Hippuric acid is formed both in the liver and kidneys by the conjugation of benzoic acid and glycine. Although it is postulated that the hippuric acid thus formed acts in a manner similar to para-aminohippuric acid, it is extremely doubtful whether the amount of circulating hippuric acid following benzoic acid administration is sufficient to inhibit by "mass action" the tubular excretion of penicillin. It is conceivable that benzoic acid may be conjugated in the kidneys and give rise to a "local" high hippuric acid concentration, but this is not subject to proof at present.

In the light of the data here reported, it is our opinion that benzoic acid has no effect upon penicillin plasma concentrations when given in the amounts described.

Conclusions. Benzoic acid given in doses of 16 and 24 g per day to patients on unrestricted diets failed to increase penicillin plasma concentrations. Caronamide given to the same patients in the same doses showed a uniform and marked enhancing effect, averaging five times the control values.

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Further Studies of Effect of Sulphur Compounds on Production of Diabetes with Alloxan.

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In previous publications [1, 2] it was reported that the intravenous injection of large doses of cysteine, glutathione, or thioglycolic acid completely protected rats against a diabetogenic dose of alloxan (40 mg/kg). This protection was observed only when the sulfhydryl compound was administered just prior to the alloxan, or within one minute after the alloxan injection. However if a large dose of cysteine was given 3 or more minutes following the alloxan, no significant protection was observed. It was further suggested that these extraneously administered compounds protected sulfhydryl enzymes from inactivation by alloxan. In this connection it is of interest to note that ascorbic acid, a compound which is reported to decrease the sulfhydryl groups in the body [3] potentiates the effect of alloxan.[4] The more effective production of diabetes in the starved rat [5] may also be related to variation in the glutathione and cysteine content of the body.

Since it was suggested [2] that alloxan may produce its diabetogenic effect by inactivating essential sulfhydryl groups of enzymes, it was desired to test other compounds with known sylfhydryl reactivating effects. The inhibiting effect of arsenic and cadmium on sulfhydryl enzymes has been reported by Peters [6] to be completely reversed by dimercaptopropanol, British Anti-Lewisite, (BAL).

1 Lazarow, A., *Anat. Rec.*, 1945, **91**, 24.

2 Lazarow, A., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 441.

3 Prunty, F. T. G., and Vass, C. C. N., *Biochem. J.*, 1943, **37**, 506.

4 Levey, S., and Suter, B., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 341.

5 Kass, E. H., and Waisbrin, B. A., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 303.

6 Peters, R. A., Stocken, L. A., and Thompson, R. H. S., *Nature, London*, 1945, **156**, 616.

It was desirable to test this compound (BAL) for its antagonistic effect against alloxan diabetes. Also a number of non-sulfhydryl sulphur compounds was tested. Preliminary results have been reported.[7]

Methods. The protection experiments were carried out in the same manner as those previously reported.[2] Alloxan and the protective agent were administered intravenously, using separate tail veins for each injection. To facilitate the intravenous injection, vasodilatation was induced by placing the rats in a 56°C oven for one to several minutes. A 2% solution of alloxan monohydrate was used. The BAL was administered in doses of .16-1.00 mM/kg. The various dilutions of the BAL were prepared in .85% sodium chloride and were injected within 5 minutes of the time of preparation. In other experiments methionine was also used in doses of 2.9 mM/kg. A solution containing 0.25 mM of methionine per cc was injected in doses of 1.15 cc for each 100 g of body weight. This solution is near saturation. Thiourea was also used in doses of 7.5 mM/kg. One cc of a solution containing .75 mM per cc was used for each 100 g of body weight. The solution of thiourea had a pH of about 6.6, and was not neutralized, inasmuch as minute amounts of N/100 sodium hydroxide brings the pH to neutrality. Serial blood sugars were taken at 0, 3, 8, 24, 48, and 72 hours. The blood was obtained by cutting the tip of the tail, the rat being heated to facilitate bleeding; and the sugars were determined by the Folin-Malmros micro method.[8]

Results. The effects of administration of various sulphur compounds are given in Table I. The per cent of animals with diabetes are given for each group, and the average 48-hour blood sugars are listed for the diabetic and non-diabetic animals. An animal was considered diabetic if the 48-hour blood sugar was greater than 200 mg per 100 cc; or if the typical blood sugar response was observed following the injection of alloxan. All of 15 animals, injected with alloxan in doses of 40

TABLE I.

