365. *Physicochemical Aspects of Bacterial Growth. Part III. Influence of Alcohols on the Growth of* Bact. Lactis Aerogenes.

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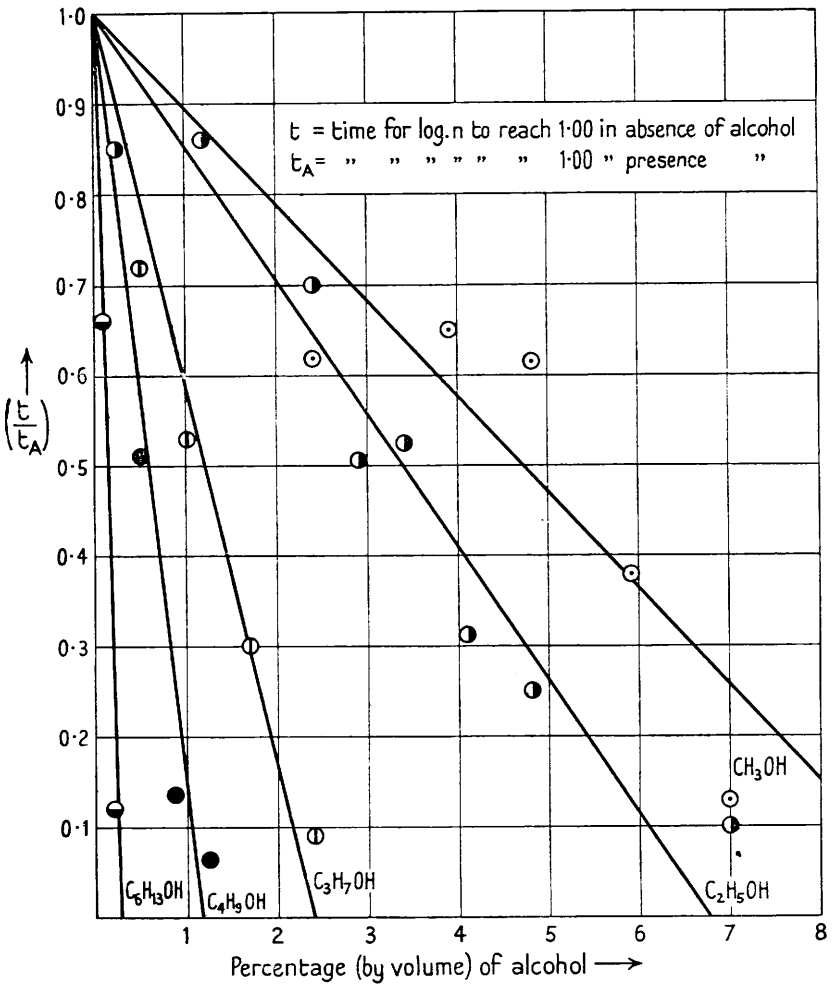
The lowering by normal aliphatic alcohols of the growth rate of *Bact. lactis aerogenes* is an approximately linear function of their concentration, and the inhibiting power of a given alcohol increases exponentially with its chain length. These observations are compared with others possessing possible physicochemical analogies and discussed in connexion with theories of toxic action.

The influence of alcohols in inhibiting bacterial growth, and in narcotising other cells, is well known, and the present paper describes a quantitative study of the effect of a series of aliphatic alcohols on the division rate of *Bacterium lactis aerogenes*.

The methods of measurement employed were the same as those described in Part I, except that the culture medium was double-strength veal bouillon of p_H 7·6, in which the cultures grew without appreciable lag period.

In general, complete growth curves were determined, and the mean generation time read off from the logarithmic slope. In the absence of lag, since $\log n/n_0 = kt$, the time for a constant inoculum to grow to a given count is inversely proportional to k, so the reciprocal of this time may be used as a measure of the growth rate. In Figs. 1 and 2 and the tables results are given for the influence of the alcohols both on the mean generation time and on the time for log n to increase to 1·0. The values of n are the actual counts in 16 small

FIG. 1.



