295. *Physicochemical Aspects of Bacterial Growth. Part VI. The Influence of Toxic Substances on Growth Rate, Stationary Population, and Fermentation Reactions of* Bact. lactis ærogenes.

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The growth rate of *Bact. lactis ærogenes*, the stationary population, n_s, which a given medium will support, and the rate at which the cells in the stationary phase cause fermentation of carbohydrates all decrease with increasing concentrations of phenol, mercuric chloride, formaldehyde, and copper sulphate, according to curves of varying type (Fig. 8), ranging from linear to one showing an abrupt drop to zero at a critical concentration. The growth rate and n_s vary in a remarkably parallel manner, which is given a theoretical interpretation.

In certain examples the fermentation rate in the stationary phase is affected by phenol to the same extent quantitatively as the mean generation time in the logarithmic growth phase. From the results it is inferred that disinfectant substances act in four separate ways. The first is some effect on the lag phase, the second on the actual processes involved in cell division, the third on processes of metabolism, and the fourth on the death rate.

The similarities and contrasts revealed in the experiments are made the basis of a tentative rough analysis of some of the underlying cell mechanisms.

This paper contains a quantitative study of the influence of various toxic substances upon the growth and activity of *Bact. lactis ærogenes*.

The quantities determined have been the mean generation time, T, the maximum population, n_s, which the medium will support, and the rate of gas evolution from fermented carbohydrates.

Under the conditions of the experiments, growth closely follows the logarithmic law $dn/dt = kn$ over a wide range : T is the time required for the total number, n, of organisms to double during this phase of growth. At the end of the logarithmic phase growth falls off abruptly (see curves in Part I; J., 1938, 1934). At this point, which is rather well determined, n is taken as n_s. (Actually a very slow growth continues for some time after this : the value of n at 24 hours, n_{24}, has also been recorded. n_s and n_{24} have always proved to vary in a parallel manner, so the question as to which is the more significant theoretically may be left open.) Before the logarithmic phase of growth there may be a lag phase, which enters into the interpretation of some of the results.

The culture was the same strain as that used in previous work (J., 1938, 1930; 1939, 1683), and the methods of manipulation and counting were similar. T and n_s have been measured for various concentrations of phenol, mercuric chloride, copper sulphate, and formaldehyde. The media used have been veal bouillon of p_H 7·6, the glucose–phosphate medium previously described, and a medium consisting of peptone (8·3 g./l.), sodium chloride (7·5 g./l.), and glucose, mannitol, or galactose (8·3 g./l.).

Mean Generation Times and Maximum Populations.—The results are shown in the various diagrams. For phenol only is a selection of the numerical data given (Tables I

and II). Counts are given in numbers per unit field : to obtain the number of organisms per c.c. these numbers must be multiplied by $1{\cdot}25 \times 10^6$.

TABLE I.

The effect of phenol on the mean generation time, stationary concentration, and count after 24 hours.

(*a*) In bouillon.

Blanks with no phenol : M.G.T. = 21·7 mins.; $n_s = 400$; $n_{24} = 770$. All other values expressed as ratios.

Phenol, %	0·042	0·100	0·108	0·116	0·125	0·133	0·142	0·150	0·158	0·166
1/M.G.T. ...	1·00	0·832	0·714	0·676	0·370	0·263	0·135	0·077	0·170	0·063
n_s	1·00	0·616	0·529	0·361	0·298	0·256	0·175	0·117	0·115	0·084
n_{24}	0·715	0·562	0·540	0·398	0·267	0·149	0·116	0·072	0·092	0·036

(*b*) In glucose–phosphate medium, p_H 7·12.

Blanks with no phenol : M.G.T. = 46 mins.; $n_s = 400$; $n_{24} = 680$. All other values expressed as ratios.

