serous edema with, in most cases, ascites and hydrothorax. The kidneys showed parenchymatous nephritis with hyaline casts in the collecting tubules. The most striking changes were in the thoracic and abdominal aortæ, and in the pulmonary arteries. These showed hyaline degeneration and calcification of the media in the form of placques. These were separated by thickened areas containing numerous fibroblasts and devoid of elastic fibres. Some of the smaller vessels near the aorta showed proliferation of the intima. There was a striking absence of fatty degeneration in any of the vessels. The controls, usually twin lambs of the same sex, did not show any of these tissue changes.

Scholz (1906) describes general edema and the kidney lesions in human cretins, without mentioning the aorta or pulmonary arteries.

Von Eiselsberg (1895) describes the changes in thyroidectomized sheep as being calcification of the intima without fat.

Experimentally, arteriosclerosis is generally produced on a mechanical basis. In this case it seems to be more of a chemical nature, perhaps due to senile changes occurring early in life as a result of complete thyroidectomy.

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285 (2817)

The reason for failure to obtain growth of an obligatory anaerobe (Actinomyces necrophorus) on plate cultures incubated in an anaerobic jar.

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In their work on *Bacillus (Actinomyces) necrophorus,* Mohler and Morse[1] reported difficulty in inducing the organism to grow on plate cultures incubated anaerobically although they were suc-

[1] Mohler, J. R., and Morse, G. B., Bull. No. 167, B. A. I., U. S. Dept. of Agric., 1905.

cessful in cultivating it on the same media in other ways. They do not discuss the cause of the difficulty.

Recently, in attempting to plate out some cultures of this organism we have experienced the same difficulty. In "hormone" agar shake cultures, good, though not vigorous, growth was easily obtained, but when the same medium was inoculated and poured into Petri dishes and incubated in a Brown anaerobic jar, failure to obtain growth has nearly always resulted.

In preparing the plate cultures, the inoculated medium necessarily is exposed to the air from the time it is poured into the plates until anaerobic conditions become established in the jar. Since the ratio of exposed surface to the volume of the medium is large, there is opportunity for the dissolving of a considerable volume of atmospheric gases, and it was thought that in this fact the cause of the failures might lie.

To test this hypothesis, fluid cultures were exposed to the air in shallow layers in flasks, and subcultures into cooked meat medium were made at intervals. It was found that, although most of the subcultures grew, there always was a period of bacterial lag, the duration of which varied directly with the time of the exposure up to 3 or 4 hours. From this time up to 8 hours, which is the limit of our observations, very little difference was noted in the subcultures. Whereas one loopful of young, normal culture sufficed to give, regularly, a vigorous subculture within 18 hours, a similar amount of the same culture exposed for 30 minutes to 1 hour gave subcultures showing a bacterial lag of 24 to 36 hours or more, and some cultures exposed for 3 to 4 hours have shown lag periods as long as 96 hours.

Cooked meat medium is the most favorable substrate for the cultivation of *Actinomyces necrophorus* which we have found. It is far superior to the plating mediums which we have used in supporting and encouraging growth and in addition, as we shall show in the following paper, it is peculiarly fitted for correcting abnormal oxidation-reduction balances. When subcultures were made from cultures exposed to air into plain infusion bouillon, a medium more nearly like the plating mediums than is cooked meat medium, it was found that growth occurred only occasionally when the exposure was as long as 30 minutes and as much as 0.1 cc. was used as an inoculum. In longer exposures growth failure was almost invariable.

The hypothesis that exposure of the medium to air was the

cause of the plating failures now seemed likely to be correct. Direct tests to prove this point were made. The experiments follow:

(a) Plates of "hormone" agar, using about 30 cc. per plate, were poured. With as little delay as possible they were placed in the anaerobe jar and anaerobiosis established. Good growth was obtained after 72 hours. All of the colonies were located in the lower third of the agar layer. Plates containing about 12 cc. of the same agar, inoculated with the same quantity of culture and incubated in the same jar failed to show growth.

(b) Plates of "hormone" agar were poured and the surfaces flooded with petrolatum as quickly as the agar congealed. After incubation in the anaerobe jar, good growth was obtained. Plates without the petrolatum in the same jar failed. Plates with the petrolatum seal, incubated aerobically, failed.

The data indicates that the anaerobic plating failures were due to injury of the bacterial cell forming the inoculum, by reason of their contact with air, so they were not able to multiply in a medium which was not highly favorable but which, nevertheless, supported growth readily from an uninjured inoculum. The following paper shows that the cause of the cell injury is probably due to the formation of hydrogen peroxide by the bacteria while under aerobic conditions.

286 (2818)

The formation of hydrogen peroxide by an obligatory anaerobe (Actinomyces necrophorus). The tolerance of this organism for peroxide.

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When difficulty was experienced in obtaining growth of *Actinomyces necrophorus* after the culture had been allowed to come in contact with air (see previous paper), the work of McLeod and Gordon, of Callow, and of Avery and his associates, deal-