

the float recorder drops down across the scale as the oxygen is used up. The time required to use from 2 to 8 cc. of oxygen is measured with a stopwatch. A series of readings are taken while the animal is quiet. The readings are accepted if there is no progressive drift in a series of 5 or more consecutive readings taken over a period of 5 to 10 minutes. These values should check each other within a maximum range of not more than 10%.

The experience of several years' steady use of this apparatus shows that no difficulty is encountered in obtaining values in good accord with those of more complicated instruments. Reference to the recently published papers on the metabolic stimulation caused by dinitrophenol will show the type of data that has been obtained with this apparatus.¹ The apparatus may be made smaller for animals below 150 gm. The one illustrated in this paper, and one about half the size, has served all needs for animals between the weights of 30 and 600 gm.

Summary. A simple apparatus is described for measuring the metabolic rates of smaller laboratory animals. It requires no unusual materials and can be made in a few hours by anyone able to solder. Its accuracy and simplicity are derived from the fact that by keeping the size down to a minimum, the contained gas volume is very small and the necessity for rigorous temperature control and circulation of the air is thereby eliminated. The values reported in the literature, with more complicated apparatus and groups of animals measured over longer intervals of time, are reproducible in this simple apparatus using single animals and observation-periods of relatively few minutes.

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Conventional Bacteriologic Technics in "Hormone" and "Vitamin" Research.*

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A fairly uniform bacteriologic technic is in current use for the demonstration of "hormones," "vitamines" and "accessory growth-

¹ Tainter, M. L., *J. Pharm. Exp. Therap.*, 1934, **51**, 45.

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factors." As a typical example of this technic, Nicolle and Césair¹ inoculated 10% broth-dilutions of specific immune serum with homologous bacteria, and invariably obtained a much more luxuriant growth in 10% homologous anti-serum than in normal or hetero-immune serum controls. From this they concluded that the specific "antibodies" in question are in reality specific "growth-stimulating hormones" for the corresponding bacteria.

In technics of this type there are numerous arbitrary experimental constants which may conceivably condition the end-result. Prominent among these arbitrary constants are the dosage, age, growth-phase and nutritional condition of the inoculum, and the time and method of reading the end-result. Most "hormone" data, for example, are recorded in terms of relative turbidity at the end of 24-hours incubation.

In order to test the reliability of this arbitrary time-factor, parallel "viable" and "total" population growth-curves were plotted for *Streptococcus hemolyticus* in 100 cc. beef-infusion broth and broth containing 0.1% beet juice. Typical curves thus obtained are recorded in Figure 1.

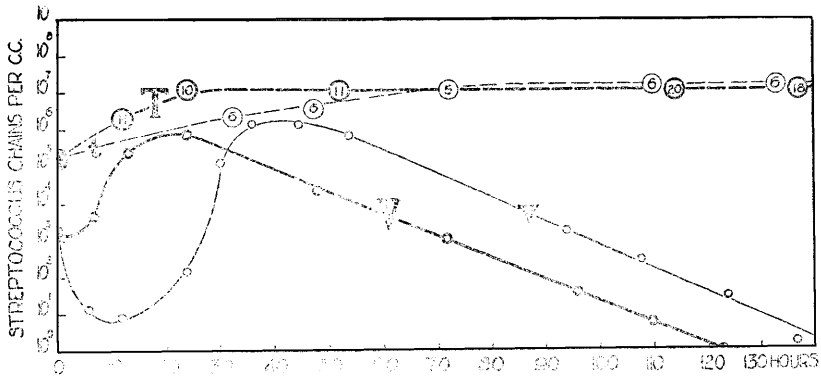


FIG. 1. Effects of Beet "Auximone" on Streptococci.

100 cc. beef-infusion broth plus 0.3 cc. 24-hour veal-infusion broth culture of *S. hemolyticus*. Total population counts (t, Spencer counting chamber) recorded in terms of chains per cc., average number of cocci per chain being shown in the recorded circles. Viable population counts (v) obtained by quantitative dilution methods, veal-infusion agar, 4-day plate-count chosen as the "viability" index. T, V: parallel "total" and "viable" population counts in beef-infusion broth plus 0.1% beet-juice (Berkefeld filtrate).

From these curves, it is seen that the conventional selection of the 24-hour turbidity (or "total" population count) as the recorded reading gives data apparently proving that beet-juice contains a powerful growth-stimulating hormone for streptococci. An arbi-

¹ Nicolle, M., and Césair, E., *Ann. Inst. Pasteur*, 1926, **40**, 43.

trary selection of the 2 to 5-day "viable" count, however, would give equally convincing evidence that beet auximone is a fairly efficient surgical antiseptic, causing premature death of 99% of the potentially "viable" streptococci.

The curves as a whole could be explained with equal plausibility, however, by assuming that 0.1% beet-juice is without direct effect upon *S. hemolyticus*, its sole indirect action being to neutralize certain unknown toxic factors in beef-infusion broth, thus shortening the usual "lag phase" in population-growth. Otherwise the 2 sets of growth-curves are practically identical.

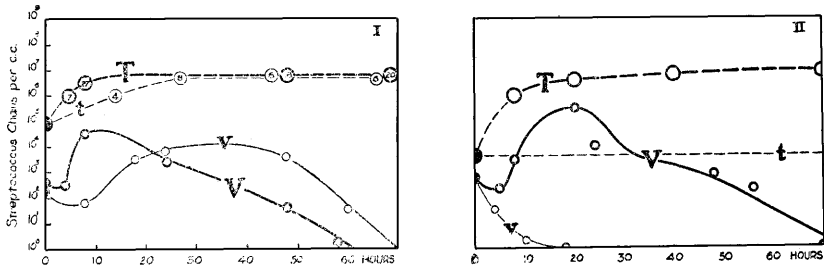


FIG. 2. "Logarithmic Phase" vs. "Lag Phase" Streptococci. Technic as in Fig. 1, except for the use of heat-sterilized beet-juice (100°C, 30 min.).

- I. "Logarithmic phase" inoculum (24-hour growth in veal-infusion broth.)
- II. "Lag phase" inoculum (10-hour growth in veal-infusion broth.)

With streptococcus inoculum in the "lag phase" instead of the "logarithmic phase" of growth, the superimposed second "lag phase" on transfer to beef-infusion broth often leads to a fairly rapid "death" of the inoculated organisms. (II, Fig. 2). The prevention of this second "lag phase" in the relatively non-toxic beet-juice-broth, therefore, gives data apparently proving that beet-juice contains a "vitamine" absolutely essential for the life of *S. hemolyticus*. Here, tested under identical conditions, 0.1% beet-juice shortens the life of a vigorous ("logarithmic phase") inoculum, but prolongs the life of a less vigorous ("lag phase") transplant.

Paradoxes and inconsistencies of this type strongly suggest that conventional bacteriologic technics are not always reliable sources of information in "vitamine" and "hormone" research.