THE RELATION OF CONCENTRATION OF FOOD SUPPLY TO THE GENERATION-TIME OF BACTERIA.

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(With 3 Charts.)

Many investigations have been undertaken to determine the generation-time of bacteria but although the effects of temperature and other factors have been considered, little attention has apparently been paid to the influence of the concentration of the culture medium employed.

In most of the papers on this subject the concentration of peptone used is not mentioned at all and hence it is difficult to compare the results obtained.

The influence of glucose and of salts has also been little investigated, the experiments on this point being designed only to determine between what limits of concentration there was any growth at all.

The question is of some interest inasmuch as exhaustion of food supply is probably a factor in bacterial selection. Thus Beyerinck considers selection to be a feature of slow growth, and this receives support from the behaviour of *B. typhosus*, in a dulcite medium, for, while growth is still proceeding rapidly according to a logarithmic law, there is but little evidence of selection, which only takes place later when the growth has become comparatively slow (Penfold, 1911).

Generation-time of B. typhosus *in Peptone-water.*

The following experiments were carried out to see to what extent the generation-time of *B. typhosus* at 37° C. was influenced by the concentration of peptone used. This varied from 0·0125 % to 1·25 % the amount of NaCl present being kept constant at 0·5 %. Tubes

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containing 5 c.c. of these various media were then inoculated with a standard drop of a suitable dilution of a 24 hour broth culture of *B. typhosus*. It was found that with such a culture diluted to 1 in 5000, the standard drop employed gave an initial bacterial count of from 200–500 organisms per c.c. of the inoculated broth examined immediately. The cultures were then incubated at 37° C. and at known intervals of time definite volumes were plated out and the number of bacteria per c.c. calculated. It was arranged that each plate should contain about 200 colonies and, where dilution was necessary, this was carried out in 0·8 % saline. The first observations of which account is taken in the curves shown below were not made until sufficient time had elapsed to ensure the period of lag being over.

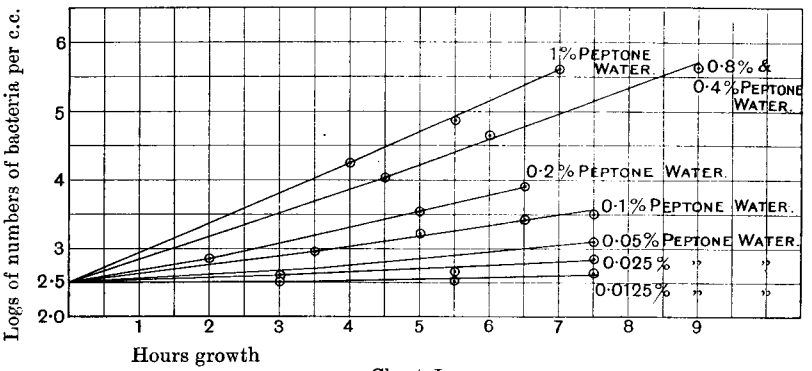

Chart I.

In Chart I the logarithms of the number of bacteria in unit volume have been plotted against the time of growth, and it will be seen that there is a rapid decrease in the rate of growth as the concentration of peptone falls below 1 %, until with 0·01 % of peptone little multiplication takes place. It will also be noticed that 0·8 % and 0·4 % peptone both gave identical results although lower than the 1 %. In a second sample of peptone, all concentrations above 0·4 % gave practically the same generation-time. From the above results the generation-times were calculated for each concentration of peptone from the formulae

$$2^n = \frac{b}{a} \text{ where } a = \text{no. of bacteria at beginning}$$
$$b = \text{ " " " end,}$$
$$n = \text{no. of generations,}$$
$$\text{and } G = \frac{T}{n} \text{ where } G = \text{generation-time and}$$
$$T = \text{time of experiment.}$$

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Only that period in which growth was proceeding according to the logarithmic law was taken into account in these calculations.

In Chart II the generation-times calculated from the above experiments have been plotted against the concentrations of peptone, the two curves representing two different samples of peptone.

