Glutathione Potentiation of Cortisone-induced Glycosuria in the Rat.* (18020)

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Glutathione, which was first shown to protect against alloxan diabetes(1,2), may play a similar protective role in other types of diabetes(3). Since the glutathione content of the blood and tissues is decreased by injecting growth hormone(4), adrenocorticotrophic hormone(5), and cortisone(6), and since it has been claimed that gluthathione, when injected into human subjects with "ACTH-induced diabetes," caused a transitory decrease in glycosuria(7), we have studied the effect of glutathione administration on cortisone diabetes in the rat(8,6).

Methods. Normal and subdiabetic Sprague-Dawley rats were used in this study. The latter were prepared by starving 100 g rats for 48 hours and injecting with alloxan (15 mg/kg). Those rats which showed a decreased glucose tolerance but normal blood sugar values (subdiabetes) were selected and allowed to reach a weight of 300 g. Rats weighing 300 g were placed in metabolism cages and a high carbohydrate liquid diet† was fed by stomach tube in the morning and early evening. Although some of the rats developed an initial glycosuria, all of the normal rats became aglycosuric within a few weeks and the subdiabetic rats became aglycosuric within one month. Aqueous suspensions of cortisone acetate (Merck) containing 1.5% benzyl alcohol were injected subcutaneously immediately preceding each feeding. The dose totaled 4 mg per day for the subdiabetic rats and 10 mg per day for the normal rats. Urines were collected daily under toluene and the glucose excretion was determined by the caramelization method of Somogyi(11) adapted to the Klett Colorimeter.

Solutions of glutathione 0.5 M, cysteine (free base) 0.5 M, ascorbic acid 0.5 M and D,L-Alanine 1.0 M were neutralized to pH 7.4 and injected intraperitoneally in doses of 0.5 cc/100 g of rat 10 minutes prior to each feeding. When cortisone was also injected it was given immediately preceding the feeding.

Results. The effect of glutathione injection (5.0 mM/kg/day) on the sugar excretion of normal rats is shown in Table I. Although glutathione induced glycosuria in the normal rat, the amount of sugar excreted following successive injections of glutathione decreased progressively as the length of time on the high carbohydrate diet increased. After two months it was insignificant. Cysteine appeared to be less effective than glutathione for equimolar doses of the former, when injected on the 25th day, induced less glycosuria than did

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† The high carbohydrate liquid diet of Reinecke *et al.*(9) as modified by Ingle(10) was diluted with an additional 25% water and administered in increasing amounts over a 5-day period. Thirty-two cc/day were fed after the fifth day and the total caloric intake remained the same(10). This dilution of the diet facilitates the passage of the food through a No. 8 French catheter.

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TABLE I.

Effect of Glutathione on the Urinary Excretion of Glucose in Normal Rats.

Seven normal rats (Nos. 22-30) were forced-fed a high carbohydrate diet. These same animals were injected at various times with the substances indicated in doses of 5.0 millimoles per kilogram per day except DL-alanine which was injected in doses of 10.0 mM/kg per day. The average 24-hr glucose excretions are recorded.

			g of urinary glucose/24 hr			
			Pre-inj. level		Increase due to inj.	
Rat No.	Days on high carbohydrate diet	Substance inj.	Avg	Range	Avg	Range
22-30	18-21	Glutathione	.03	.00-.05	.66	.06-1.58
,,	32	,,	.03	.00-.08	.37	.02-1.31
,,	45	,,	.02*	.00-.04	.11*	.00- .27
,,	63	,,	.01†	.00-.03	.03†	.00- .11
14-17	47-54	,,	.01‡	.00-.03	.06‡	.01- .13
22-30	25	Cysteine	.04	.00-.07	.20	.00- .58
,,	37	Ascorbic A.	.03	.00-.06	.09	.05- .17
,,	29	Alanine	.02	.00-.07	.10	.00- .19

* Average of only 6 rats.

† Average of only 4 rats.

‡ Represents the average of 6 determinations on 3 new rats which received their first glutathione injection on day 47 and 2nd injection on day 54.

glutathione given on the 32nd day.

