments revealed an unsuspected picture of magnesium deficiency since this element was not added to the diet. The classic signs of patchy hair loss and extreme irritability were seen after about 14 days. When these animals were given magnesium chloride (2%) in the drinking water their coats regained the usual texture and became normal within a few days. For the experiments reported here 1.5 gm% magnesium chloride was added routinely to the diet by Nutritional Biochemicals Corp.

The rats receiving the low potassium diet were found to have a hypokalemic, hypochloremic, metabolic alkalosis when compared to the animals receiving the control diet. This pattern was consistently demonstrated in each group of animals prior to experiment (Table III).

The serum levels of potassium and chloride were significantly reduced by the diet while the serum carbon dioxide content was significantly elevated. The serum sodium was not appreciably changed (Table III).

Muscle biopsies in the low potassium group showed significantly lower levels of potassium when compared to the animals on the control diet (Table III).

Urine values showed a decreased 24-hour excretion of potassium in the animals on the low potassium diet as compared to those on the control diet. The excretion of sodium and

[5] Courtesy of Schering Corp., Bloomfield, N. J.

[6] Courtesy of Professor F. Simon, Weitzman Institute, Jerusalem, Israel.

TABLE III. Summary of Serum, Muscle, and Urinary Electrolytes in Rats Maintained on Low Potassium or Control Diet for 25 Days.

Exptl. group	Diet	Serum electrolytes (mEq/liter)				Urinary electrolytes (μeq/day)			Muscle potassium (wet wt. mEq/100 gm)
		Sodium	Potassium	Chlorides	CO_2 content	Sodium	Potassium	Chloride	
1	Low K	133 ± 1.8	3.9 ± 0.2	72 ± 6.0	28.5 ± 2.7	348	195	695	6.7 ± 0.3
	Control	139 ± 0.8	7.4 ± 0.3	104 ± 0.8	20.7 ± 1.4	536	631	656	10.9 ± 0.9
	p Value	>.5	<.05	<.05	<.05				<.05
2	Low K	132 ± 1.7	4.3 ± 0.1	86 ± 4.1	31 ± 1.5	226	42	817	6.4 ± 0.7
	Control	129 ± 2.6	8.9 ± 1.4	100 ± 1.2	23 ± 0.7	240	240	431	10.8 ± 0.2
	p Value	>.5	<.05	<.05	<.05				<.05
3	Low K	158 ± 9.3	3.3 ± 0.4	82 ± 3.1	25 ± 2.4	474	83	282	5.5 ± 0.5
	Control	156 ± 8.2	7.0 ± 0.7	99 ± 1.0	18 ± 1.0	853	808	593	9.3 ± 0.2
	p Value	>.5	<.05	<.05	<.05				<.05
4	Low K	139 ± 2.3	2.9 ± 1.1	80 ± 1.0	28 ± 1.4	520	100	180	4.3 ± 0.4
	Control	142 ± 1.0	6.8 ± 0.8	102 ± 1.4	20 ± 1.9	1000	1010	500	8.6 ± 0.8
	p Value	>.2	<.05	<.05	<.05				<.05
5	Low K	135 ± 1.1	5.3 ± 0.4	89 ± 1.3	28 ± 2.2	269	15	676	8.2 ± 0.5
	Control	137 ± 6.5	7.9 ± 0.7	102 ± 0.4	24 ± 1.3	241	225	623	9.9 ± 0.6
	p Value	>.2	<.05	<.05	<.15				<.05
6	Low K	126 ± 5.1	4.3 ± 0.5	81 ± 6.3	24 ± 1.4	103	180	186	6.6 ± 0.7
	Control	135 ± 4.4	8.2 ± 0.8	105 ± 0.6	22 ± 0.7	229	561	464	10.2 ± 1.0
	p Value	>.2	<.05	<.05	<.15				<.05

chloride was variable (Table III).

