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.

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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

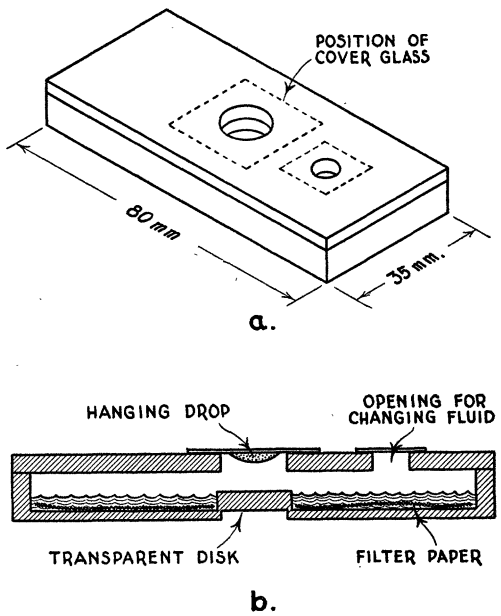

FIG. 1. Transparent plastic chamber used in observing effect of cyanide on behavior of organisms in a hanging drop. A. Isometric view. B. Longitudinal section.

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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without altering the position of the organism or disturbing its ionic environment. The saturation of a 0.03 ml drop depletes the 3 ml of solution in the chamber by only 1 per cent. Fig. 2, curve A, shows

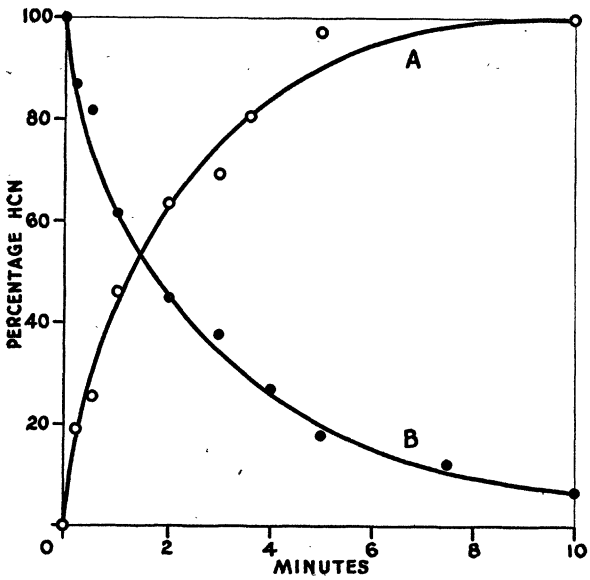

FIG. 2. Curve A. Ordinate shows the percentage saturation of a 0.03 ml hanging drop exposed to 3 ml of 0.01 M. HCN solution in the plastic chamber. Curve B. Ordinate shows the percentage of the original concentration remaining after the 0.01 M. HCN solution had been replaced by 1 per cent. KOH. Abscissas indicate time in minutes.

the rate of saturation of a drop after HCN solution is placed in the chamber. It is apparent that within 2 minutes the drop is almost two thirds saturated, and that after 5 minutes exposure to the HCN solution its concentration is almost the same as that of the larger volume of liquid. (All cyanide measurements were made by the phenolphthalin method.[2])

It is an equally simple process to watch the recovery from cyanide treatment. When the HCN solution is removed from the chamber with a pipette and replaced with a 1 per cent. KOH solution, the cyanide in the drop quickly passes into the alkali and leaves the drop in its original condition. As shown by curve B in Fig. 2, the concentration in the drop decreases 50 per cent. within 2 minutes after the replacement with KOH, and is less than one fifth of the original level in 5 minutes.

The cyanide solutions are prepared by dissolving a weighed amount of KCN in a volumetric flask, neutralizing it with dilute HCl (with a drop of phenol red as indicator), and diluting this stock solution to make the concentrations desired. Although it is theoretically preferable to make these solutions from liquid of the same pH and osmotic pressure as the medium of the hanging drop, there is actually very little difference in the HCN tension within a range of pH 6.8 to 7.4 and a salinity of 0 to 5 per cent. With sea water experiments the cyanide solutions should be prepared with sea water.

Three sample experiments suggest the possible uses of the method. (1) *Vorticella:* These protozoons placed in a hanging drop of pond water and exposed to 0.04 M. HCN were immediately stimulated and most of them became free-swimming, but in spite of the high concentration the majority of the animals were still alive after an hour. (2) *Artemia:* Brine shrimp in artificial sea water showed a marked variation in sensitivity with age, perhaps associated with the type of substrate being metabolized. Young animals were still living after an hour in 0.01 M. HCN, but older ones became inactive in 10 minutes with only 0.001 M. HCN. (3) *Ciliated Epithelium:* Cilia on sections of rat trachea suspended in buffered saline will beat for hours at room temperature. A concentration of 0.0003 M. HCN stopped most of this action in 5 minutes, but with 0.0001 M. it was only slightly slowed after half an hour. After the activity had been arrested by 0.01 M. HCN, the solution in the chamber was replaced with 1 per cent. KOH. Enough recovery occurred in 15 minutes to permit motion to start again, and by half an hour the ciliary activity appeared normal.

WILBUR ROBBIE

SCIENTIFIC BOOKS

MINERALOGY

Dana's System of Mineralogy. By CHARLES PALACHE, HARRY BERMAN and CLIFFORD FRONDEL. Seventh Edition, Volume I, Elements, Sulfides, Sulfosalts, Oxides. New York: John Wiley and Sons, Inc., 1944. $10.00.

DANA'S System of Mineralogy is America's most important compilation of information on mineral species, and has been a reference standard for about a century. It holds a place of authority in mineralogy somewhat analogous to that of Gray's Manual in the field of botany. The last edition of the System appeared in 1892, and supplements to it were published in the form of Appendices in 1899, 1909 and 1915. Since these appeared x-ray diffraction has been developed into a new tool with which to study crystals generally, and has been intensively applied to the study of minerals. The science of mineralogy has been greatly enriched by its aid, not only in sheer

[2] W. A. Robbie, *Arch. Biochem.*, 5: 49, 1944.