Blood Glutathione in Mild Diabetes Mellitus Before Treatment and During Sulfonylurea-Induced Hypoglycemia.* (23125)

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To ascertain whether arylsulfonylurea compounds influence blood glutathione concentrations, determinations of reduced glutathione were made following oral administration of tolbutamide (Orinase)† or carbutamide (BZ-55)† to patients with normal carbohydrate metabolism and patients with mild diabetes mellitus. Glutathione values were much lower in the diabetic patients both before and after administration of the sulfonylureas. However, no change in glutathione levels was observed in either group during the acute hypoglycemic response to these compounds, nor in diabetic patients who received therapeutic doses of tolbutamide for several days.

Material and methods. The patients were all men. Each of 9 control patients had a normal oral glucose tolerance test. Twelve of the 15 diabetic patients were eating at least 200 g of carbohydrate daily, while the others received 150 g daily. Insulin had never been given to 12 of the diabetics, and had been discontinued in the others at least one week before the test. The ages of control patients ranged from 24 to 62 years, with a mean of 37 years. The diabetic patients were older, from 46 to 68 years of age with a mean of 59 years, since most of them were selected for probable susceptibility to sulfonylureas. Liver function tests were normal in all 24 individuals. Following an overnight fast 3 g of tolbutamide were given orally to all control patients and to 11 of those with diabetes. The other 4 diabetics received 3 g of carbutamide orally. Oxalated specimens of venous blood were obtained before administration of sulfonylurea and at hourly intervals for 5 hours. Duplicate determinations on whole blood were done for glucose by the Somogyi-Nelson method(1), and for reduced glutathione by the method of Woodward and Fry (2). Hematocrits obtained at each interval on heparinized blood enabled the blood glutathione concentration to be expressed in terms of 100 cc of packed red blood cells.

Results. In analyzing the findings the diabetic patients are discussed as a group because, regardless of magnitude of individual hypoglycemic response or sulfonylurea agent administered, the glutathione data yielded by each diabetic were entirely similar.

In Table I the desultory hypoglycemic response to tolbutamide in the control group contrasts sharply with the marked depression of the blood glucose level induced in most of the diabetic patients by tolbutamide or carbutamide. The average maximum depression from the fasting glucose value was 14% in the former group and 43% in the latter.

However, in neither group was the fall in blood glucose accompanied by an important change in blood concentration of reduced glutathione (Table I). In control patients the glutathione level remained essentially constant throughout the test period. In the diabetic group the mean 5-hour glutathione value of 70.5 ± 7.6 (S.D.) mg/100 cc of packed red blood cells was insignificantly higher ($p = 0.1$) than the mean fasting concentration of 66.6 ± 5.6.

At the same time, the actual blood glutathione concentration of diabetic patients was distinctly subnormal. The difference between the mean fasting blood glutathione concentration in the normal group (81.3 ± 6.7 mg/100 cc of packed red blood cells) and the respective value in the patients with diabetes (66.6 ± 5.6 mg/100 cc of packed red cells) represents a significance of $p = <.001$ (Table I). Equally significant differences between respective values persisted throughout the test period except at the 1-hour interval, where $p = <.01$.

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† Tolbutamide was kindly supplied by Upjohn Co., and carbutamide by Eli Lilly and Co.

TABLE I. Blood Concentrations of Glucose and Reduced Glutathione after Administration of 3 g of Tolbutamide or Carbutamide to Non-Diabetic and Diabetic Patients.

Patients	Fasting	1 hr	2 hr	3 hr	4 hr	5 hr	Max fall from fasting level
	Blood glucose (mg %—Somogyi)						
Non-diabetics (9)*	77 ± 4†	75 ± 4	72 ± 4	72 ± 5	68 ± 5	68 ± 4	14 ± 6%
Diabetics (15)	177 ± 59	167 ± 62	149 ± 60	127 ± 56	115 ± 57	107 ± 51	43 ± 11%
	Reduced blood glutathione (mg/100 cc packed red blood cells)						
Non-diabetics (9)	81.3 ± 6.7	78.6 ± 8.5	81.9 ± 9.1	81.0 ± 7.6	81.4 ± 7.5	83.1 ± 7.5	
Diabetics (15)	66.6 ± 5.6	67.9 ± 4.9	67.3 ± 5.3	68.1 ± 7.4	69.3 ± 6.1	70.5 ± 7.6	
p‡	<.001	<.01	<.001	<.001	<.001	<.001	

* No. of patients. † Stand. deviation. ‡ Derived from comparison of respective glutathione values at each interval.