Levels of glycogen showed significantly increased glycogen stores in both muscle and liver in the animals on the low potassium diet when compared with the control group (Fig. 1).

When diazoxide was administered, the animals on the low potassium diet showed a significantly increased hyperglycemic response when compared to the animals on the control diet (Fig. 2 and Table IV). This increased response could be reduced to the usual hyperglycemic response to diazoxide by the addition of potassium chloride (2%) in the drinking water for 4 days.

Epinephrine, glucagon, and mannoheptulose, however, produced hyperglycemic responses in the animals on the low potassium diet which were identical to the responses of the animals on the control diet (Tables V and VI).

Hydrocortisone, like diazoxide, produced a hyperglycemic response in the low potassium group which was statistically greater than the animals receiving the control diet (Table IV).

Discussion. Many investigators have used low potassium diets, but the degree of potassium deficiency has not been accurately de-

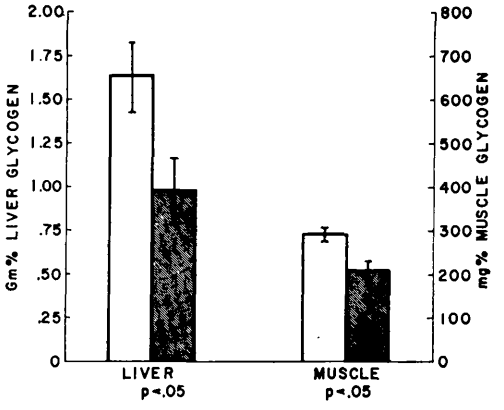

FIG. 1. Muscle glycogen (mg%) and liver glycogen (gm%) of rats maintained on low potassium (open columns) or control diets (shaded columns) for 25 days with their *p* values. (Bars represent standard error of the mean, SEM.)

TABLE IV. Effect of Diazoxide and Hydrocortisone and Their Solvents on Blood Sugar Levels in Rats Maintained on a Low Potassium or Control Diet for 25 Days.

Drug	Time (min)	Low potassium diet	Control diet	p value
Diazoxide	0	126 ± 2.8[a]	130 ± 3.9	<0.40
	90	286 ± 15.5	212 ± 4.5	<0.001
	180	464 ± 27.3	349 ±25.6	<0.001
	300	497 ± 58.0	309 ±29.0	<0.001
Solvent	0	119 ± 2.6[a]	123 ± 3.2	<0.40
	90	105 ± 3.1	118 ± 2.1	<0.05
	180	105 ± 2.5	115 ± 2.0	<0.05
	300	108 ± 3.7	113 ± 3.5	<0.40
Hydrocortisone	0	122 ± 2.8[a]	127 ± 1.4	<0.20
	60	188 ± 17.2	144 ± 4.4	<0.05
	150	166 ± 14.5	134 ± 3.9	<0.05
	240	120 ± 5.7	139 ± 4.3	<0.05
Solvent	0	120 ± 3.0	117 ± 3.2	<0.50
	60	120 ± 4.8	126 ± 5.8	<0.40
	150	109 ± 4.1	117 ± 7.4	<0.35
	240	118 ± 6.4	122 ± 5.1	<0.60

[a] Mean value of 11 animals ± standard error of the mean (SEM).

termined, but rather assumed (7,13,14). At first we used the technique of oral administration of a sodium polystyrene resin (*Kayexelate*) by intubation. This was done in order to accelerate the potassium deficiency by removing all potassium from the diet. The technique was soon abandoned because of the high death rates in the intubated animals.

TABLE V. Effect of Epinephrine and Its Solvent on Blood Sugar Levels of Rats Maintained on a Low Potassium or Control Diet for 25 Days.