Effect of Various Sulphur Compounds on Production of Diabetes with Alloxan. Both alloxan and the protective agent were given intravenously.

Protective agent	No. of animals	Dose of alloxan mg/kg	Dose of protective agent mM/kg	Substance given first	Time between injections minutes	% diabetic	Avg 48-hr blood sugar	
							Diabetic mg/100 cc	Nondiabetic mg/100 cc
None	15	40	0	0	—	100	444	—
BAL	4	40	.16	BAL	1-2	50	396	154
,,	2	40	.36	,,	1-2	0	—	150
,,	2	40	.72	,,	1-2	0	—	148
,,	2	40	1.00	,,	1-2	Dead	—	—
,,	4	0	.16	—	—	—	—	154
,,	1	0	.36	—	—	—	—	150
,,	2	0	.72	—	—	—	—	140
,,	1	0	1.00	—	—	Dead	—	—
,,	4	40	.16	Alloxan	5	100	404	—
,,	4	40	.75	Alloxan	5	100	403	—
,,	6	40	2.9	Methionine	1-2	100	412	—
Methionine	4	40	7.5	Thiourea	1-2	100	408	—
Thiourea	3	40	7.5	,,	1-2	Death 1-2 hr	—	—
,,	4	40	7.5	—	—	0	—	164
,,	4	0	7.5					

[7] Lazarow, A., *Anat. Rec.*, 1947, **97**, 37.

[8] Folin, O., and Malmros, H., *J. Biol. Chem.*, 1929, **83**, 115.

mg/kg, developed diabetes. The average 48-hour blood sugar was 444 mg per 100 cc. When BAL was injected in doses of .16-1.0 mM/kg, immediately preceding a diabetogenic dose of alloxan, the response varied with the amount of BAL administered. Two of 4 rats were protected at a dose of .16 mM/kg of BAL. All animals were protected when the dose was .36-.72; whereas, at a dose of 1.0 mM/kg, the animals died within 2 to 6 hours. BAL alone produced no significant effect in doses of .16-.72 mM/kg, but one animal given a dose of 1.0 mM/kg also died. When the BAL was injected 5 minutes after the alloxan administration, no protection was observed, even with a dose of 0.75 mM/kg which is near the toxic dose for rats.

Methionine, in doses of 2.9 mM/kg (or thiourea in doses of 7.5 mM/kg) did not modify the diabetogenic dose of alloxan. Some animals given thiourea, in doses of 7.5 mM/kg, preceding a diabetogenic dose of alloxan, died within the first 1 to 2 hours, whereas all animals given thiourea without alloxan survived.

Discussion. Mole per mole, BAL, which is a disulfhydryl compound, is at least twice as effective in protecting rats against alloxan diabetes, as is cysteine or glutathione. Doses of .16 mM/kg of BAL protected half of the rats, and doses of .36 mM/kg protected all the rats. With cysteine doses of 1.0 mM/kg, it was previously reported [2] that 12% of the animals became diabetic; whereas, with a dose of .5 mM/kg, 66% of the rats became diabetic. However, allowing for the 2 SH groups in BAL, this compound is at least as effective per mole of SH as is cysteine. The small number of animals used in the BAL experiments do not permit more quantitative comparisons.

It will be noted that the diabetogenic effect of alloxan cannot be modified by BAL when the latter compound is given 5 minutes after the alloxan. This is in marked contrast to the effect of BAL on arsenic and cadmium inactivation of sulfhydryl compounds. Peters [6] has reported that BAL is effective when administered after the heavy metal poisoning has already taken place. Thus if the diabetogenic effect of alloxan is due to inactivation of the sulfhydryl groups of essential enzymes, these sulfhydryl groups cannot be reactivated by BAL. Very little information is available as to the mechanism of inactivation of sulfhydryl groups which takes place with alloxan. If the inactivation consists of merely oxidizing SH groups to S-S groups, one might expect BAL, cysteine, and glutathione to reactivate these groups. However, if the alloxan formed an addition product with the SH groups of enzymes, (as do quinones) this type of inactivation would not be expected to be reversed by glutathione or BAL. Further studies on the combination of alloxan with SH groups are needed.

