typical for pheohemin proteins. The values obtained by these workers[3] for rat retina represent points on the spectrum of the Pasteur enzyme (Stern and Melnick[4]). The respiratory ferment in animal tissues is generally identified with cytochrome oxidase, which catalyzes the oxidation of cytochrome c.

For an investigation of the spectrum of cytochrome oxidase in mammalian tissue, phosphate extracts (pH 7.3) of rat heart muscle were chosen; succinate served as substrate. The extracts contained an excess of cytochrome c. Although the overall reaction is the oxidation of succinate to fumarate, there is ample evidence to show that this reaction is mediated by the cytochrome-cytochrome oxidase system.[5] CO is a strong inhibitor of cytochrome oxidase in the absence of cells[6]; this inhibition may be relieved by light. Such extracts exhibit a vigorous O_2 uptake in the presence of succinate at temperatures as low as 10°, and consequently lend themselves to the photochemical technique.

The arrangement of the photochemical apparatus and the method of charting photochemical absorption spectra have already been described.[2,4] In the present case the photochemical effect consists of an increase in O_2 uptake when rat heart muscle extract, in the presence of succinate and a gas phase of 95 per cent. CO and 5 per cent. O_2, is subjected to strong monochromatic illumination. The relative light absorption coefficients as referred to a standard wave-length ($\beta\lambda/\beta_{436}$) were calculated for twenty-three wavelengths.

The data show that cytochrome oxidase from a mammalian source, like the respiratory ferment in yeast and in bacteria, exhibits a spectrum characteristic of pheohemin compounds. There is a steep Soret or γ-band in the blue at 450 mμ, and two secondary maxima, the β-band in the blue-green at 510 mμ and the α-band in the yellow at 589 mμ. The thermolability of the enzyme suggests that the hemin grouping is combined with a protein. In spite of the similarity of the spectral patterns of these enzymes, there exist significant differences in details, indicating that they are not identical. Thus the position of the main absorption band is at 450 mμ in the instance of the enzyme of heart muscle and at 430 mμ for that in acetic acid bacteria and in yeast.[2,7]

It is of interest to note that the Pasteur enzyme of rat retina also has its main absorption band at 450 mμ[4]; however, its non-identity with rat heart muscle cytochrome oxidase is indicated by the fact that the α-bands are located at different positions, namely, at 578 mμ for the Pasteur enzyme and at 589 mμ for cytochrome oxidase. A similar situation exists in the yeast cell where the γ-bands of the Pasteur enzyme and the respiratory ferment coincide, whereas the structure of the α-bands differs significantly.[7]

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[3] O. Warburg and E. Negelein, *ibid.*, 214: 101, 1929.
[4] K. G. Stern and J. L. Melnick, *Jour. Biol. Chem.*, 139: 301, 1941.
[5] D. Keilin and E. F. Hartree, *Proc. Roy. Soc. Series B*, 127: 167, 1939.
[6] *Ibid.*, 125: 171, 1938.
[7] J. L. Melnick, *Proc. Am. Soc. Biol. Chem., 35th Annual Meeting*, 1941, p. 90.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

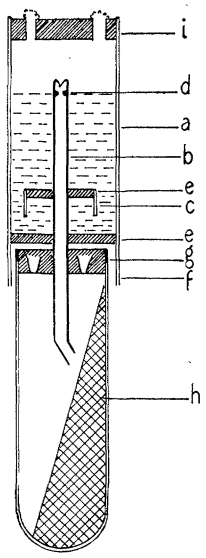
THE EXAMINATION OF CONTAMINATED WATERS[1]

A RAPID method has been devised to speed up the routine bacteriological examination of water from an average of 48 to 96 hours to 8 to 10 hours. The sample is inoculated into routine presumptive lactose broth, then transferred at the optimum time to a confirmation media, either liquid or solid. No difficulty has been experienced in obtaining discrete colonies. The ordinary laboratory glassware is used to assemble this very simple apparatus. Both presumptive and confirmation media can be sterilized and handled as a single unit. Other types have been developed for special purposes.