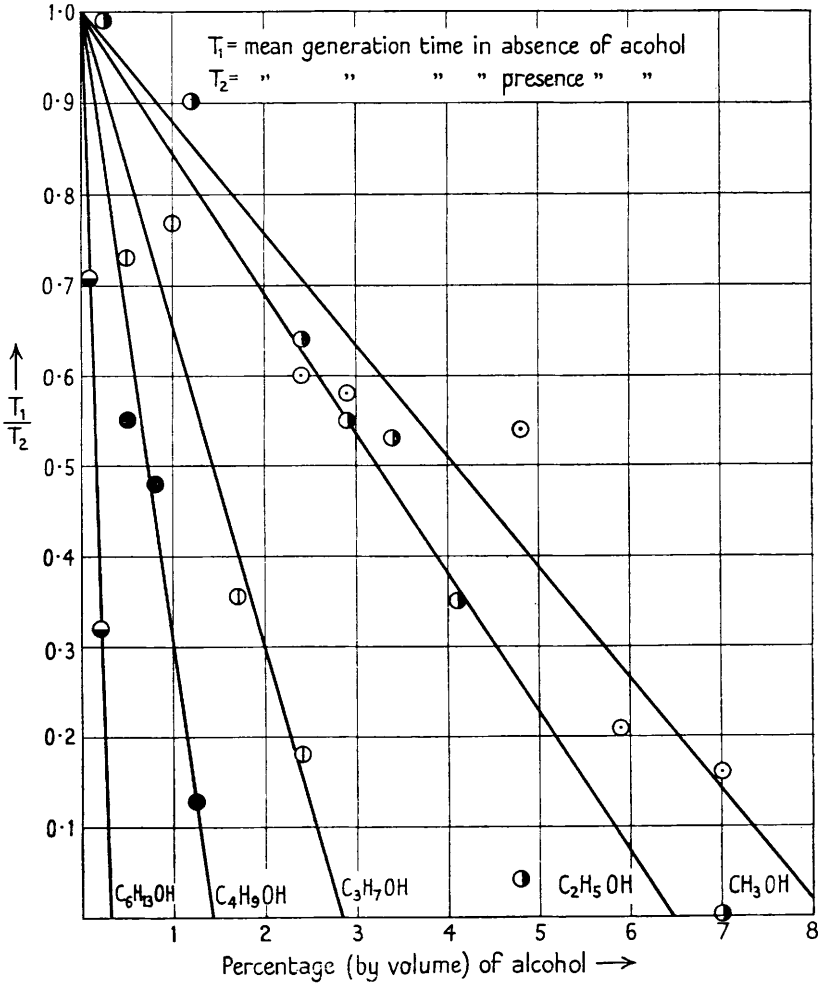
hæmocytometer squares: the total population in organisms per c.c. is obtained by multiplying n by $1·25 \times 10^6$.

The alcohols used were all pure specimens, introduced in the required amount into the culture medium by sterile pipettes just before inoculation. The most important facts which the figures and tables reveal are: (1) The inhibiting effect of the alcohol increases almost linearly with the concentration for small additions. (2) The inhibiting effect increases very rapidly with the length of the hydrocarbon chain of the alcohol. (3) The curves obtained by plotting the ratio of the mean generation times are closely similar to those obtained by plotting the ratio of the times to log $n = 1·0$; this shows that the

alcohols have little influence on the lag period, which remains negligible throughout all the experiments.