A complete and irreversible inactivation of the sulfhydryl groups of the beta cells in the pancreas may be requisite to the production of diabetes with alloxan. Reversible sulfhydryl inactivators, such as arsenic, cadmium, etc., might not be expected to produce diabetes because of their reversibility.

The selective inactivation of essential sulfhydryl groups in the beta cells could be a result of a low glutathione content. Since glutathione can protect sulfhydryl enzymes from inactivation, the local concentration of glutathione might determine the sensitivity of cells to sulfhydryl inactivators. Unfortunately, the glutathione content of the beta cells in the islets has not yet been determined.

Inasmuch as alloxan produces its diabetogenic effect within the first 5 minutes of injection, the amount of alloxan reaching the beta cells within this time is a critical factor. Inasmuch as administered glutathione protects against alloxan diabetes, the glutathione, which normally is present in the beta cells, must also act as a protective factor. When this protective amount of glutathione disappears, the alloxan would then be free to act on the cell enzymes. The blood supply of a tissue will also determine the total amount of alloxan reaching the cells during the critical period following its injection, for in a given time, a highly vascular region, such as the islet tissue, may receive a much greater amount of alloxan than other less vascular tissues.

Methionine was also studied because it is important as a methyl donator, and because it contains sulphur. Unfortunately, the solubility of methionine is limited to about .22 mM per cc at 25° C, and in order to inject a dose of 7.5 mM/kg, each rat (average weight of 350 g) would have to have been injected with about 10 cc of fluid intravenously. Consequently, the dose of methionine used in the present experiment was not greater than 2.9 mM/kg. At this dose, no effect was observed; whereas, at corresponding doses of cysteine and glutathione,[2] 100% protection was noted. Thiourea was also studied because of its sulphur content, inasmuch as its sulphur is not in the SH form. Although thiourea did not protect against alloxan damage, some animals given thiourea and alloxan died within the first 1-2 hours. Thiourea alone was not toxic. The mechanism of death in these animals given both thiourea and alloxan has not been determined.

Conclusion. BAL, in doses of .36-.72 mM/kg completely protected rats against a diabetogenic dose of alloxan (40 mg/kg intravenously) when the protective agent was given immediately preceding the alloxan injection. However, no protection was observed when the disulfhydryl compound was given 5 minutes following the alloxan. Methionine, in doses of 2.9 mM/kg, and thiourea, in doses of 7.5 mM/kg, injected immediately preceding a diabetogenic dose of alloxan did not alter the course of alloxan diabetes.

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The Relation of Pteroylglutamic Acid to Metal-Porphyrin Enzymes.*

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Data was reported recently [1, 2] and interpreted as suggesting that pteroylglutamic acid (PGA) and its conjugates are involved in the synthesis of the porphyrin portions of metal-porphyrin enzymes. This communication offers additional data in support of that hypothesis.

For the experiments recorded here the organism *Streptococcus faecalis* (8043) was grown in the usual pteroylglutamic acid assay medium,[3] but with the addition of either potassium cyanide, hydrogen peroxide, or caffeine. The H_2O_2 was diluted, filtered through a Berkefeld filter and added aseptically in the required quantity to sterile culture tubes containing appropriate volumes of medium. KCN was treated similarly, after neutralization with acetic acid. The caffeine was added before autoclaving. The final concentrations of the inhibitors in the culture tubes were as follows: H_2O_2, 0.012%; KCN, 0.05%; caffeine, 0.5% (Table I). After inoculation, turbidity was determined at various intervals with a Coleman spectrophotometer. Responses of the organism to stimulation with varying quantities of PGA as the limiting factor was determined with and without the added inhibitor. As a control procedure, similar experiments were conducted by varying the amount of Ca pantothenate and keep-

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