entirely possible for gross fecal pollution to be present in the absence of recoverable colon bacilli or even of any members of the coli-aerogenes group. This fact may explain certain explosive outbreaks of water-borne disease, such as have occasionally been reported —outbreaks with gastro-intestinal symptomatology and probable etiology but unsolved as to microbial causation. These data explain discrepancies in existing data on the coli-aerogenes distribution in normal feces. Changes in flora of the type reported have been noted, though rarely, in pathological conditions. That such change may occur in normal subjects has not been emphasized and renders less significant such reports in cases of disease. These findings make it increasingly clear that there is no possibility, as yet, of distinguishing between fecal and non-fecal forms of the coli-aerogenes group in any specific instance, despite the probability that in general non-citrate utilizing coli are typically fecal and the other forms of the group less so. If a presumably healthy individual may for days pass feces from which the bacillus nominated as the fecal indicator par excellence can not be recovered one may ask whether there is not still work to do in sanitary bacteriology. If the coli-aerogenes group is our best indicator of intestinal contamination the need for rigid control of water and food processing is emphasized from a new angle. Not the least interesting of the points raised is the question of the mechanism whereby the lactose-fermenting, gram-negative, non-sporing aerobes of the bowel are so grossly disturbed in their relationships or indeed temporarily eliminated in the apparently healthy adult. Bacterial antagonisms and inhibitions, bacteriophage activity, and sub-clinical infections involving disturbances of bowel conditions are a few only of the points which may well be looked into in this connection.

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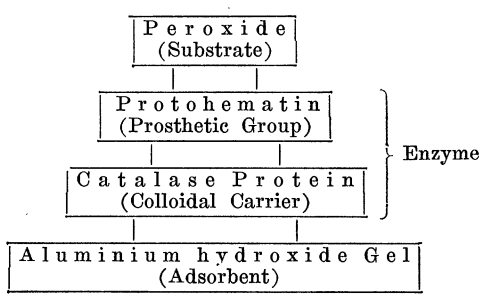
MODE OF COMBINATION OF AN ENZYME WITH AN ADSORBENT AND WITH A SUBSTRATE

IF a purified and highly active solution of liver catalase[1] is adjusted to pH 5 and stirred with a suitable amount of aluminium hydroxide gel or of silicic acid, a large fraction of the enzyme is deposited on the adsorbent. The adsorbate is washed with and suspended in distilled water. When placed in an optical trough of 1 cm thickness and examined in the condensed beam of a 500 watt projection lamp by means of a pocket spectroscope, a three-banded absorption spectrum is observed which proves to be exactly the same as that of the enzyme in solution.[2] The adsorbate is catalytically active towards hydrogen peroxide and monoethyl hydrogen peroxide. With the latter substrate, the two-banded spectrum of the intermediate enzyme-substrate compound and the whole reversible spectral cycle, as reported for the dissolved enzyme,[3] may be seen. Likewise, cyanide will combine with the hematin grouping of the enzyme in the adsorbed state.

Inasmuch as it is known that invertase is eluted from an adsorbate by its substrates, it was necessary to ascertain whether the catalytic activity of the adsorbate in the present case is not due to a preceding desorption of catalase by the peroxide. However, when an adsorbate suspension to which an excess of monoethyl hydrogen peroxide had been added was subjected to filtration while the enzymatic reaction was in progress, a colorless, catalase-free filtrate was obtained, proving that the catalysis and the spectral changes occur directly on the surface of the adsorbate.

The extent of adsorption of the enzyme depends on the type of adsorbent used. Kaolin, finely powdered quartz and activated charcoal did not give satisfactory results under similar experimental conditions.

The enzyme catalase consists of protohematin[4] and of a specific protein.[5] During the heterogeneous catalysis the following arrangement exists:



Free hematin or pyridine-parahematin will not form compounds with peroxides of the type obtained in the case of catalase or methemoglobin. Considering the much smaller activity of methemoglobin compared with that of the enzyme, it follows that not only is a protein component apparently necessary for the formation of the intermediate compound but also the specific nature of the protein determines the decomposition rate of the intermediate. The protein may provide for proper spacing of the hematin groups on the enzyme surface.

It is suggestive to depict the enzyme-substrate compound in the manner preferred by Haurowitz[6] for the

[1] Prepared according to Zeile and Hellstroem.[2] The monomolecular velocity constant of the solution used for these experiments was k = 4525.

[2] K. Zeile and H. Hellstroem, *Z. physiol. Chem.*, 192: 171, 1930.

[3] K. G. Stern, *Nature*, 136: 335, 1935.