Phenol, %	0·02	0·035	0·05	0·07	0·09	0·10	0·11	0·125	0·15
1/M.G.T.	0·94	0·832	0·706	0·572	0·5	—	0·267	0·232	no growth
n_s	1·00	0·925	0·750	0·70	0·60	0·575	0·345	0·326	—
n_{24}	0·765	0·560	0·510	0·452	0·415	0·375	0·213	0·253	—

(*c*) In glucose–peptone medium.

Blanks with no phenol : M.G.T. = 50 mins.; $n_s = 260$; $n_{24} = 296$.

Phenol, %	0·004	0·013	0·021	0·033	0·033	0·042	0·058	0·067	0·071	0·083	0·10	0·133	0·167
1/M.G.T. ...	1·00	0·73	0·70	0·73	0·65	0·57	—	0·57	0·57	0·532	0·42	—	—
n_s	0·690	0·525	0·455	0·455	0·77	0·415	0·327	0·318	0·246	0·208	0·135	—	—
n_{24}	0·87	0·50	0·42	0·41	0·83	0·37	0·34	0·28	0·233	0·186	0·15	0·12	0·02

TABLE II.

The effect of various concentrations of phenol on gas evolution by bacteria growing in various media.

(*a*) Carbohydrate–peptone medium.

	Glucose.				Galactose.		Mannitol.	
Phenol, %.	Rate of gas evoltn., ml./min.	Ratio to blank.	Final vol. of gas, ml.	Ratio to blank.	Ratio to blank (rate of gas evoltn.).	Ratio to blank (final vol.).	Ratio to blank (rate of gas evoltn.).	Ratio to blank (final vol.).
0·003	0·0012	0·92	0·56	1·03	0·92	0·95	0·69	0·84
0·066	0·0010	0·77	0·46	0·81	0·77	0·77	0·63	0·81
0·100	0·00087	0·67	0·40	0·70	0·54	0·57	0·51	0·59
0·133	0·00055	0·42	0·21	0·34	0·43	0·52	0·32	0·28
0·166	0·00033	0·25	0·17	0·28	0·19	0·17	0·21	0·22
0·200	0·00013	0·09	0·08	0·09	No gas evolution		No gas evolution	
0·233	—	No gas evolution		—	No gas evolution		No gas evolution	
0·266	—	No gas evolution		—	No gas evolution		No gas evolution	

(*b*) Glucose–phosphate medium, p_H 7·12.

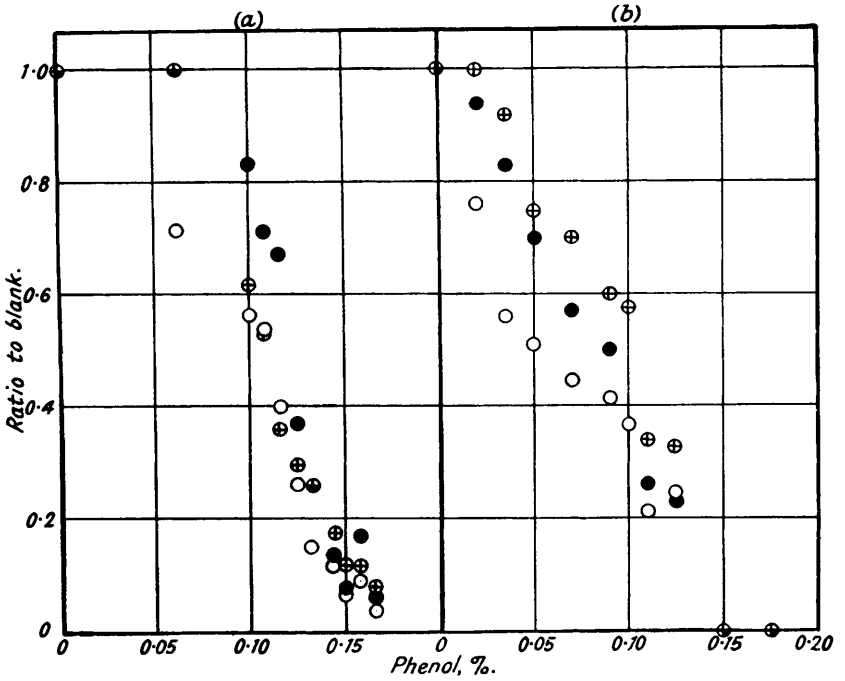
Phenol, %		0·015	0·025	0·040	0·05	0·07	0·08	0·12	0·16	0·20
Ratio to blank	Rate of gas evoltn....	0·88	0·83	0·40	0·37	0·24	0·23	—	—	—
	Final vol. of gas	0·90	0·77	0·29	0·18	0·11	0·07	0·03	0·01	nil

