1. The presence of reversibly oxidizable substances in melanoblasts that accelerate oxidation of dopa by atmospheric and dissolved oxygen.

2. The presence of tyrosinase in melanoblasts along with an inhibitor. Such melanoblasts could oxidize dopa in air rapidly, but tyrosine would be oxidized so slowly as to be imperceptible over a short period of time because of the inhibitor.

That a positive dopa reaction indicates the presence of inhibited tyrosinase seems most likely and experiments are in progress to test this hypothesis.

11963

Electron Microscopic Studies of Biological Reactions. I. Reduction of Potassium Tellurite by *Corynebacterium diphtheriae.*

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Since the beginning of the science of bacteriology, investigators have pursued the study of the chemical reactions brought about by bacteria. As a result there has accumulated a tremendous mass of knowledge concerning the types of reactions induced by bacteria, and the particular reactions which characterize various species. However, in all this work it has not been shown definitely whether the reactions in question take place exclusively on the cell surface, as some investigators maintain, or inside the cell, or both.

With a powerful new tool, the electron microscope[1] now available, it was thought desirable to attempt to determine the site of some chemical reaction brought about by a bacterial cell. Since the electron microscope detects variations in density, such a reaction should involve as products large particles or crystals resolvable with the microscope; *i. e.*, the particles must have diameters of at least 50 Å (5 mμ). Another possibility would be to select for study reactions which involve substances which are capable of adsorbing particles

* RCA Fellow of the National Research Council.

1 Marton, L., *Phys. Rev.*, 1940, **58**, 57; *J. Bacteriology*, in press; and other references quoted in these papers; Zworykin, V. K., Hillier, J., and Vance, A. W., *Electrical Engineering*, in press.

or substances such as large protein molecules, organic compounds, or inorganic salts, any of which would give rise to a region of high density within or upon the cell. The addition of material to a suspension would, however, give rise to the possibility of the production of artifacts. We have consequently chosen for initial study a reaction of the first type, one brought about by an organism in a medium on which it produces an insoluble substance.

The reaction chosen for study was discovered in 1900 by Klett.[2] He observed that many microörganisms, including the diphtheria bacillus, when grown in or upon culture media containing tellurite or selenite salts, reduces them to the free metals. Conradi and Troch[3] proposed a medium containing a tellurite salt for an aid in the diagnosis of *Corynebacterium diphtheriae* because on this medium the colonies of this organism have a characteristic black color due to the reduced tellurium, and because the medium exerts a selective bacteriostatic effect, allowing the diphtheria bacillus to grow but inhibiting certain other microörganisms. Although Klett observed gray particles inside and outside the cells of *Bacillus anthracis* grown on tellurite media, it is not known where the reaction occurs in the case of *C. diphtheriae*.

We have obtained micrographs with the RCA electron microscope of cells from diphtherial colonies growing on blood extract agar and on potassium tellurite chocolate agar. Fig. 1 shows un-

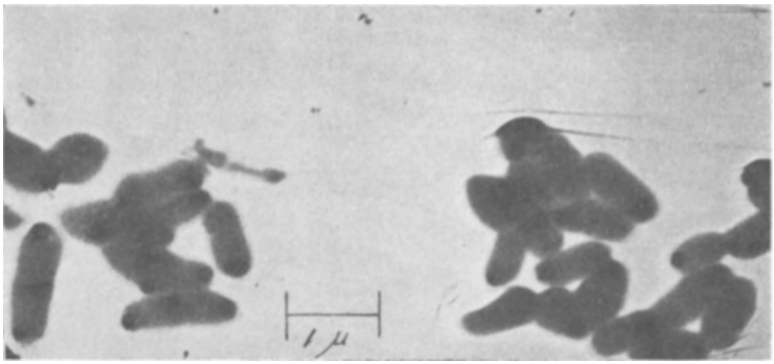

FIG. 1.

C. diphtheriæ, XIII, × 20,000. Grown on extract agar, pH 7.2, containing 1% Parke, Davis peptone and 5% normal horse blood, for 48 hours at 37°C and an additional 48 hours at room temperature. Preparations made from a suspension of the bacteria in distilled water.

[2] Klett, Ad., *Z. f. Hyg.*, 1900, **33**, 137.

[3] Conradi and Troch, *Münch. med. Wochenschr.*, 1912, **59**, 1652.

stained cells of *C. diphtheriae* from blood extract agar. The characteristic polar granules appear as black circles, while the remainder of the cell is light gray. Since, as previously mentioned, electron micrographs reveal differences in densities, this is to be interpreted as indicating that the polar granules are spheres or possibly plates of high density, while the remainder of the cell is of a relatively low density, in the dry state.

Fig. 2 and 3 are of unstained cells grown on potassium tellurite chocolate agar. Inside the cells, and in addition to the polar granules, are seen minute needle-like crystals of high density. If these crystals are actually tellurium metal they should dissolve in the presence of an oxidizing agent such as bromine. To test this point,

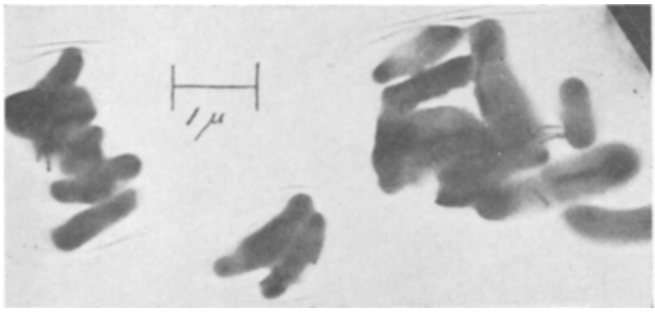

FIG. 2.
C. diphtheriæ, XIII, × 18,000. Grown on potassium tellurite chocolate agar (Difco) for 48 hours at 37°C and an additional 96 hours at room temperature. Preparations made from a suspension of the bacteria in distilled water.

