acter. To determine this a number of infected allantoic fluids were centrifuged until the supernates no longer gave positive cultures. With one exception bacteria-free allantoic fluids yielded reactions similar to those obtained prior to centrifugation.

The data are summarized in Table 1.

CONCLUSIONS

(1) Specific oxydase reactions may be obtained with solutions of either dimethyl or tetramethyl paraphenylene diamine hydrochloride when the following are infected with Neisseria: the surface of the chorioallantoic membranes of chick embryos, allantoic fluids both before and after the removal of the infecting bacteria and suitable broth media.

(2) The enzyme responsible for the positive oxydase reaction elicited with the Neisseria is soluble and diffuses into the surrounding liquid environment.

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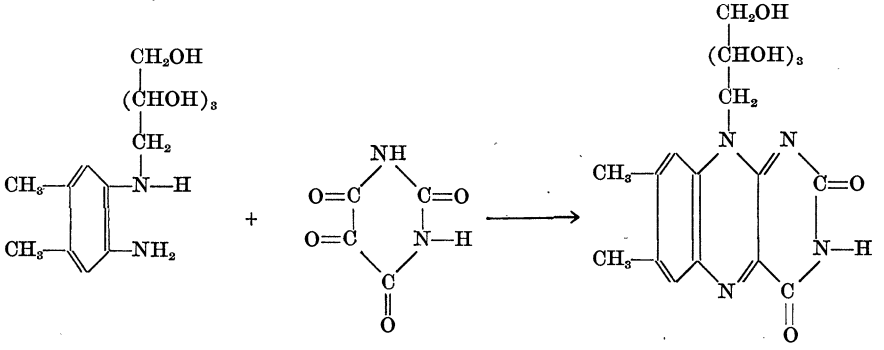
SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MICROBIOLOGICAL AND FLUOROMETRIC TEST FOR MINUTE AMOUNTS OF ALLOXAN[1,2]

THE work of Dunn and coworkers[3,4] has revealed that alloxan administered parenterally produces in rats and rabbits an acute specific necrosis of the islets of Langerhans. This has been amply confirmed in rats, rabbits, dogs and monkeys by various workers.[5–10] The production of experimental diabetes by alloxan and the importance of these experiments has been reviewed by Joslin.[11,12] It has been suggested by Dunn and his coworkers[4] that possible defects in the metabolism of purines or of alloxan in man may play some role in the etiology of diabetes mellitus. For the study of the metabolism of alloxan, a specific method for the determination of alloxan would obviously be advantageous. In the present note, we wish to report our preliminary studies leading to a sensitive and specific test for the quantitative estimation of alloxan in pure solution, which we hope may eventually lead to a specific method applicable to biological materials.



I Alloxan Riboflavin

At the time this work was undertaken, no specific method for alloxan was available. While the work was underway, we learned through private communication from Dr. R. M. Archibald[13] that he had a paper in press which contained several methods for the determination of alloxan. We would like to thank him for his courtesy in allowing us to read his manuscript before it appeared in print. Of those methods described by Archibald the most specific and sensitive is based on the condensation of alloxan with *o*-phenyl-

[1] The authors wish to express their appreciation to the Lederle Laboratories for a research grant that has aided greatly in this work. We are indebted to Dr. R. O. Roblin, Jr., of the American Cyanamid Company, for the sample of alloxan monohydrate used in these studies, and to Dr. J. A. Aeschlimann, of Hoffmann-La Roche, Inc., for a supply of 1,2-dimethyl-4-amino-5(*d*-1′-ribityl-amino)-benzene.

[2] The authors also wish to acknowledge with appreciation the technical assistance of Miss Rachel Jewett and Miss Martha Fuchs.

[3] J. S. Dunn, H. L. Sheehan and N. G. B. McLetchie, *Lancet*, 244: 484, 1943.

[4] J. S. Dunn, J. Kirkpatrick, N. G. B. McLetchie and S. V. Telfer, *Jour. Path. Bact.*, 55: 245, 1943.

[5] J. S. Dunn and N. G. B. McLetchie, *Lancet*, 245: 384. 1943.

[6] C. C. Bailey and O. T. Bailey, *Jour. Am. Med. Asn.*, 122: 1165, 1943.

[7] M. G. Goldner and G. Gomori, *Endocrinology*, 33: 297, 1943.

[8] H. Hughes, L. L. Ware and G. F. Young, *Lancet*, 246: 148, 1944.

[9] J. H. Ridout, A. W. Ham and G. A. Wrenshall, SCIENCE, 100: 57, 1944.

[10] S. Banerjee, *Lancet*, 247: 658, 1944.

[11] E. P. Joslin, *New Eng. Jour. Med.*, 230: 425, 1944.

[12] E. P. Joslin, *New Eng. Jour. Med.*, 232: 219, 1945.

[13] R. M. Archibald, *Jour. Biol. Chem.*, 158: 347, 1945.

enediamine to yield alloxazine, which is measured by its fluorescence.

