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Peroxidase-like Activity of Alloxan and Aldehyde.

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Alloxan has recently been found to cause hypoglycemia in normal rabbits.[1] The manner in which this hypoglycemia is brought about should now be explained. This paper presents a reaction of alloxan which might take place in tissues and thus could be concerned with the hypoglycemic effect.

If alloxan in aqueous solution is mixed with a simple aldehyde and hydrogen peroxide, the resulting solution will oxidize benzidine, *p*-phenylenediamine, pyrogallol, iodides, etc. A mixture of alloxan and aldehyde therefore has *peroxidase-like* activity.

The reaction is easily demonstrated. To a few cc of a dilute aqueous solution of an aldehyde add a little alloxan and a few drops of 5% hydrogen peroxide. Then add the substance to be oxidized. For example, 5 drops of a saturated glacial acetic acid solution of benzidine turns the mixture deep blue at once. The reaction will not occur when one of the 3 reagents: alloxan, peroxide, aldehyde, is absent. (Except that lower aldehydes after standing exposed to air and used in strong solution themselves oxidize benzidine.[2]) The reaction will not occur if alloxan is briefly treated with excess alkali, then acidified again. This procedure, which destroys alloxan, eliminates the possibility of metallic catalysis, by traces of copper or iron for example.

All of the aliphatic aldehydes tested and most of the aromatic aldehydes react positively. All substances found to react positively are aldehydes.

Positive. Formaldehyde, hexamethylene tetramine, trioxymethylene (slowly), acetaldehyde, paraldehyde, propionaldehyde, n-butyl aldehyde, crotonaldehyde, aldol, glyceric aldehyde, glyoxal, methyl glyoxal, benzaldehyde, piperonal.

Negative. Ethyl alcohol, acetone, ether, chloroform, glycine, alanine, glycine anhydride, sodium butyrate, glycerol, sodium glycerophosphate, urea, glucose, galactose, sucrose, glycogen, arabinose, xylose, taurine, creatine, creatinine, phlorhizin, chloranil, xanthine, chloral hydrate, *p*-dimethylaminobenzaldehyde, salicylaldehyde, and many others.

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1 Jacobs, H. R., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 407.

2 Woker, G., *Z. Allg. Physiol.*, 1914, **16**, 341; Gallagher, P. H., *Bioch. J.*, 1924, **18**. 29.

As substitutes for alloxan, barbituric acid, isobarbituric acid, barbital, nembutal, alloxantin, murexide, parabanic acid, uric acid, diketopiperazine, chloranil, uramil and cyanuric acid were worthless. No substitutes for simple peroxides were found.

The reaction is very sensitive for the simpler aldehydes. Formaldehyde can be detected in 1-50,000 dilution. The reaction is prevented by very dilute solutions of hydrazines, hydroxylamine, cyanide, bisulfite and cysteine. It is not affected by alcohols and amino-acids. In many respects, therefore, the resemblance to plant and animal peroxidase preparations is great.

Whether the peroxidase-like activity of alloxan and aldehyde is the basis for the observed hypoglycemic effect of alloxan is not known. All of the required reagents are in the tissues. Hydrogen peroxide is formed by the oxidases and by the direct oxidation of sulphydryl compounds and other substances. Aldehydes are present normally.[3] The substrates to be oxidized remain undistinguished in a large group. One surmises that per-acids are formed from aldehydes *in situ* and that they are active in an oxidative process which removes sugar from the blood. Crude peroxidase from milk[4] causes hyperglycemia only, as might be anticipated from its high protein content.

The reaction here described may help explain the acceleration of oxygen uptake by minced liver when treated with alloxan as noted by Bernheim,[5] the peculiar reactivity of aldehydes in biological reactions, and a possible function of hydrogen peroxide in the tissues.

Summary. Alloxan, aldehydes, and hydrogen peroxide together appear to form an oxidizing substance more reactive than any one or two of these reagents alone. This peculiarity results in a peroxidase-like oxidizing system and a specific test for aldehydes.

[3] Supniewski, J. V., *J. Biol. Chem.*, 1926, **70**, 13.

[4] Elliott, K. A. C., *Bioch. J.*, 1932, **26**, 10.

[5] Bernheim, F., *J. Biol. Chem.*, 1938, **123**, 741.