n_s, n_{24}, and $1/T$, which measures the growth rate, are plotted for each concentration of the toxic substances as fractions of the value they would have in the pure medium. All three ratios fall off with increasing concentration of the poison according to almost identical curves, but the form of the curve varies in a very characteristic manner with the medium and the poison. The main types of curve are shown schematically in Fig. 8. Type 1, a linear decrease of the ratio with concentration, has already been found for the influence of alcohol on the growth rate (Dagley and Hinshelwood, J., 1938, 1930). It is further illustrated by the results for phenol in glucose–phosphate (Fig. 1*b*), and for formaldehyde, mercuric chloride, and copper sulphate in bouillon (Figs. 3*a*, 2*a*, 4*a*).

Type 1*a* is illustrated by glucose–peptone containing phenol (Fig. 6*a*), Type 2 by

FIG. 1.

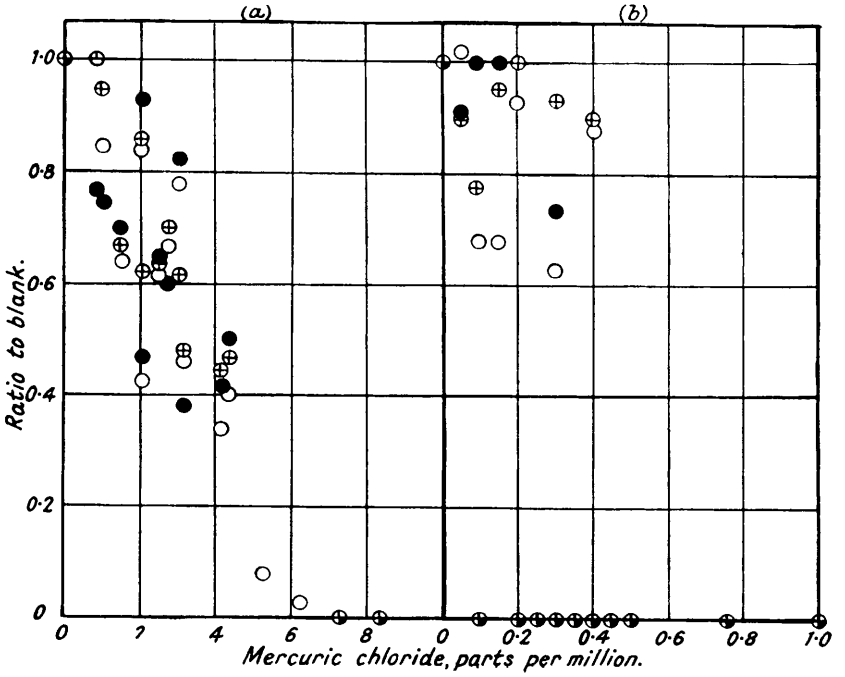
The effect of phenol upon growth : (a) *in bouillon,* (b) *in glucose–phosphate medium,* p_H 7·12. *Ratio to blank plotted against % of phenol.*



Key : ● *Mean generation times.* ⊕ *Stationary populations.* ○ *Counts after 24 hours.*

FIG. 2.

The effect of mercuric chloride upon growth. Media as for Fig. 1. *Ratio to blank plotted against parts per million of mercuric chloride. Key as in Fig.* 1.