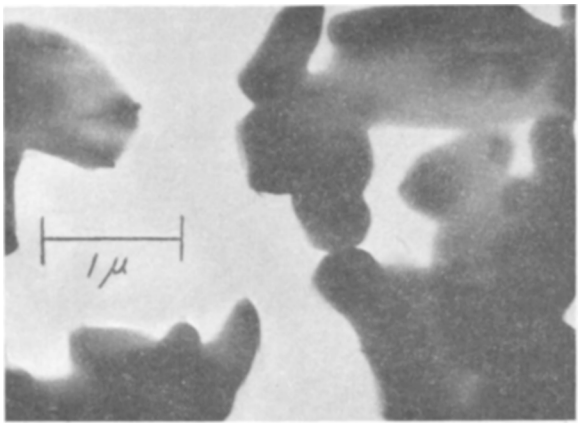

FIG. 3.
C. diphtheriæ, XIII, × 30,000. Conditions of growth same as Fig. 2.

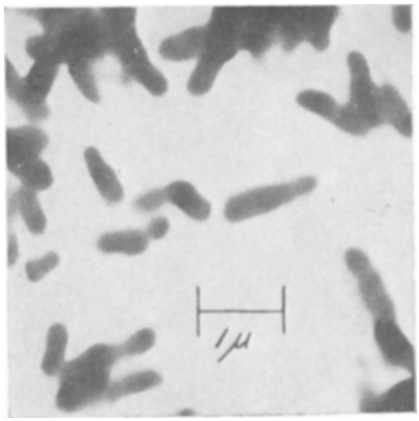

FIG. 4.

C. diphtheriæ, XIII, × 18,000. Same as in Figs. 2 and 3, except that the cells, originally containing crystals of tellurium, were treated with bromine water to dissolve the tellurium metal.

a drop of bromine water was added to 1 cc of a suspension of cells from black colonies on potassium tellurite chocolate agar. The black color of the mass of cells immediately disappeared; likewise, the needles were no longer observed in electron micrographs of cells so treated (Fig. 4). Thus it appears highly probable that the black color is due to tellurium metal which exists, at least in part, in the needle-like forms shown in the pictures.

More important than the identification of the crystals is the location of the site of the reduction of the tellurite. In a few exceptional cases (Fig. 3) crystals actually penetrated beyond the cell boundary, possibly during the drying of the preparation. In other cases the crystals distort the cell outline without penetrating. In no cases were crystals observed to lie wholly outside the cells. Since the majority of the crystals are contained wholly within the cells, it is to be inferred that the tellurite or tellurous ion is able to diffuse through the cell wall and is there reduced to tellurium metal which is precipitated inside the cells.

Similar crystals have been observed within cells of *C. xerosis* grown on tellurite chocolate agar.

Summary. (1) Typical polar granules appear as dense spherical masses, or possibly plates, in electron micrographs of unstained preparations of *C. diphtheriae* grown on blood infusion agar. (2) In addition to polar granules, needle-like crystals appear in electron micrographs of unstained preparations of *C. diphtheriæ* cells grown on potassium tellurite chocolate agar. (3) The needle-like crystals,

as well as the black color, of cell masses of *C. diphtheriae* grown on potassium tellurite chocolate agar disappear upon the addition of small amounts of bromine water. It is inferred, therefore, that the black color is due to the tellurium metal which occurs in the form of needles. (4) It is to be further inferred that the tellurite ion is able to diffuse through the cell wall and is reduced with the precipitation of tellurium metal within the cell boundaries. (5) With the aid of the electron microscope it is now possible to obtain pictorial records of the location of sites of certain chemical reactions incident to the metabolism of the bacterial cell.

Acknowledgments. We wish to thank Dr. V. K. Zworykin, Associate Director of the Research Laboratories of the RCA Manufacturing Company, for his kind advice and support, and Dr. L. Marton for assistance in obtaining the pictures.

11964

Inactivation of Estrone by Liver After Exclusion of Reticuloendothelial System.*

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It has previously been established[1] that estrone injected in rats disappears rapidly from the organism without being excreted in the urine and feces. To recover the estrone injected the animals were minced, and from the resulting pap the hormone was extracted by organic solvents. Three hours after the injection less than 5% of the estrone could be extracted, after 20 hours, only 0.2%. Hydrolysis permitted the extraction of as much as 20%. No transformation of the estrone into the less active estriol takes place, the hormone being inactivated in the organism. As has been demonstrated by experiments *in vitro*, this inactivation takes place mainly in the liver, and to a small extent in the spleen. Other organs have no inactivating effect. From the following it was concluded that the inactivation in the liver is a fermentative process (estrinase):

* Aided by a grant from the Rockefeller Foundation.

1 Zondek, B., *Lancet*, 1934, **227**, 356; *Skand. Arch. Physiol.*, 1934, **70**, 133; *Hormone des Ovariums und des Hypophysenvorderlappens*, 2nd edition, Springer, Vienna, 1935, p. 124.