[4] K. G. Stern, *Nature*, 136: 302, 1935. *J. Biol. Chem.*, 112: 661, 1936.

[5] K. G. Stern, *Z. physiol. Chem.*, 208: 86, 1932; 217: 237, 1933.

[6] F. Haurowitz, *Z. physiol. Chem.*, 232: 159, 1935.

hydrogen peroxide-methemoglobin, namely as a coordinative linkage between the porphyrin-bound trivalent iron and the peroxide molecule. The stoichiometry in the two cases may, however, differ.[3] In contrast to methemoglobin, catalase will combine with the substrate at a pH where both, at least in part, exist as anions. This may be of some significance in explaining the behavior of the two catalysts.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

PIPETTING DEVICE FOR DISINFECTANT TESTING

IN the determination of phenol coefficients by the F.D.A. method, it is necessary to inoculate suspensions of pathogenic microorganisms into medication tubes with quantitative accuracy. Pipettes are the prescribed equipment for measuring the dosage of culture, which must be introduced without the tip of the pipette touching the disinfectant. The tip may be allowed to rest against the side of the tube just above the surface of the liquid. It is obvious that greater precision will be obtained if uniform and accurately measured samples of culture are used and if organisms are not left on the sides of the tube where they might escape adequate contact with the solution.

We have found that a simple device regulates the spatial relations of pipette and test fluid in the medication tube, irrespective of the fatigue of the worker, and permits an unusual precision of technique in this test, which in our experience greatly reduces irregularities of results. Furthermore, our arrangement reduces the danger of infection of the technician or of the laboratory.

Fig. 1 shows the device, assembled for use. It consists of a graduated pipette A, actuated through suitable tubing by a heavily greased, 10 ml syringe B. An essential feature is a 90° V-shaped guide trough C, fixed in a vertical position to a convenient support. We have used a supporting block of wood fastened to a ring stand with ordinary burette clamps D. This trough is conveniently formed from No. 18 gauge aluminum, 2″ × 12″, with its long edges rolled back. (This size is useful for medication test-tubes, 25 × 150 mm). A screw clamp on one edge of the guide trough serves as a stop E to fix the position of the test-tube in use. The spring clamp F, attached by clamp fasteners G and short pieces of steel rod to the ring stand, is adjusted to hold the pipette in a position equidistant from and parallel to the walls of a test-tube H held against the trough. A beaker I directly below the pipette and containing a small volume of 1:1000 $HgCl_2$ serves as a trap for any accidental dripping of culture.

When a test is to be made, the guide trough is

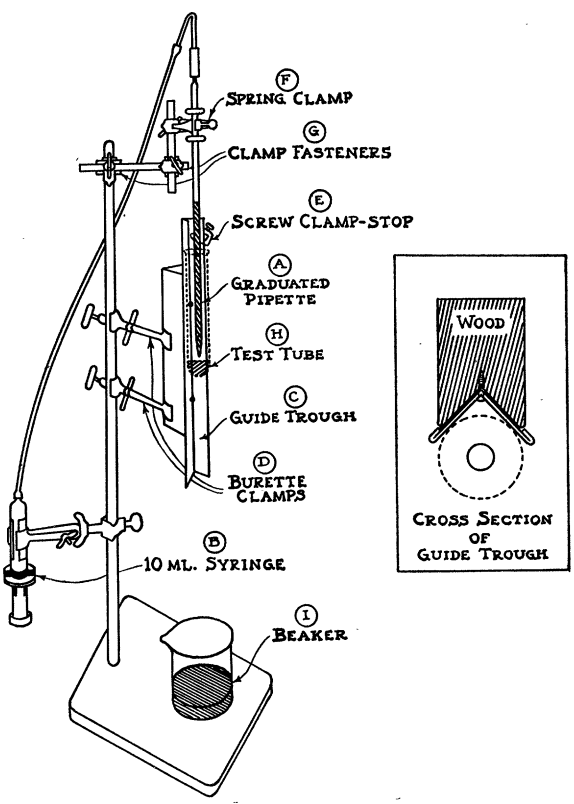

FIG. 1

flamed with a bunsen, the free end of the rubber tubing is slipped over the mouth end of a sterile, cotton-plugged pipette which is then clamped into position. A test-tube of bacterial culture is slid along the trough to a point where the pipette is just above the sediment, and the pipette is then charged with inoculum by withdrawing the syringe plunger. Inoculation is effected into a medication tube slipped into place where the level of its contents will be substantially 1 cm below the pipette tip. By means of the syringe the desired volume of inoculum is dispensed smoothly. The last drop may be withdrawn or dislodged, provided the same technique is always followed.

This device has the following advantages: