

plasia is limited to the cells of the zona glomerulosa and the outer zona fasciculata. There is a definite hyperemia proportional to the amount of adrenocorticotrophic extract injected.

The adrenal cortices of hypophysectomized rats treated with adrenocorticotrophic extracts (Fig. 4) show a restoration of the cytoplasm of the cortical cells. The nuclei of the cells are no longer pycnotic. There is a great increase in the fat content of the cortical cells.

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Studies on the Enumeration of Marine Anaërobic Bacteria.

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Considerable difficulty has been experienced in estimating the abundance of marine anaërobic bacteria although they have been demonstrated¹ in nearly all samples of water or mud examined. Most of the conventional procedures² such as the incubation of plates in anaërobic jars have failed to yield reproducible results and moreover, the use of such complicated, space- and time-consuming apparatus is entirely impracticable aboard a rolling boat at sea. The application of oval tubes as described by Anderson³ for the enumeration of anaërobic bacteria has exceeded expectations.

Ordinary round glass tubes with sealed ends as used by Roux⁴ and Burri⁵ are satisfactory for the cultivation of anaërobic bacteria but the curvature of the glass makes it virtually impossible to count the colonies which develop, particularly when it is necessary to use a hand lens. The Kimball Glass Company fabricated special oval tubes for us with flat, parallel sides, thereby obviating this difficulty. The oval tubes are 6x14 mm. in cross-section and 380 mm. long with one end permanently sealed and the other end flared to facilitate the introduction of the medium. The tubes are sterilized in a pipet can. A test-tube containing 10 cc. of nutrient agar recently heated to nearly 100°C. to expel oxygen and cooled to 45° is inoculated

¹ ZoBell, C. E., and Anderson, D. Q., *Am. Assn. Petrol. Geol.*, 1936, **20**, 258.

² Hall, I. C., *J. Bact.*, 1929, **17**, 255.

³ Anderson, D. Q., In press.

⁴ Roux, E., *Ann. de l'Inst. Past.*, 1887, **1**, 49.

⁵ Burri, R., *Centralbl. f. Bakt., Abt. 1, Orig.*, 1902, **8**, 533.

with the proper dilution of the sample to be analyzed for anaërobes. Without undue agitation (shaking has been found to be unnecessary to insure an even distribution of bacteria) the inoculated medium is poured into the oval tube. As soon as the medium has solidified, it is covered with a deep layer of reduced methylene-blue agar. The seal excludes oxygen and indicates the degree of anaërobiosis, Hall⁶ having shown that conditions are suitable for the growth of anaërobes in an environment in which methylene blue remains colorless. The inoculated tubes are then incubated at the desired temperature and they may be observed from time to time without interrupting the experiment. The colonies are counted in reflected light against a dark background. The technic is applicable not only to the enumeration of total anaërobes, but by employing appropriate differential media, the abundance of various physiological types can be estimated. The oxidation-reduction potential of any stratum can be measured by the insertion of long slender microelectrodes.

In several series of 50 duplicate determinations in which the oval-tube count was as low as 30 per cc., the standard deviation was only 7%. This is practically the same as the standard deviation obtained in plate-counts on aërobes. The standard deviation of the anaërobic-jar count was 2 or 3 times as great.

Besides being more reproducible than other methods of determining anaërobes, the oval tube count is more representative of the anaërobic population. Tests on picked colonies revealed that all of the bacteria which develop in the oval tubes are either strict or facultative anaërobes, whereas due to incomplete anaërobiosis, many of the colonies which develop in anaërobic jars prove to be either microaërophiles or even strict aërobes.

Oval-tube counts on samples of sea water collected from various depths were between 10 and 20% as high as the total plate-counts (aërobic). A larger percentage of viable bacteria in bottom muds were anaërobes, the proportion of anaërobes to aërobes increasing with core-depth. Many of the bacteria from the mud which developed in oval tubes proved to be strict anaërobes.

⁶ Hall, I. C., *J. Bact.*, 1920, 6, 1.