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-

ing with hydrogen peroxide production by bacteria immediately came to mind. Using the benzidine test with a bit of raw potato to supply the necessary oxidases, as suggested by Avery in his work on the *Pneumococcus,* the production of peroxide by this organism when in contact with air was readily demonstrated. After exposure to the air in a shallow layer, distinct positive tests for peroxide were obtained in from 1½ to 2 hours in plain bouillon cultures which had been grown under petrolatum seal for 24 to 48 hours. The fluid part of cooked meat cultures, when fairly free from meat fragments, usually gave a positive reaction after two hours exposure but if a considerable quantity of meat fragments was present the reaction was delayed or prevented. When meat fragments were present, the reaction, if it occurred at all, could be obtained over only a short period of time. In most cases a reaction could not be obtained after 8 hours under such conditions, whereas in plain bouillon it could sometimes be obtained for as long as 24 hours.

The inhibiting effect of the meat fragments suggested the presence of catalase. It was also found that when titrated solutions of hydrogen peroxide were introduced into sterile meat medium, the peroxide rapidly was destroyed, or combined in some way so that it would not react to benzidine. The introduction of 1 cc. of a 3 per cent solution of hydrogen peroxide through a melted petrolatum seal and the rapid congealing of the seal formed a means of detecting small amounts of gas formation readily. In this way it was shown that the meat medium actually possessed some oxidizing substance, while the plain bouillon did not. From ¼ to ½ cc. of gas always appeared below the seal in the first case, but there was none in the latter. The cooked meat medium had been sterilized at 120° C. for 1 hour.

A rough idea of the concentration of peroxide formed by the bacteria was obtained by making up various dilutions of titrated hydrogen peroxide in sterile media and comparing the color reactions of the benzidine test with those obtained in the cultures. It was found that, in bouillon, benzidine would detect 1 part of hydrogen peroxide in 100,000 to 200,000. The reactions from the cultures generally were much stronger, at the height of the reaction, than the reaction obtained by the 1-100,000 dilution but not as strong as that obtained in a concentration of 1-10,000.

In meat medium dilutions of hydrogen peroxide as low as

1-1,000 were rapidly destroyed by the meat fragments so that practically no inhibition was noticed in subcultures. Subcultures from tubes in which the meat fragments had been visibly bleached by the peroxide grew readily.

In plain bouillon, however, distinct inhibition was noticeable. Hydrogen peroxide in dilutions of 1-10,000 and 1-100,000 was added to 24 hour cultures. In the 1-100,000 dilution, some lag was noted in subcultures, this period being in proportion to the length of time the organism was subjected to the action of the peroxide. All subcultures, made at intervals up to 6 hours, were growing at 48 hours. In the 1-10,000 dilution the lag period extended from 24 hours to 96 hours, the longer lag periods being in those cultures which were made after longer contact with the peroxide.

In the previous paper a record of the behavior of fluid cultures of this organism after exposure to air in shallow layers was made. This behavior was practically duplicated by those cultures which were in contact with a 1-10,000 dilution of hydrogen peroxide.

287 (2819)

The effect of thyroxin on the cutaneous system in the sheep.

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On January 11th, of this year, a thyroidectomized lamb, about eight months old, showed great muscular depression and in order to save its life the subcutaneous administration of thyroxin was begun. Half a milligram was injected every second day for one week; then as recovery began the dose was reduced to ¼ mg. and later to ⅛ mg. In about ten days the animal showed great improvement as was anticipated but after about a month's treatment it was observed that the wool was beginning to fall out in patches and this effect was not expected. The shedding of the wool was first noticed on February 7th; photographs were taken every second or third day to show the progress of the denudation and