In the test we are reporting in the present communication, we have employed the condensation of alloxan with 1,2-dimethyl-4-amino-5(*d*-1′-ribitylamino)-benzene(I) to yield riboflavin.[14]

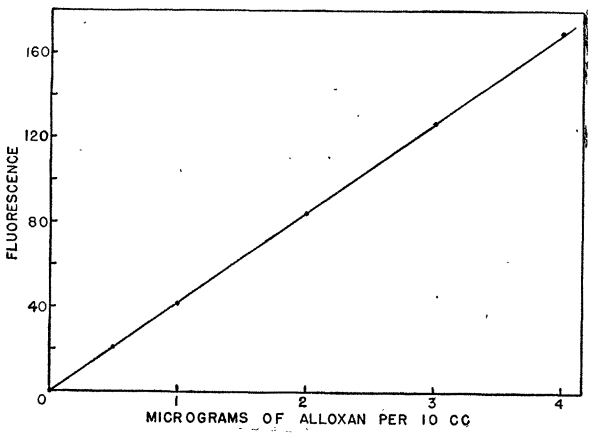


FIG. 1. Fluorescence of riboflavin plotted against the amounts of alloxan which were treated to yield riboflavin.

The riboflavin so produced was measured quantitatively by its fluorescence and by its growth-promoting properties for *Lactobacillus casei*. This method thus offers the high degree of specificity of the requirements for riboflavin by a microorganism as a check for the easily measured fluorescence of riboflavin. Furthermore, the already well-known methods for the determination of riboflavin are utilized.

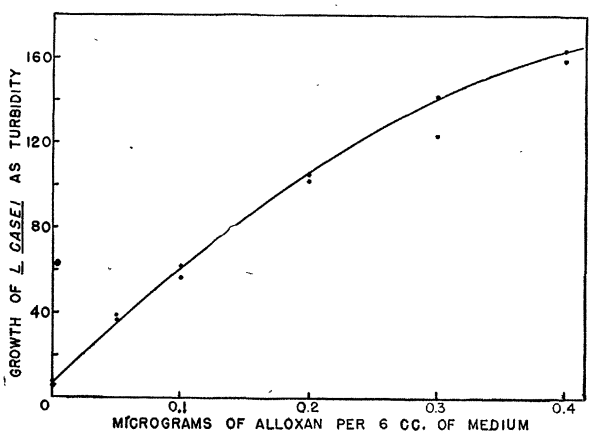


FIG. 2. Growth of *L. casei* plotted against the amounts of alloxan which were treated to yield riboflavin.

When alloxan in amounts from 0.2 to 4 micrograms per cc was treated as described under "Procedure," the fluorescence obtained was proportional to the amount of alloxan present (Fig. 1). A typical riboflavin growth-response with *L. casei* was obtained with the riboflavin formed in the condensation. Amounts from 0.05 to 0.4 micrograms of alloxan converted to riboflavin could be measured by this microbiological procedure (Fig. 2).

Using our fluorescent method, we were able to confirm the results of Leech and Bailey[15] on the stability of alloxan at various hydrogen ion concentrations (Fig. 3). Alloxan remained stable in glacial acetic

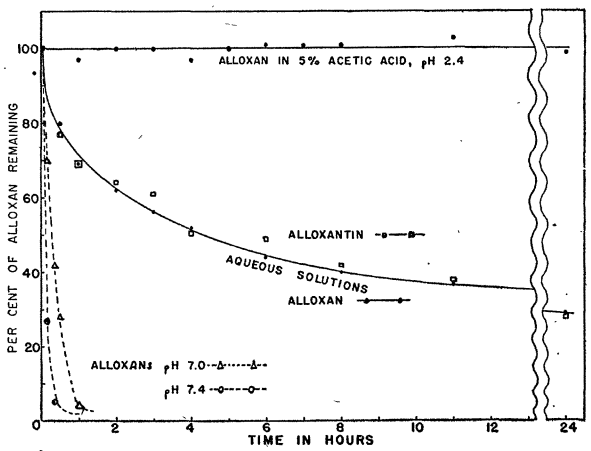


FIG. 3. Curves illustrating the stability of alloxan at various hydrogen ion concentrations, and a comparison of the stability of alloxan with alloxantin in aqueous solutions.

acid and in solutions of 5 per cent. acetic acid in water for a period of several weeks. Alloxantin, when treated with 1,2-dimethyl-4-amino-5(*d*-1′ribityl-amino)-benzene, yielded the same amount of riboflavin as did alloxan. The stability of alloxantin in water (10γ/cc) was also found to be identical with that of alloxan in water (Fig. 3).

Procedure: A stock solution of alloxan, containing 100 micrograms of alloxan per cc of 5 per cent. acetic acid in water, was prepared weekly. Solutions containing 10 and 1 micrograms per cc, respectively, were prepared daily by diluting the stock solution with 5 per cent. acetic acid. A sufficient amount of 1,2-dimethyl-4-amino-5(*d*-1′-ribitylamino)-benzene was dissolved in glacial acetic acid to give a concentration of 2 mg per cc.

