STUDIES ON CULTURAL REQUIREMENTS OF BACTERIA

IV. QUANTITATIVE ESTIMATION OF BACTERIAL GROWTH

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In carrying forward experiments on nutritional requirements of certain pathogenic bacteria, notably the diphtheria bacillus, the necessity for some fairly accurate method for determining the amount of growth became increasingly evident to the writer. It seemed wise to attempt a direct estimation, either of total dry weight of bacterial substance formed in a given amount of medium, or to estimate quantitatively some fairly constant constituent of the bacterial cell. The dry weight method, which has been used to some extent for other purposes by some workers, was ruled out because of the technical difficulty of filtration and washing of considerable numbers of cultures through bacteria-tight filters of a form suitable to be dried and weighed accurately. Nitrogen is a fairly uniform component of the living cell, and was selected as the simplest substance capable of rapid and careful determination. It has been found entirely practicable to centrifuge, wash and carry out micro-kjeldahl determinations on cultures of diphtheria bacilli. The method is somewhat tedious when many daily cultures must be dealt with, but the results obtained and information acquired have so far exceeded those possible with less quantitative records that we venture to hope others working in the same field may find the method of value. There is also every reason to believe that vaccines could be standardized by the same method. The difficulty of obtaining uniform dosage over periods of time by any of the usual methods

of counting or turbidity measurements, is well known, while dosage expressed in terms of bacterial nitrogen should be both easy to determine and constant in value. The attempt is being made to coördinate such values with those now employed in standardizing vaccines at the Massachusetts State Antitoxin Laboratory through the courtesy of Dr. Elliott S. A. Robinson.

Cultures are grown in fluid medium. Exactly 10 cc. of each lot to be tested are pipetted into a pyrex test tube 150 × 20 mm. in size with the end drawn to a blunt point like a centrifuge tube such as Pyrex no. 2185. The open end is without rim, but may conveniently be supplied with a small "pour out." The medium, after sterilization, is inoculated and incubated in a slanted position, in the case of the diphtheria bacillus, in order to allow maximum pellicle formation. In the accompanying chart, it will be seen that determinations carried out on duplicate tubes, during the early period of incubation, show rather wide differences in bacterial nitrogen, but that as maximum growth is attained, checks between duplicate tubes become fairly good, and in many cases, almost incredibly close. Later, there is a slow diminution of nitrogen, depending probably on ageing, death and partial autolysis of the cells. However, on the chart shown, at a temperature of 35°, the values are practically constant between forty-four hours and sixty-four hours. Routinely, therefore, we have grown our cultures for approximately fifty-four hours. Obviously, such a time curve would have to be made for any other type of organism which might show both more rapid growth and more pronounced autolysis.

The cultures, after removal from the incubator, are shaken and each tube is tilted repeatedly to wash down as much of the growth adhering to the wall as possible. They are then either autoclaved at 10 pounds for ten minutes, or steamed for fifteen minutes, the cotton plugs removed, and the tubes centrifuged in 100-cc. cups (International Equipment Co., no. 340), the space around the tube being about two-thirds filled with water to minimize risk of breakage. Five minutes' centrifugation at 2600 R.P.M. proves adequate for the diphtheria bacillus, although a longer time may be required for other organisms. The supernatant fluid is care-

fully pipetted off, and the sediment washed with 0.05 per cent acetic acid. If distilled water is used in the washing, a suspension is formed which does not readily come down in the centrifuge. The washing is done by stirring the sediment with a few drops of the dilute acetic acid, using a glass rod, which is then carefully rinsed with 2 cc. more of the acetic solution, and enough of the latter run in to fill the tube about two-thirds full, altogether about 20 cc., so that any pellicle adhering at the upper end of the original

TABLE 1

TIME OF INCUBATION	NITROGEN IN GROWTH ON 10.0 CC. MEDIUM	TIME OF INCUBATION	NITROGEN IN GROWTH ON 10.0 CC. MEDIUM
hours	*mgm.*	*hours*	*mgm.*
16	0.36 0.37	44	1.95 2.00
20	Lost Lost	48	1.98 1.99
24	0.84 1.03	52	2.00 2.04
28	1.36 1.52	56	2.00 2.00
32	1.62 1.82	60	2.00 2.02
36	1.97 1.95	64	2.01 2.02
40	2.05 1.91	72	1.94 1.97

slanted culture is covered. A second five minutes centrifugation is carried out, and the wash fluid drawn off. Calculation and determination of nitrogen in this wash water show that not enough remains in the tip of the tube to necessitate a second washing.