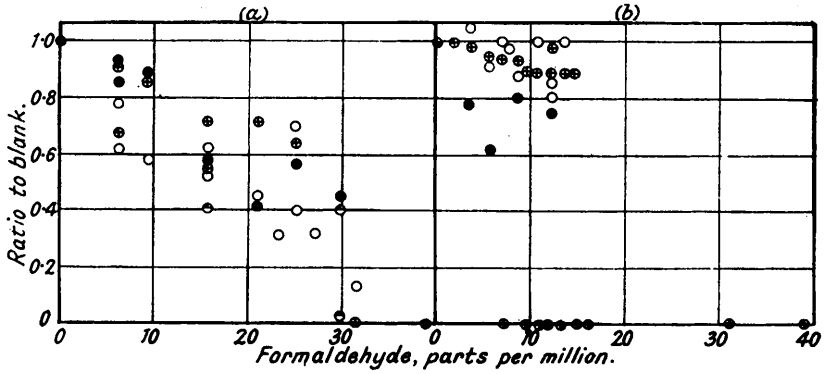


bouillon containing phenol (Fig. 1*a*), Types 3 and 3*a* by glucose–phosphate containing mercuric chloride, formaldehyde, or copper sulphate, of which mercuric chloride approximates most nearly to the idealised form 3 (Figs. 2*b*, 3*b*, and 4*b*).

Types 3 and 3*a* call for special comment. Either growth is nearly normal or it will not occur at all. No values of the ratio, either for T or for n_s, which were below about 70% of normal could be measured. Where they might have been expected there was no growth at all. An analogous effect has already been observed (Lodge and Hinshelwood, J., 1939, 1692) in the experiments on the relation between growth and magnesium content in a glucose–phosphate medium: with the magnesium above a certain limit growth was constant and normal; below the limit, zero.

FIG. 3.

The effect of formaldehyde upon growth.



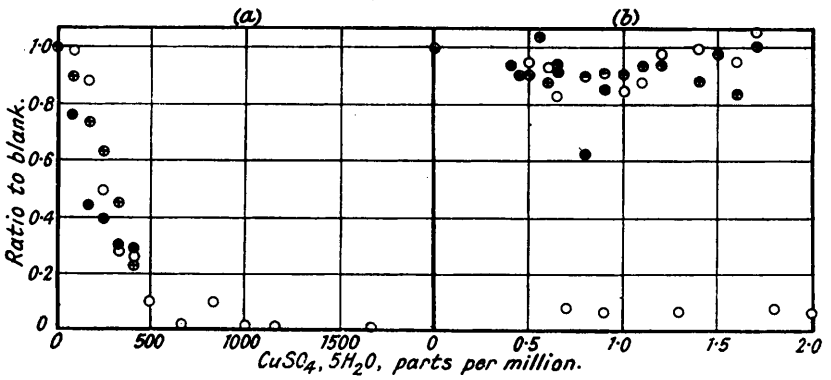


FIG. 4.

The effect of cupric sulphate upon growth.

Keys and media as for Fig. 1.

Ratio to blank plotted against parts per million of toxic substance in each case.

The explanation appeared to be that the magnesium was necessary for some reaction occurring in the lag phase which precedes logarithmic growth. In the absence of magnesium, the lag phase was so prolonged that the inoculum all died before the cells began to divide. There was accordingly, for a given magnesium concentration, a critical inoculum size below which no growth occurred and above which it was normal. A similar effect was found here with mercuric chloride in the glucose–phosphate medium. For a given inoculum size there is a critical concentration of mercuric chloride above which no growth occurs, and for a given mercuric chloride concentration there is a critical inoculum size below which no growth occurs. In contrast with this result, the inoculum size is found to have no effect with phenol, which does not give a curve of Type 3. We may therefore assume that, just as magnesium shortens the lag phase, so mercuric chloride lengthens it,

and that the critical concentration corresponds to a lag phase during which all the inoculum can die before division begins.