Blood glutathione concentrations were determined in 6 diabetic patients who received therapeutic doses of tolbutamide for 6 days or more. Table II demonstrates that the low pre-treatment glutathione values remained unchanged whether or not the drug was effective in restoring normal fasting blood sugar levels.

Discussion. The strikingly subnormal blood glutathione concentrations observed in these diabetic patients disagrees pointedly with recent reports(3,4) of normal glutathione values in such patients. Two major considerations may account for this apparent discrepancy.

Woodward and Fry(2) originally expressed concentration of reduced blood glutathione in terms of mg/100 cc of whole blood. Since blood glutathione exists entirely within the erythrocyte, however, it has subsequently been emphasized(5,6) that precision requires its expression in terms of red cell mass, the procedure used in the present study. In reports employing the former and less accurate means of presenting glutathione concentrations in diabetic patients(3,4) blood glutathione levels which are actually depressed may have appeared normal when masked by the hemoconcentration accompanying severe glycosuria.

TABLE II. Persistence of Subnormal Blood Glutathione Concentrations after Several Days of Tolbutamide Therapy in Patients with Diabetes.

Patient	Fasting blood glucose (mg % -Somogyi)	Blood glutathione (mg/100 cc packed RBC)	Daily tolbutamide dosage and duration of treatment
1	329	62.4	P.t.*
	281	57.8	5 g for 6 days
2	152	60.8	P.t.
	148	62.5	4 g for 2 mo
3	142	67.4	P.t.
	68	61.5	5 g for 6 days
4	139	72.6	P.t.
	87	70.8	4 g for 6 days
5	132	69.3	P.t.
	80	65.3	5 g for 7 days
6	113	74.0	P.t.
	82	70.9	4 g for 8 days

* P.t. = Pretreatment.

The opinion that blood glutathione concentrations are normal in diabetes has also been propagated by the tendency of most workers to consider their diabetic populations as homogeneous groups. Such an assumption ignores the possibility that glutathione concentration bears a relationship to degree of diabetic control. However, experimental(7) and clinical studies(8,9) strongly suggest that normal glutathione metabolism, in both diabetic and non-diabetic patients, requires the existence of normal carbohydrate tolerance. Although blood levels of glutathione are known to be low in frank ketosis(10), no analysis has yet appeared of glutathione concentrations in controlled compared to uncontrolled but non-ketotic diabetics. In the present study each diabetic patient exhibited an elevated fasting blood sugar as evidence of mild to moderate uncontrol of his disease, but none had ketosis.

Conversely, factors which are known to be associated with true depression of the blood glutathione concentration were not present in these diabetic patients. Such factors include starvation, protein depletion, liver disease and

ketosis(6).

The insignificant rise in blood glutathione concentrations coincident with sharply falling blood glucose levels in the diabetic patients indicates that this convenient potential index of intracellular sulfhydryl activity is unaffected by the arylsulfonylurea compounds. Nevertheless, the essentially unchanged glutathione values do not entirely erase the possibility that metabolic activity of these agents may be mediated by altering the intracellular availability of sulfhydryl groups. Experimental work(11) demonstrates that profound changes in tissue concentrations of glutathione may or may not be accompanied by variations in the blood glutathione level. A similar situation may also exist in man.

Summary. 1. Blood glucose and reduced glutathione determinations were performed at intervals on 9 non-diabetic patients and on 15 patients with moderately uncontrolled diabetes following oral administration of 3 g of tolbutamide or carbutamide. 2. Mean maximal decrease from fasting blood glucose value was 14% in patients with normal carbohydrate metabolism, and 43% in the diabetic group. 3. In control patients there was no change in glutathione levels during the test period. In diabetic patients a minimal, insignificant rise had occurred 5 hours after ingestion of the hypoglycemic agent. 4. The mean blood reduced glutathione concentration was strikingly lower in patients with diabetes than in those whose carbohydrate metabolism was intact. In 4 patients subnormal values persisted despite restoration of normal fasting blood glucose levels by daily therapeutic doses of tolbutamide.

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Human Chorion Cells: Cultivation and Susceptibility to Viruses.* (23126)

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The ready availability and usefulness of amnion cells in tissue culture virology have been demonstrated since the original publication of Zitcer *et al.*(1). Modifications of the amnion cell culture, virus spectrum, and application to primary isolation have been reported by several groups(2,3). The chorion is a placental by-product of every amnion trypsinization, and therefore the usefulness of the chorion was explored.

Materials and methods. Chorion cell cultures. The methods employed were essentially similar to procedures used for the amnion(2). Fresh membranes were collected within one

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