The first point follows from a simple theoretical treatment. The interior of a cell presents a complex structure in which the distinction between the surface and any continuous phases present is difficult to define. We shall refer to it simply as an assemblage of structural elements. Let s be the number of the structural elements concerned in the growth and division of the organism. In the absence of alcohol the growth rate is given by $dn/dt = k_1sn$ or $\log n = k_1st +$ const. We may suppose that the alcohol puts a certain

FIG. 2.



number of the elements out of action, and that the number affected is proportional to the concentration of the alcohol. The growth rate is now

$$dn/dt = k_1s(1 - k_2c)n \text{ or } \log n = k_1s(1 - k_2c)t + \text{const.}$$

and the mean generation time in presence of alcohol is

$$T_2 = 0{\cdot}693/k_1s(1 - k_2c)$$

and that in absence of alcohol is

$$T_1 = 0{\cdot}693/k_1s$$

whence

$$T_1/T_2 = (1 - k_2c)$$

This relation is approximately fulfilled, as illustrated by the figures.

The question arises whether the action of the alcohol is confined to a simple inhibition of cell division, or whether it is more drastic, causing the actual death of the organisms. If x and y are respectively the numbers of living and dead cells at the time t, we may write

for reproduction $\quad dx/dt = k_a x - dy/dt$

for death $\quad dy/dt = k_b x$

whence $\quad dx/dt = (k_a - k_b)x$

If k_b is proportional to the alcohol concentration, this equation is of the same form as that derived above, so that a formal distinction between the two hypotheses is difficult when using the technique of total counting. But on quite general grounds we know that reproduction is a function much more sensitive to adverse influences than is mere survival. Thus, since the organisms do in fact grow under the conditions of these experiments, it seems much more probable that the first action of the alcohol is to reduce the division rate, rather than to leave it unchanged while balancing it with a greatly increased death rate. Taken over longer periods, of course, it is clear that the alcohol will actually kill the organisms, but its first effect, which is what we are measuring here, is that of inhibiting division.

The mode of action of the alcohols must now be discussed. The characteristic relation which should provide some clue to this mechanism is the remarkable increase of the constant k_2 with the chain length of the alcohol, a relation paralleled in the case of other toxic actions of alcohols. Two main theories have been advanced to explain the action of a narcotic on a cell (cf. *Trans. Faraday Soc.*, 1937, **33**, 1057, 1062) : the first postulates that the narcotic is adsorbed on certain active centres, and the second that it dissolves in the lipines of the cell. The distinction between adsorption and solution is, however, indefinite for a cell with its complex internal organisation, and it is difficult to say whether phenomena such as the penetration of substances between oriented micellar units, and the change in protein structure which occurs on stretching fibres are to be termed surface effects or not. It seems simpler to speak of the action as due to penetration and attachment to structural units of the organism without attempting to draw any rigid distinction between adsorption and solubility.