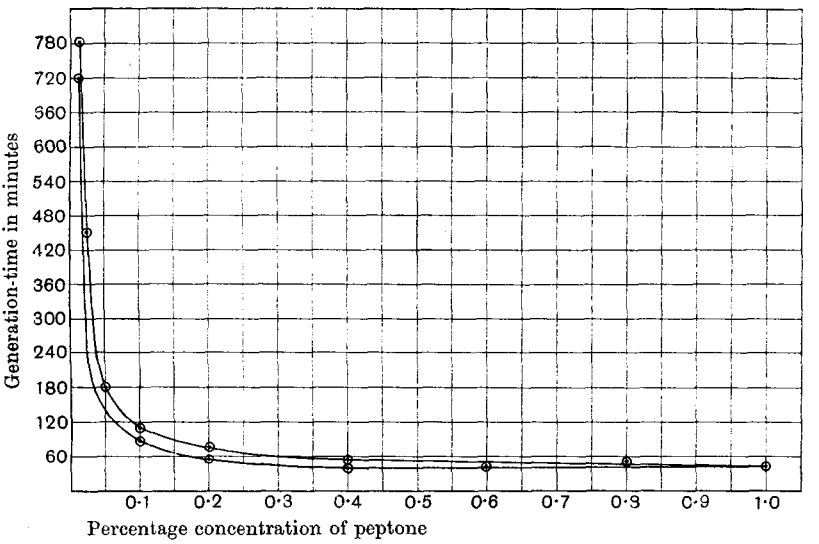

Chart II.

The generation-time increases but slowly until the concentration of peptone falls below 0·4 %. After this however it becomes rapidly greater and with 0·012 % is almost infinite.

The following table gives the generation-times for each sample of peptone examined.

TABLE I.

Percentage concentration of peptone. (C.)	Generation-time in minutes. (T.) Sample I.	$C \times T$	Sample II.	$C \times T$
1·25	—	—	42	—
1·0	39	—	—	—
0·8	49	—	—	—
0·6	—	—	42	—
0·4	49	—	40	—
0·2	77	—	51	10·2
0·1	111	11·1	84	8·4
0·05	181	9·05	—	—
0·025	450	11·2	—	—
0·0125	783	9·8	723	9·04

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It will be noticed that with concentrations below 0·2 % in one case and 0·4 % in the other the product of concentration of peptone and generation-time is a constant within the limits of the experimental error, this being about 10 %. It will be seen that there is a considerable variation in the generation-times when different samples of peptone are used. In each case however the shortest time obtained was the same namely from 39–40 minutes.

Hehewerth's (1901) result was 45 minutes at 37° C. and Lane-Claypon (1909) found 33 minutes at 34·3° C. so that our value lies between the two.

Effect of addition of Glucose to the Peptone.

We next tried the effect of adding small amounts of glucose to the peptone tubes. It was to be expected that with low peptone concentrations the generation-time would be reduced by this procedure and this proved to be the case. The amount of glucose added was 0·175 %.

The experiments are at present incomplete but the results so far obtained are shown in Chart III.

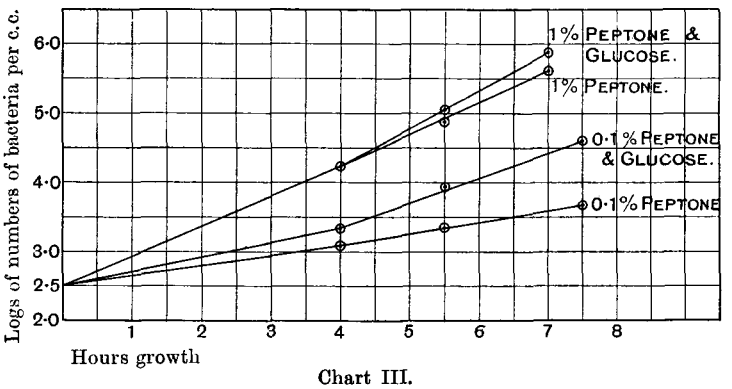

Chart III.

In this chart is shown the effect of adding 0·175 % of glucose to tubes containing 1·0 % and 0·1 % peptone respectively. In the case of the 1·0 % peptone the rate of growth is not much increased. Only the later part of the curve is affected, the generation-time being decreased from 39 minutes to 34 minutes. In the other case, however, the rate of growth is greatly increased by the small amount of glucose added and the generation-time decreased from 111 minutes to 50 minutes or about 50 %. In further experiments it is proposed to determine the

effect on the generation-time of concentrations of peptone higher than 1 % and of larger additions of glucose.

We desire to express our indebtedness to Dr J. C. G. Ledingham for help in this research.

SUMMARY.

1. The generation-time of *B. typhosus* in 1 % peptone at 37° C. is 40 minutes.

2. The rate of growth of *B. typhosus* is greatly influenced by the concentration of peptone in nutrient medium when the peptone is below 0·4 %.

3. *Below* 0·2 % *the generation-time is inversely proportional to the concentration of peptone used.*

4. The addition of 0·175 % of glucose to a medium containing only 0·1 % of peptone lowers the generation-time by about 50 %.

With 1·0 % peptone this effect is less marked.

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