One normal rat (No. 18), was injected with cortisone (10 mg/day) beginning on the 33rd day of the high carbohydrate diet; glycosuria appeared on the fourth day, reached a maximum of 1.9 g/day on the 7th day and thereafter stabilized at a value of 0.3 to 0.5 g/day. Glutathione, injected on 3 separate days induced a ten-fold increase in glycosuria (up to 5.2 g/day). (Table II). In 4 additional normal rats injected with cortisone (10 mg/day), glutathione increased the glycosuria from 2 to 4½ times. In 2 cortisone diabetic rats (10 mg/day) in which the glycosuria had leveled off at about 2 to 3 g/day, glutathione injection caused death within 1 to 2 hours.

Smaller doses of cortisone induced glycosuria in subdiabetic rats. One such rat (No. G) which received cortisone (4 mg/day) beginning on the 57th day developed glycosuria after 4 days; this reached a maximum of 0.9 g after 6 days, and leveled off at about 0.5 g/day thereafter. At various times indicated in Table II this same rat was injected with glutathione, cysteine, ascorbic acid and alanine and the effects on sugar excretion were determined. Four sub-diabetic rats, which did not receive cortisone showed insignificant responses to cysteine and glutathione.

Discussion. In normal rats fed a high carbohydrate diet the injection of glutathione appears to bring out latent abnormalities in tolerance to carbohydrate which ultimately disappear after 6 to 8 weeks of the diet. The decreased effectiveness of successive doses of glutathione is not due to the development of a tolerance to injected glutathione because when the first glutathione injection is delayed until the 47th day of feeding, the glycosuria was nevertheless insignificant. The increased glycosuria which follows glutathione injection into cortisone-diabetic rats, occurs at a time when the control rats showed insignificant responses to glutathione. An increased excretion of cysteine or glutathione following sulfhydryl injection would not be measured as glucose since these compounds will not caramelize with sodium carbonate. Direct conversion of the injected glutathione to glucose could account for only a small fraction of the glucose excreted. Since preliminary studies indicated that the blood sugar of cortisone-diabetic rats is markedly increased following glutathione injection, the glycosuria is not simply the result of a decreased renal threshold.

Insulin is inactivated by cysteine and glutathione *in vitro*(12) and presumably also *in*

12 du Vigneaud, V., Fitch, A., Pekarek, E., and Lockwood, W. W., *J. Biol. Chem.*, 1931-1932, v94, 233.

TABLE II.

Effect of Glutathione on Urinary Glucose Excretion in Cortisone-Diabetic Rats.

Rats forced-fed a high carbohydrate diet. Test substances were injected in doses of 5 millimoles per kilo (except DL-alanine which was injected in doses of 10 mM/kg) on the days indicated.

Condition of rat	No. of rats	Dose cortisone		Days on high carbohydrate diet	Test substance	g of urinary glucose/24 hr			
						Pre-inj. level		Increase due to inj.	
		mg/day	days			Avg*	Range	Avg*	Range
Normal	3	0	0	47-54	Glutathione	.01	.00- .03	.06 (6)	.01- .13
"	1 (No. 18)	10	11, 14, 21	44, 47, 54	"	.38	.30- .48	3.75 (3)	1.84-5.17
"	4	10	10-23	18-31	"	.51	.40- .73	1.39 (4)	1.17-1.59
Sub-diabetic	1 (No. 6)	4	10, 22, 37, 51	67, 79, 94, 108	"	.55	.34- .71	1.80 (4)	1.45-2.44
"	" "	4	16, 32	73, 89	Cysteine	.82	.61-1.03	1.15 (2)	.50-1.80
"	" "	4	19, 44	76, 101	Ascorbic A.	.42	.20- .64	.61 (2)	.51- .72
"	" "	4	28	85	Alanine	.77		.35 (1)	—
"	4	0	0	89-116	Glutathione, cysteine, or ascorbic a.	.03	.00- .06	.05 (16)	.00- .20

* No. of determinations are given in parenthesis.

