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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20 July 1954.

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

alfalfa meal or bovine feces (*2*). It was shown to be stable to heat, acid, alkali, and drying and was not a known B vitamin, amino acid, peptide, purine, pyrimidine, oleic acid, or mineral, although some of these substances are undoubtedly also required. Hungate (*3*) concluded that the factors required for growth of this organism are not all present in the feed consumed by the animal but are in part produced by the accompanying microorganisms. The purpose of the present work was to determine the nature of this unknown factor.

The assay procedure involved the use of an anerobic culture method previously described (*1*). The basal culture medium contained minerals, B vitamins, purines, pyrimidines, casein hydrolyzate (enzymatic), a carbonic acid-bicarbonate buffer system, and either glucose or cellulose (0.3 percent Whatman No. 1 filter paper) as carbon source. Growth in mediums containing cellulose was estimated by the visible loss of cellulose from the tube and growth in glucose mediums was estimated by amount of turbidity after about 18 to 20 hr of incubation using the Cenco-Sheard Spectrophotelometer set at 420 mμ.

The factor was found in the ether extract of rumen fluid extracted at *p*H 2 but not in the residue. The reverse was true on ether extraction at *p*H 10. The factor was found in the volatile acid fraction on acid steam distillation and was not volatile when distilled under alkaline conditions. Fractionation of the volatile acids by chromatographic technique (*4*) showed most of the activity to be present in the valeric acid fraction.

The assay of commercially available volatile fatty acids showed that none of the acids, C_2 through C_6 and their isomers, n-heptanoic, or n-caprylic, allowed a significant amount of growth when added singly to the basal medium. However, when all combinations of two of the acids isobutyric, n-valeric, isovaleric, DL-α-methyl-n-butyric, or n-caproic were assayed, good growth occurred if one was a straight-chain acid and the other, a branched-chain acid. This observation was confirmed by assaying all possible combinations of three of these acids. In this case the only combination not allowing growth was that containing the three branched-chain acids.

Studies to date show that the branched-chain component can be any one of the acids isobutyric, isovaleric, or DL-α-methyl-n-butyric. Isocaproic, trimethyl acetic, or the straight-chain acids C_2 to C_8 will not replace these acids. The straight-chain component can be any one of the acids C_5 to C_8. Acetic, propionic, n-butyric, or lauric acid will not replace these acids. A small amount of activity for the straight-chain component was found in palmitic and stearic acid.

Minimum concentrations for good growth when n-valeric and isovaleric acids were used were about 3 and 1.5 μM, respectively, per 10 ml of medium.

It seems probable that the acids chiefly concerned with the growth response due to rumen fluid are isobutyric, isovaleric, and DL-α-methyl-n-butyric acid for the branched-chain component, and n-valeric and n-caproic acid for the straight-chain component. These have all been found in significant amounts in rumen fluid (*5, 6*). The probable origin of these acids by Stickland reactions from amino acids has been discussed by El-Shazly (*7*).

Details of this work will be reported elsewhere.

Note added in proof: After this work was submitted for publication, O. G. Bentley *et al.* reported that the volatile fatty acids, n-valeric, isovaleric, isobutyric, and n-caproic, or their amino acid precursors stimulated cellulose digestion and the conversion of urea nitrogen into protein by rumen microorganisms as measured by the "artificial rumen" technique (*8*).

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30 July 1954.

In vitro Differentiation between Auto- and Isoimmune Antibodies by Protamine and Trypsin

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It has been shown that low concentrations of heparin inhibit the agglutination of red cells coated with autoantibodies (*1*). This phenomenon was believed to be either competitive, similar to the mechanism of hemagglutinating virus antagonists (*2*), or due to the strongly negative charge of heparin. Although autoantibodies were selectively inhibited, no interaction with isoantibodies was observed (contrary to virus antagonists). The effect of a positively charged colloid on these hemagglutinins was therefore investigated (*3*). Protamine sulfate (Lilly) in concentrations from 0.01 percent (*p*H 6.11) to 1.0 percent (*p*H 5.98) was used.

It was found that saline suspensions of normal cells, cells sensitized with anti-D (albumin) typing serum (*4*), and cells from patients with idiopathic acquired hemolytic anemia were agglutinated by protamine sulfate. Normal, trypsinized cells (*5*) were not affected by any of the concentrations used. Control series showed that acidification alone (*6*) will not cause agglutination in the absence of protamine, nor will neutralization of a protamine solution (*p*H 7.1) affect its agglutinating ability. The effect of cell fragility was also negated, since the resistance to hypotonic saline was the same before and after trypsinization. The possibility of protamine inactivation by minute amounts of trypsin adsorbed on the cell surfaces (*7*)