Drug (i.p. dose)	Time (min)	Low potassium diet	Control diet	p value
Epinephrine	0	114 ± 2.0[a]	113 ± 2.6	>.9
	90	216 ±31.5	239 ±16.9	>.5
	180	158 ±12.8	159 ± 3.9	>.9
	270	123 ± 6.5	116 ± 6.5	>.4
Vehicle	0	118 ± 2.7	120 ± 3.2	>.5
	90	104 ± 4.4	112 ± 4.1	>.2
	180	109 ± 3.9	106 ± 2.8	>.6
	270	99 ± 3.2	109 ± 3.1	<.05

[a] Mean value of 8 rats ± SEM.

However, 25 days on the low potassium diet alone produced a measurable potassium deficiency with a low death rate. The diet produced a hypokalemic, hypochloremic metabolic alkalosis. This was repeatedly demonstrated in each group of animals before experiment.

Our observations of the results of potassium deficiency are in agreement with Gardner *et al.* (2) and with Spergel *et al.* (5) who also found retarded growth rate, decreased tissue contents of potassium and increased glycogen levels in both liver and muscle.

The increased hyperglycemic response to diazoxide in potassium deficient animals supports the previous work of Kvam and Stanton

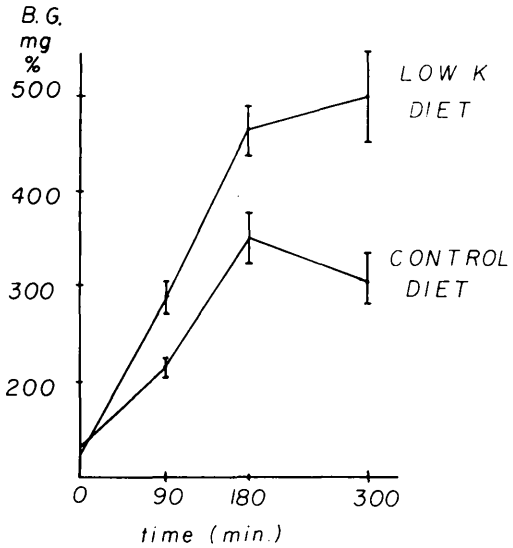

FIG. 2. The response to diazoxide dose and route in rats maintained on a low potassium or control diet for 25 days. (Bars indicate standard error of the mean SEM.)

(7) but these investigators used a low potassium diet for only 10 days prior to experiment, and did not document the degree of potassium deficiency. It was this hyperglycemic response of diazoxide which led us to the investigation of other hyperglycemic agents in potassium deficiency.

Four groups of mechanisms have been proposed for the hyperglycemic effect of diazoxide: (i) Inhibition of the release and/or the synthesis of insulin by the pancreas (15, 16). (ii) Catecholamine release (17,18). (iii) An effect of diazoxide on the cyclic adenylic

TABLE VI. Effect of Glucagon and Mannoheptulose on Blood Sugar Levels in Rats Maintained on a Low Potassium Diet for 25 Days.

Drug (i.p. dose)	Time (min)	Low potassium diet	Control diet	p value
Mannohep-	0	129 ± 5.6[a]	132 ± 1.8	>.6
tulose	90	239 ± 8.9	240 ± 6.1	>.9
	180	171 ± 7.3	168 ± 5.1	>.7
Glucagon	0	108 ± 3.7[b]	107 ± 2.3	>.9
	15	122 ± 7.1	125 ± 3.8	>.8
	35	114 ± 3.9	107 ± 3.0	>.2
	55	118 ± 6.3	114 ±11.0	>.8
	75	127 ±23.2	111 ± 3.7	>.8

[a] Mean value of 8 rats ± SEM.

[b] Mean value of 10 rats ± SEM.

acid system in the liver (19–21). (iv) Peripheral mechanisms—decreased utilization of glucose in peripheral tissues (22).

Hydrocortisone causes hyperglycemia by gluconeogenesis increasing hepatic glucose output from precursor substances (23). Secondary effects include a reduced entry of pyruvate into the tricarboxylic acid cycle and a decreased glucose uptake in adipose tissue.