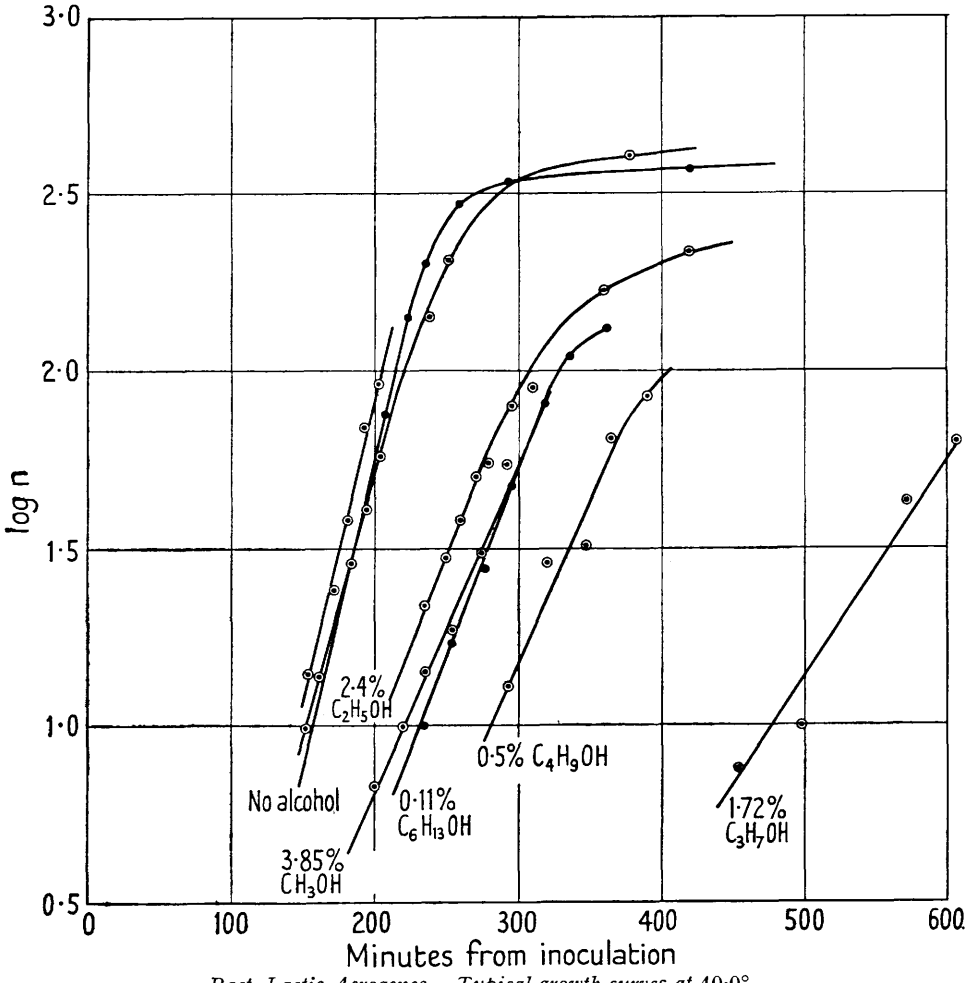
Even so, two possibilities arise in connexion with the growth-inhibiting action of the alcohols. It might be due to an attachment of the alcohol to some active centre by means of its hydroxyl group, the marked effect of increasing chain length being due to the greater area (or volume) throughout which the long, anchored chain, possibly waving about, could exert its disturbing influence on the vital functions. It might, on the other hand, be due to the affinity of the hydrocarbon groups of the alcohols for certain lipoid elements in the structure. According to the first hypothesis, the inhibitory action of the alcohol should be proportional to the area (or volume) which the chain shields, and should thus vary as the square (or cube) of the chain length. This picture of toxic action is realised in the experiments of Maxted and Evans (J., 1937, 603, 1004) on the poisoning of catalytic surfaces of platinum by aliphatic thiols and sulphides. If k_0 and k_c are respectively the activities of the catalyst when unpoisoned and when in presence of a concentration c of the sulphur compound, then $k_c = k_0(1 - \alpha c)$, which may be compared with the equation given above for the action of alcohols on growth. But the variation of α with the chain length of the compound is quite different from that observed in the growth experiments. If the slopes of the curves in Figs. 1 and 2 are plotted against the square of the chain length there is not even a rough proportionality. The only relation which expresses the results is one which makes the inhibiting action increase exponentially with the chain length.

Now, not only is the linear relation between the logarithm of the inhibiting power or toxicity and the chain length a general one, but it appears that the slopes of the curves are usually the same if the same units are employed, the effect of adding one carbon atom to the chain being to make the alcohol about three times as toxic. For example, in Fig. 4 the present results are plotted on the same graph as those of Styles and Stirk (cf. Styles, " Introduction to the Principles of Plant Physiology," 1936, p. 81) on the toxic effect of alcohols on potato tissue and those of Tilley and Schaffer (*J. Bact.*, 1926, **12**, 303) on the organism *Bact. typhosum*. The latter authors did not determine growth curves but found

the relative amounts by weight of alcohol and of a phenol standard required to kill all the cells in a given time.