In all the rest of this work the inoculum had been 2 or 4 loops of a 12-hour old culture. For the experiments on inoculum size small volumes of a similar culture were pipetted into 20 c.c. of medium. The results are given in Tables III and IV.

TABLE III.

The effect of varying inoculum size on the mean generation time, stationary concentration, and count after 24 hours, in glucose–phosphate medium, p_H 7·12, *containing* 0·05% *of phenol.*

Inoculum size, ml. × 10^2	20	5	1·2	0·6	0·06
M.G.T., mins.....	71·7	72	—	75	—
n_t	360	360	365	—	345
n_{24}	475	460	400	410	415

The count of the inoculum, which had just reached its stationary concentration, was 400.

TABLE IV.

The effect of inoculum size on the growth limit in glucose–phosphate medium, p_H 7·12, *containing mercuric chloride.*

$HgCl_2$, p.p.m.	Inoculum size, ml. × 10^2. 20.	2·4.	1·2.	0·12.	Count of inoculum.	$HgCl_2$, p.p.m.	Inoculum size, ml. × 10^2. 20.	2·4.	1·2.	0·12.	Count of inoculum.
0·70	—				500	0·35	+				435
0·65	+				500	0·30	+	—	—	—	495
0·60	+				500	0·25	+	—	—	—	435
0·55	+				500	0·20	+	+	—	—	470
0·50	+				435	0·15	+	+	+	—	410
0·45	+				435	0·10	+	+	+	+	470
0·40	+				435						

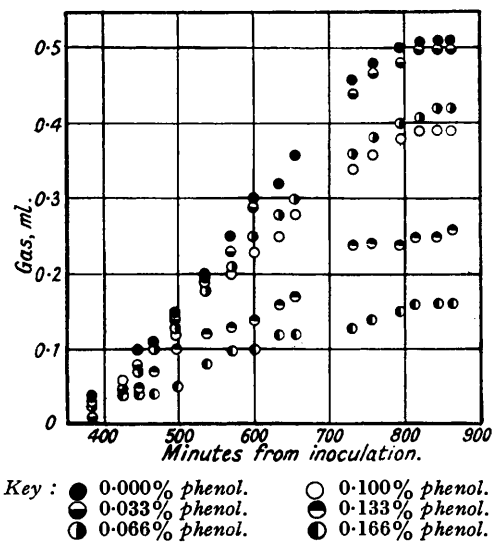
+ = Growth. — = No growth.

Fermentation of Carbohydrates.—Many bacteria of the Coli-Typhosum group ferment media containing various carbohydrates, with the evolution of gas. It was proposed to compare the influence of a toxic substance upon the rate of this process with its influence on the growth rate.

Small graduated glass tubes of about 1 c.c. were inverted in a larger tube containing the culture medium, all the air in them except about 0·02 c.c. being removed by evacuation under sterile conditions. The medium was then inoculated. Parallel readings of the number of bacteria and of the total volume of gas evolved were made. No measurable amount of gas began to collect until the bacteria had attained their stationary population. From this point the gas volume increased linearly with time, then abruptly ceased to increase. This circumstance made it possible to separate the two effects of a toxic substance, *viz.*, that upon the growth rate and that upon the fermentation reaction. All cultures could be grown to the stationary phase, evolving hardly any gas: the poison could then be added, and the gas evolution observed from then on. A typical series of results is shown in Fig. 5. For the medium containing peptone with glucose, the rate of gas evolution and the total final volume are both decreased in the same proportion in the

FIG. 5.

Gas evolution curves for glucose–peptone media containing various amounts of phenol.