For the actual determination of the nitrogen, the Pregl (1930) micro-kjeldahl method has been used. Any other type of micro-kjeldahl would prove equally satisfactory, including colorimetric

methods, for the accuracy of the Pregl method is beyond the requirements of the rest of the procedure. However, since the

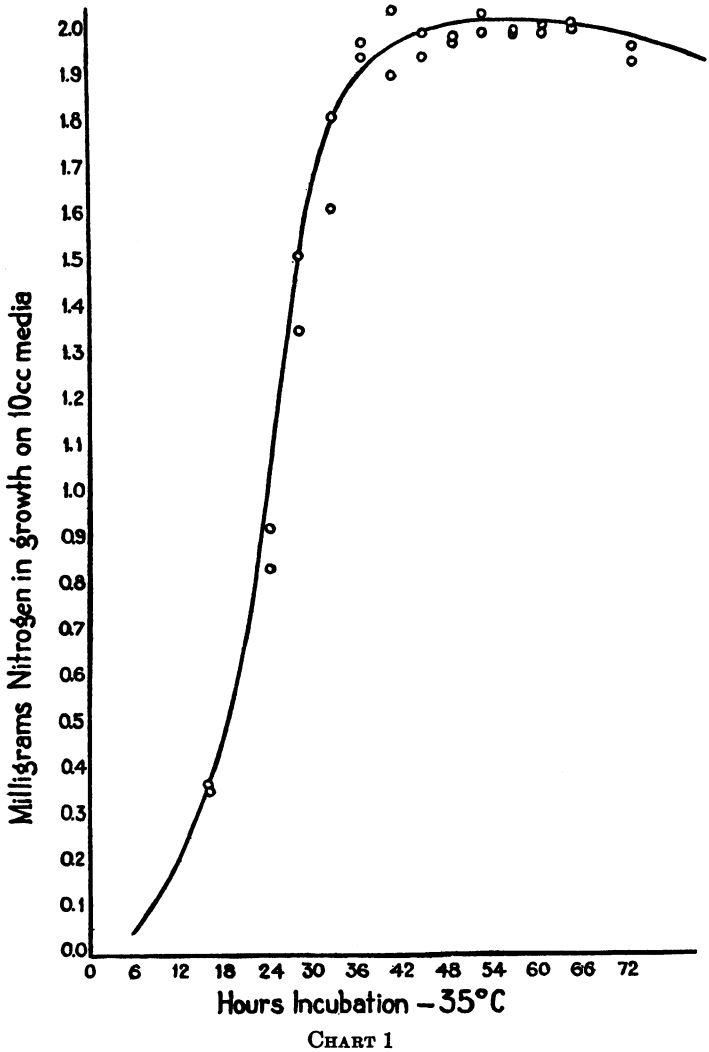

CHART 1

apparatus for this method was already assembled, it has been used and is probably as rapid as any other. For details of the method, the original description should be consulted. The digestion is

carried out directly in the culture tubes, and there is no other variation whatever from Pregl's procedure, except that we find it advantageous to make four additions of three drops each of 30 per cent H_2O_2 during the course of the digestion. The quantities of nitrogen dealt with in the studies made lie between about 0.2 and 2.0 mgm., or somewhat more, calling for from 5 to 15 cc., and very occasionally 20 cc. of 0.01 N HCl. It is possible to carry through determinations on about 20 cultures in a day.

The chart and table 1 show the type of result which may be expected.

SUMMARY

A method for the estimation of total quantity of bacterial growth by means of the micro-kjeldahl method is described. Its utility as a means of recording growth in studies on bacterial culture requirements is pointed out, and the suggestion is made that it would be found useful in vaccine standardization.

REFERENCE

PREGL, F. 1930 Quantitative Organic Microanalysis. P. Blakiston's Son & Co., Philadelphia.