Epinephrine causes hyperglycemia by promoting the formation of 3′,5′-cyclic AMP which mediates the conversion of phosphorylase B to active phosphorylase A which in turn stimulates glycogenolysis (24); epinephrine also has a secondary effect of decreasing the peripheral utilization of glucose. Similarly, glucagon has been shown to cause short-lived hyperglycemia by increasing the synthesis of 3′,5′-cyclic AMP (24).

Mannoheptulose, a seven carbon sugar extracted from the avacado pear, has been shown to cause hyperglycemia by a stimulation of gluconeogenesis in the periphery (25, 26), and also by inhibition of insulin release (27).

An hypothesis which would explain the responses to both diazoxide and hydrocortisone in the presently reported series of experiments, is that potassium deficient rats are more responsive to the action of glucocorticoids, and that diazoxide causes an increased secretion of the adrenal hormone which was not seen with mannoheptulose, glucagon and epinephrine. This is supported by the results of Gardner *et al.* (2), who found increased adrenal gland weights in potassium deficient animals as well as decreased peripheral eosinophilia, (an indirect reflection of 11-17 oxy steroids). The observation of Zarday *et al.* (17), who found that chronic diazoxide administration increased adrenal weight, would also support this hypothesis.

Summary. The hyperglycemic effect of diazoxide, hydrocortisone, epinephrine, glucagon, and mannoheptulose was studied in Sprague-Dawley rats maintained on a low potassium diet for 25 days, or on a control diet. The low potassium diet produced a hypokalemic, hypochloremic, metabolic alkalosis. There were increased glycogen levels in both liver and muscle with the low potassium diet compared to controls. Diazoxide and hydrocortisone produced an increased hyperglycemic effect in the potassium deficient animals.

The response to mannoheptulose, glucagon, and epinephrine was identical in control and potassium depleted groups. The significance of these results is discussed.

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Detection of *in Vivo* Protein Photooxidation by Means of Tritium Loss from the Histidine Moieties* (32735)

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In vitro protein photooxidation with methylene blue was discovered to involve preferentially the histidine moieties (1). To detect photodamage to histidine and other amino acids, proteins were acid-hydrolyzed, and assays for the various amino acids were undertaken by chemical and microbial methods. These methods are laborious and are inconvenient when many samples are required as, for example, in cell survival-time studies. A rapid and sensitive presumptive test for the photooxidation of protein histidine is desirable. Such a test has been devised and is described in this report. It takes advantage of the tritium lost from tritiated histidine during photooxidation. Tritium detected in cell filtrates correlates with photodamage to the cellular protein histidine, if the suspensions are kept cold and are compared with suitable controls. Furthermore, this tritium loss is a more sensitive test for photodynamic action than is cell death.

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Materials and Methods. Escherichia coli was cultivated in MYE medium, was harvested during exponential growth (log cells), was washed in buffer, and was suspended in buffer, usually at a cell concentration of 5×10^8/ml. Photosensitizer (methylene blue at 5 μg/ml, acridine orange at 5 μg/ml, or benzo(*a*)pyrene at 1 μg/ml) was added, the suspension was placed in a small petri dish, and was allowed to equilibrate for 0.5 hour at 6°C before illumination. Media composition and other details have been given elsewhere (2). Illumination and all subsequent manipulations were at 6°C. Two controls were employed: cells held in darkness with photosensitizer (dark control), and cells illuminated in absence of photosensitizer (illumination control). The dyes were illuminated with two 17-inch, 15-watt "daylight" fluorescent tubes (G. E. F15T-D) in a desk type reflector 28 cm above the suspension. This gave an incident dose of 80 ergs per mm^2 per sec according to a YSI-Kettering model 65 radiometer. The hydrocarbon was administered in colloidal form and was illuminated with 355 mμ ultraviolet also at 80 ergs per mm^2 per sec (2).

Bacterial protein was labeled during growth by including in the MYE medium a tritiated