The following theory of the inhibiting action embodies the salient features both of the adsorption and of the solution theories. Since all aliphatic hydroxyl compounds do not inhibit growth, we may conclude that the unsubstituted hydrocarbon chain is the active agent. On the other hand, the presence of the hydroxyl group attracted to the aqueous medium is necessary, since otherwise only an infinitesimal concentration of the substance

FIG. 3.



Bact. Lactis Aerogenes.—Typical growth curves at 40·0°.

could be contained in the solution. The affinity of the alcohol for the aqueous medium may be expressed by an energy E, while that of each CH_2 group for the lipoid elements of the cell may be expressed by an energy Q. Consider the equilibrium between the aqueous medium and the structural elements of the cell which hold the alcohol.

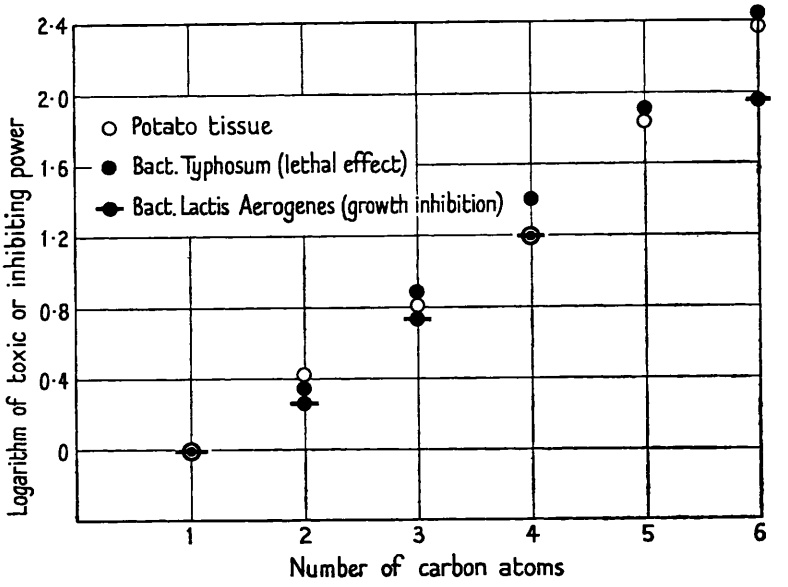
To leave the aqueous medium we may suppose that an energy E, practically independent of the chain length, is required by the molecules : the number which have sufficient energy to overcome the attraction and escape is, therefore, proportional to $e^{-E/RT}$. Similarly, to detach an alcohol molecule from the material of the organism an amount of work, nQ, proportional to the chain length will be needed, and the number which can escape back into the aqueous medium is proportional to $e^{-nQ/RT}$. If c_1 and c_2 respectively are the concentrations of alcohol in water and in the organism, then for equilibrium

$$A_1c_1e^{-E/RT} = A_2c_2e^{-nQ/RT}$$

where A_1 and A_2 are constants. Hence,

$$c_2/c_1 = \text{const.} \times e^{-E/RT} \cdot e^{nQ/RT}$$

FIG. 4.



For a given value of c_1, the toxicity will be greater the greater c_2. Thus, representing the inhibiting power by θ, we have

$$\theta \propto e^{nQ/RT} \text{ or } \log \theta \propto n.$$

The value to be assigned to Q according to this theory is quite reasonable. Let θ_n and θ_{n+1} be the inhibiting powers of two successive alcohols in the homologous series. Then

$$\theta_{n+1}/\theta_n = e^{Q/RT} = 3$$

since on the average each alcohol is about three times as potent as its predecessor. Since T is 313° abs., $Q = 313 \times 1{\cdot}98 \times 2{\cdot}303 \times 0{\cdot}477 = 680$ cals.