To 20 × 150 mm pyrex test-tubes were carefully added the following amounts of alloxan: 0.2, 0.5, 1.0, 2.0, 3.0 and 4.0 micrograms; the volume in each tube was adjusted to 1.0 cc with water. Unknown solutions to be analyzed are likewise made up to 1 cc of volume. Then to each tube was added 1.0 cc of glacial acetic acid and 1.0 cc (2.0 mg) of 1,2-dimethyl-4-amino-5(*d*-1′-ribitylamino)-benzene. The tubes were

[14] R. Kuhn, K. Reinemund, F. Weygand and R. Ströbele, *Ber.*, 68: 1765, 1935.

[15] R. S. Leech and C. C. Bailey, *Jour. Biol. Chem.*, 157: 525, 1945.

heated simultaneously in a water bath (90–100°) for 3 hours. At the end of this period of heating, if the amount of alloxan present was to be measured fluorometrically, 8.0 cc of 1 M sodium acetate-acetic acid buffer, pH 5.5, were added to each tube. The fluorescence of the formed riboflavin was measured by a Coleman photofluorometer, Model 12, using the filters for riboflavin determinations supplied with the instrument. The degree of fluorescence is plotted against concentration of the standard alloxan solutions as shown in Fig. 1.

The concentration of alloxan in the unknown solutions, run simultaneously with the standards, is obtained from the plotted curve. If the amount of riboflavin formed from the alloxan was to be measured by its effect on the growth of *Lactobacillus casei,* then the contents of the tubes after heating on the water bath were carefully concentrated to dryness. This was accomplished rapidly by evaporating the solutions under vacuum of a water pump while at the same time the tube was shaken with a swirling motion in a hot water bath. Standard and unknown samples were treated under identical conditions. Immediately after the solutions were concentrated to dryness, the residue was dissolved in exactly 10 cc of 0.2 M sodium acetate-acetic buffer, pH 6.6. One cc of each of these solutions was then added to 5.0 cc of the medium developed by Landy and Dicken,[16] which needed only riboflavin for good growth of *Lactobacillus casei.* After sterilization, each tube was inoculated with one drop of a 1:20 dilution of a 24-hour culture of *L. casei* grown in the basal medium; the cells were centrifuged and resuspended in a sterile saline solution before the final inoculum dilution was made. After 40 hours of incubation at 37°, the growth of *L. casei* was measured turbidimetrically in a Klett-Summerson photoelectric colorimeter. The growth response of *L. casei* to increasing amounts of alloxan is plotted in Fig. 2.

SUMMARY

A microbiological and fluorometric test for the determination of minute amounts of alloxan has been described. The test involves the conversion of the alloxan to riboflavin which is measured by microbiological or fluorometric techniques.

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[16] M. Landy and D. M. Dicken, *Jour. Lab. Clin. Med.,* 27: 1086, 1942.

[17] Ghosh Traveling Fellow of the University of Calcutta.

A HANGING DROP METHOD FOR CONTINUOUS OBSERVATION OF THE ACTIVITY OF ORGANISMS IN CYANIDE[1]

INTERPRETATION of cellular oxidative processes may be aided by correlating the oxygen consumption of organisms exposed to cyanide with observations of their visible activity. With the method described it is possible to observe cell division, muscle contraction and other phenomena, while the concentration of HCN is varied quantitatively and the organisms remain otherwise undisturbed. The procedure is possible because of the rapid diffusion of HCN gas and the consequent rapid attainment of equilibrium between a large volume of cyanide solution of a known concentration and a hanging drop exposed to it.

Fig. 1 illustrates the transparent plastic chamber

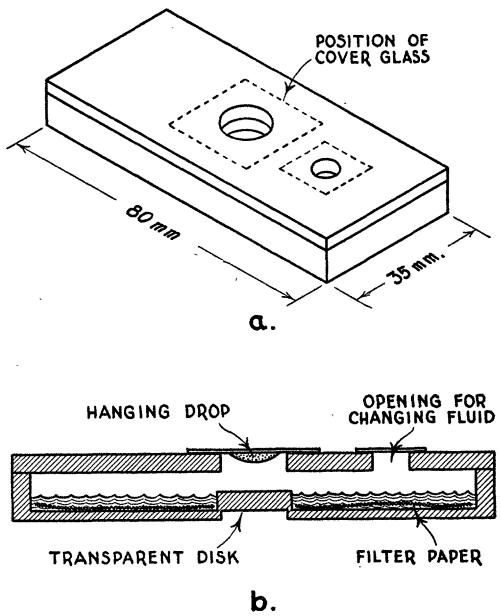

FIG. 1. Transparent plastic chamber used in observing effect of cyanide on behavior of organisms in a hanging drop. A. Isometric view. B. Longitudinal section.

designed for these experiments. The top and bottom plates are sealed together with liquid petrolatum and a cover glass with a hanging drop containing the tissue or organism is set over the opening as shown. After a control period in which the normal behavior is noted, 3 ml of HCN solution made up in the same medium as the hanging drop is placed on the filter paper in the chamber and the small opening is closed. In a short time the HCN concentration in the drop becomes the same as that in the larger volume of fluid, and since the plastic is relatively impermeable it will stay at this level for hours without change. It is thus possible to attain a given level of cyanide

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