Technical Papers

Prevention of Alloxan Diabetes by Sodium Nitrite and Paraminopropiophenone

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The injection of alloxan into laboratory animals causes a rapid decline in the glutathione content of the blood (*1*). Excess of glutathione, injected intravenously just before alloxan is given, prevents alloxan diabetes (*2*). This and other evidence, summarized by Houssay (*3*), suggest that the level of sulfhydryl in blood is an important factor in the etiology of alloxan diabetes. Recently it was found that sodium nitrite and paraminopropiophenone (PAPP) increased blood sulfhydryl as much as 50 percent in rats and guinea pigs when given in doses producing substantial methemoglobinemia (*4*). This led to experiments, described here (*5*), to determine the effects of nitrite and PAPP upon alloxan diabetes.

Male Long-Evans rats weighing 130 to 160 g were injected subcutaneously with the methemoglobin-formers and intraperitoneally, ½ hr later, with alloxan. In the first experiment they received 4 mg of sodium nitrite in 0.1 ml of phosphate buffer (*p*H 7.2), or buffer alone, and then alloxan at 250 mg/kg. In the second experiment they received 3 mg of PAPP in slightly acidified saline, or acidified saline alone, and alloxan at 250 mg/kg; and additional treatment 2 days later with 2 mg of PAPP and 100 mg/kg of alloxan. The animals were kept in individual metabolism cages with water and their usual food (*6*) until the end of the experiments. Sugar determinations (*7*) were made on urine samples on the 5th and 11th days after alloxan and on blood the 12th day. An animal was counted as diabetic if it showed blood sugar in excess of 180 mg/100 ml (*8*) on the 12th day and at least one previous urinary sugar measurement exceeding 1 g per 20 hr. (Actually only one "diabetic" in each experiment had blood sugar below 300 mg/100 ml or less than the two possible high urinary sugar values). Table 1 summarizes the results.

Table 1. Diabetes and mortality after alloxan, in rats pretreated with nitrite or paraminopropiophenone.

Rats	Number	Dead	Diabetic	Nondiabetic
Nitrite-treated	20	1	1	18
Controls	20	7	8	5
*P**		*0.05*	*< 0.01*	
PAPP-treated	20	1	1	18
Controls	24	5	10	9
*P**		*> 0.20*	*< 0.01*	

* Based on χ^2, Goulden's corrected formula (*9*).

Under the conditions of the experiments, the doses of alloxan used approximated the LD_{50} and the ED_{50} for diabetes. It is clear that both nitrite and PAPP gave marked protection against the effects of alloxan, whether these were manifested by deaths (presumably due to hepatorenal damage) or by diabetes (damage to the pancreatic islets). Both experiments therefore were consistent with the hypothesis that suggested them—that is, that the determining factor in prevention of this type of diabetes is the level of blood sulfhydryl at the time alloxan is injected. This implies chemical reaction between the alloxan and blood sulfhydryl. The sulfhydryl is largely intraerythrocytic, and the alloxan remains in the blood only for a few minutes (*1*): therefore this reaction should be investigated by diffusion techniques. Until such a reaction is clearly demonstrated to be possible, it would be well to reserve judgment concerning the mechanism(s) by which these two methemoglobin-formers prevent alloxan diabetes.

References and Notes

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4. E. P. Vollmer and M. M. Carey, *J. Pharmacol. Exptl. Therap.* **111**, 114 (1954).
5. The opinions expressed are those of the authors and do not necessarily represent official naval opinion.
6. Rockland Rat Diet.
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Factors Necessary for the Growth of *Bacteroides succinogenes* in the Volatile Acid Fraction of Rumen Fluid

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Bacteroides succinogenes has been shown to be one of the more important of the cellulolytic bacteria that are essential to normal digestion in the bovine rumen (*1–3*). This organism is one of several kinds of rumen bacteria that have not been grown without rumen fluid in the medium.

The factor(s) required by *B. succinogenes* was not detectable in several materials commonly used to grow nutritionally fastidious bacteria or in extracts from