The above argument could be applied *mutatis mutandis* to explain why the partition coefficients of alcohols between aqueous and non-aqueous media, the surface activity, and other properties sometimes obey an analogous logarithmic relation. When a physical property is determined by the presence of molecules in a given phase or region, and when the work done in removing the molecule from this is a function of the number of CH_2 groups, then the property in question will vary logarithmically with the chain length. It follows, as has been said by Kurt Meyer (*Trans. Faraday Soc.*, 1937, **33**, 1067), that no determination of a physical property within the limits of a given homologous series can distinguish between an adsorption theory and a solution theory, even if any such rigid distinction can be drawn.

In determining the growth curves, it was observed that the stationary population of organisms decreased as the alcohol concentration increased. For example, the population of 20 c.c. of bouillon inoculated with two loops of stock culture reached the following " final " values, depending upon the amount of ethyl alcohol added :

Alcohol, c.c.	0	0·25	0·5	0·6	0·7	1·0
n_s	397, 460, 450, 429	400	259	196	178	75

The reduction in the stationary concentration cannot be due simply to death of the organisms : for there is nothing in the equation

$$dx/dt = (k_a - k_b)x$$

where k_a is the reproduction rate and k_b is the death rate, to indicate that a stationary concentration will be reached at all; and the influence of k_b is merely to diminish the rate of attainment of any final concentration which the other factors may determine.

Two explanations seem possible. As shown in Part I, under normal conditions, exhaustion of foodstuff is the principal factor limiting growth. Now, all the foodstuff is not necessarily consumed in the actual growth processes: non-dividing organisms can presumably cause fermentation or other destructive reactions of various substances present in the medium. The amounts removed in this way will be a function of the time as well as of the number of organisms. If, then, growth is slowed down by the addition of an inhibiting agent, the balance will be shifted in favour of destructive processes using up the foodstuff, but not directly concerned in growth. Thus the exhaustion will occur before the population has reached the size it would normally reach when division is more active.

Another possible explanation is that successive generations produced in presence of the alcohol become more and more enfeebled so that k_a itself decreases and k_b increases until finally the difference no longer has a positive value and division ceases altogether. Since each new cell contains initially a large part of the content of the parent cell, it is clear that

(a) *Growth rates.* (T = mean generation time.)

Vol. % of alcohol.	T (mins.).	Time to log n = 1·00 (mins.).	Vol. % of alcohol.	T (mins.).	Time to log n = 1·00 (mins.).
	Methyl alcohol.			Propyl alcohol.	
0	18·0	144	0	18·0	144
2·4	30	232	0·50	24·5	200
3·9	31	221	1·0	23·5	272
4·8	33·5	234	1·72	50·5	480
5·9	85	380	2·4	102	1570
7·0	112	1126			
	Ethyl alcohol.			Butyl alcohol.	
0	18·0	144	0	18·0	144
0·25	18·2	170	0·50	32·5	282
1·2	20	168	0·88	37·5	1060
2·4	28	206	1·24	140	2300
2·9	32·5	286			
3·4	34	274		Hexyl alcohol.	
4·1	51	460	0	20·5	153
4·8	405	580	0·11	29	233
7·0	2000	1470	0·20	64	1270

(b) *Relative retardation caused by different alcohols.*

	For a given volume per cent. of alcohol.			For equal weights.	For equal molecular concns.
Alcohol.	Calc. from mean generation times.	Calc. from times to log n = 1·00.	Mean.		
Methyl	1·00	1·00	1·00	1·00	1·00
Ethyl	1·31	1·39	1·35	1·36	1·96
Propyl	2·92	4·3	3·61	3·6	6·8
Butyl	5·7	8·3	7·00	7·1	16·5
Hexyl	25·4	34	29·7	31	99

the impairing of the vital processes, unless it occurs by a rapid and reversible mechanism, may progress through a number of generations and not by any means attain its full effect in the mean generation time of a single organism. In this connexion it is of interest to note that the abnormal growth forms frequently reported when organisms exist in adverse circumstances were occasionally observed in the experiments with alcohol, well-defined branching of the cells to Y-shaped forms occurring (cf. Gardner, "Microbes and Ultramicrobes," 1931, p. 23).

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