vivo(13). If the increased glycosuria resulting from sulfhydryl administration were the result of *in vivo* inactivation of insulin, one would expect the glycosuria following cysteine injection to be greater than that following glutathione, since cysteine is a more potent inactivator of insulin than is glutathione(12). This does not seem to be the case. Although the greatest sugar excretion was observed when glutathione was injected into cortisone-diabetic rats, it cannot be argued that these diabetic rats should show more glycosuria as a consequence of insulin inactivation than do normal rats, because Ingle has shown that cortisone-diabetic rats are highly resistant to insulin(14). Since glutathione can reduce the disulfide groups of protein to sulfhydryl, it may produce glycosuria through its effect on some disulfide dependent enzyme. Insulin, which requires disulfide groups for activity (15), may have an opposite effect.

Since cortisone injection results in an increased ketosteroid excretion(16) part of the injected steroid is destroyed by oxidation of the side chain. Reducing agents such as cysteine and glutathione, by protecting cortisone from destruction, may thereby potentiate its diabetogenic effect. Cysteine and probably glutathione have also been found to potentiate the carbohydrate storage (liver glycogen) effects of cortisone(17). Since glutathione also induced glycosuria in normal animals during the early period of high carbohydrate feeding, it may have an effect on endogenous steroids.

The increased glycosuria found in "cortisone-diabetic" rats treated with glutathione is contrary to the observations reported by

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Conn(7). He found that when glutathione was injected into man with "ACTH diabetes" there was a transitory decrease in glycosuria lasting 1 to 2 hours. Studies with ACTH are now in progress.

These results emphasize that although glutathione protects the beta cell against physiologically occurring toxic compounds(3) it may also have diabetogenic effects due to its action on steroids, enzyme systems, etc. They furthermore suggest the possibility that glutathione might potentiate the therapeutic effect of cortisone in the treatment of rheumatic disease.

Summary. Glutathione, when injected into normal animals which had been force-fed a high carbohydrate diet, induced glycosuria. The amount of glycosuria decreased with increasing adaptation to the high carbohydrate diet and after feeding the diet for 2 months, glutathione produced insignificant effects. By contrast the injection of glutathione into cortisone-diabetic rats caused a marked (in some cases up to 10 fold) increase in glycosuria which has been observed even after 3 months of the high carbohydrate diet. Cysteine and ascorbic acid were also studied. Glutathione potentiates the diabetogenic effect of cortisone. The mechanism of this potentiation has been discussed and its possible significance in the treatment of rheumatic disease has been suggested.

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Chemoprophylactic Effectiveness of Aureomycin and Terramycin in Murine Bartonellosis.* (18021)

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The bartonellae resemble the rickettsiae in many of their characteristics. Previous work in this laboratory (unpublished) has shown, however, that murine bartonellosis is not prevented or made less severe by para-aminobenzoic acid or penicillin, both of which are highly effective against murine typhus infection in mice(1,2). We have also obtained negative chemoprophylactic results in murine bartonellosis with sulfanilamide, sulfathiazole, sulfapyridine, folic acid, atabrine, and several other antimalarial drugs. The purpose of this paper is to report the chemoprophylactic effectiveness of aureomycin and terramycin in this disease.

Material and methods. Two strains of white rats were used, one of which was bartonella-free while the other, like most laboratory strains, was a so-called "carrier" strain, latently infected with *Haemobartonella muris*. It is well known(3) that rats rarely develop clinical or hematological evidence of bartonellosis unless splenectomy is carried out. When bartonella-free rats are splenectomized and then injected with the organism, they become severely ill in a few days as a result of extensive parasitization and destruction of erythrocytes, and the mortality is usually 100%. When ordinary carrier rats are splenectomized, they also become severely ill, and the mortality usually ranges from 50 to 75%.

In one experiment, 19 bartonella-free rats (Sprague-Dawley strain) were splenectomized, and injected 3 weeks later with 1 cc of rat blood containing *H. muris* of high virulence. Twenty-four hours after injection, 5 of these rats were started on aureomycin, 4 on terramycin and 5 on chloramphenicol, the remaining 5 serving as controls. The aureomy-

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