The principle of this method is the utilization of gas produced by fermentation in the presumptive media to cause a small amount of enriched inoculum to overflow into a conductor tube, automatically inoculating the

FIG. 1

"a" Presumptive tube containing Lactose Broth.
"b" Conductor tube.
"c" Fermentation vial.
"d" Limiting level mark; = height of water + media.
"e" One hole rubber stoppers.
"f" Glass skirt; continuation of presumptive tube.
"g" Rubber stopper notched along edge to admit air.
"h" Confirmation media; solid E.M.B. slant, or liquid B.G.B.
"i" Two-hole rubber stopper with cotton plugs.



[1] Preliminary report.

confirmation media. It should be understood that since the success of this method depends upon the formation of certain minimum amounts of gas those samples having very few organisms, or predominatingly slow lactose fermenters, must necessarily take a longer period of time for completion of the test. Speed is enhanced by using larger samples for only slightly contaminated waters.

The governing factors are: (1) concentration of organisms in inoculum; (2) size of fermentation vial which regulates the amount of liquid which will overflow into conductor tube; (3) height of limiting level mark; (4) diameter and shape of conductor tube; (5) length of time inoculum is enriched before passing into conductor tube, 4 hours found to be optimum time. All these factors are controllable.

With this method, mobile laboratories are enabled to collect a flock of samples on one day and are ready to move again the next morning when the tests are completed. Positive tests on ships' supplies may be accomplished overnight as compared with 48 to 96 hours. In a large distribution system, water leaving the reservoirs may be tested with ensuing results obtained sufficiently early to regulate the supply before it reaches the end of the distribution system.

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PERMANENT MOUNTS OF VIRUS-INFECTED CHORIOALLANTOIC MEMBRANES

THE chorioallantoic membrane of chick embryos has become an important tissue for the cultivation of viruses. The lesions produced are in many cases characteristic of the infecting virus. There is a need for an easy method of permanently mounting such membranes. A method is here described which has proven itself to be satisfactory.

The mounting material is prepared by slowly pouring, with constant stirring, 50 gm of powdered isobutyl methacrylate polymer[1] into 100 cc of xylol. The mixture is placed in the incubator and stirred at intervals until it becomes clear. This takes about an hour. A higher concentration of the plastic is less good, as air bubbles do not rise well to the surface.

The membranes are harvested in the usual manner and rinsed in physiological saline or Tyrode's solution. They are then passed through a series of dilutions of ethyl alcohol, 5, 10, 15, 20, 25, 30, 40, 50, 65, 85, 95 per cent. and, finally, absolute alcohol. They are spread out and left in each dilution for 15 minutes or longer, except for the absolute alcohol in which they are left for at least half an hour. Just before mounting they are transferred from the latter to xylol, where they are left for five minutes. About 5 cc of the solution of plastic is poured into the bottom of a Petri dish. The membranes are drained slightly and spread out in this. A paper label may be embedded beside them. This may be typewritten or marked with pencil, ink or india ink. The Petri dish is set aside to dry in a dust-free place. A second layer of plastic is added to cover all irregularities. When this has hardened, the cover of the Petri dish is put on to protect the surface from dust and injury.

When the membranes are passed through fewer dilutions of alcohol or more rapidly, the normal parts do not remain as clear and the lesions do not show as well. Other solvents were tried, but none gave better results than xylol.

No difficulty is experienced from curling of the membranes. In membranes with considerable edema there is a shrinkage of 10 to 15 per cent., but in normal ones or in those with little edema there is no shrinkage.

This method produces a solid mount which can be easily handled and examined. The areas of hyperplasia due to virus infection stand out in sharp contrast to the surrounding tissue.[2]

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[1] Manufactured by E. I. du Pont de Nemours and Company, Wilmington, Del.

[2] I wish to express my appreciation for the valuable suggestions of Dr. Maurice N. Richter. This work was conducted under a grant for virus research from the Lambert Pharmacal Company, St. Louis, Mo.

CORRECTION

IN Table 2 of the article "Prevention of Tumor Growth (Carcinoma 2163) by Intravenous Injections of Yeast and Vitamins" (SCIENCE, July 18, 1941) the per cent. figures for non-takes should read: Yeast + Riboflavin 62%, Yeast 21%, Riboflavin 14%, Yeast + Thiamin 18%, Thiamin 3%.

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