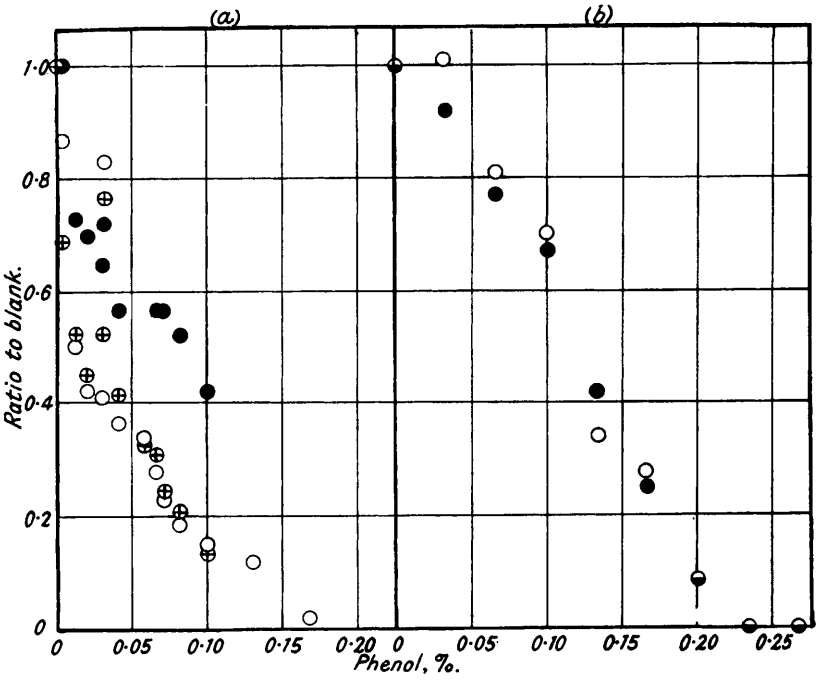
presence of a given quantity of phenol. The ratio of either quantity to the corresponding value for the phenol-free medium gives a straight line when plotted against the phenol concentration.

Similar results are found for mannitol– or glucose–peptone media and for a glucose–phosphate medium, except that in the last case the gas evolution is less vigorous, and the curve of gas evolution against phenol concentration is no longer linear.

Comparison of Figs. 6*a* with 6*b* and 7*a* with 7*b* shows that the effect of phenol on the fermentation occurring during the stationary phase is quantitatively very nearly identical with its effect on the growth rate itself.

FIG. 6.

Glucose–peptone medium. The effect of phenol upon (a) *growth,* (b) *gas evolution. Ratio to blank plotted against phenol, %.*



Keys : (*a*) *As in Fig.* 1.
(*b*) ● *Rates of gas evolution.* ○ *Final volumes of gas.*

Parallel Variations in Mean Generation Time and Stationary Population caused by Toxic Substances.—One might have expected a toxic substance to affect the growth rate alone, leaving n_s unchanged, but there is, in fact, a rather striking parallelism between the two, which is illustrated in Figs. 1—4. It stands in sharp contrast to what is found when the p_H of the medium is varied. An adverse p_H may reduce n_s almost to zero while hardly changing the rate at which the sparse residual growth occurs. From this the conclusion was drawn in Part IV that the cell division process involved a series of changes, one at least of which was shielded from the influence of the p_H of the medium. The present parallelism between growth rate and n_s also requires an explanation, which may be given as follows. We assume that under the conditions of the experiments growth ceases when the *combined actions* of the added poison and of toxic products from the cells themselves reduce the growth rate to zero. (Since the *total* count and not merely the *viable* count becomes stationary it is not enough to assume that division rate balances death rate.)

During the logarithmic phase the numbers are given by the equation $dn/dt = kn$. At the onset of the stationary phase k falls rather rapidly to zero. Since growth rate decreases linearly with increasing concentration, p, of a toxic substance such as phenol, we may write for the logarithmic phase $k = k_0 - ap$, where a is constant.

The amount of toxic products from the cells themselves present at time t will be proportional to the quantity

$$\int_0^t n \,.\, \mathrm{d}t$$

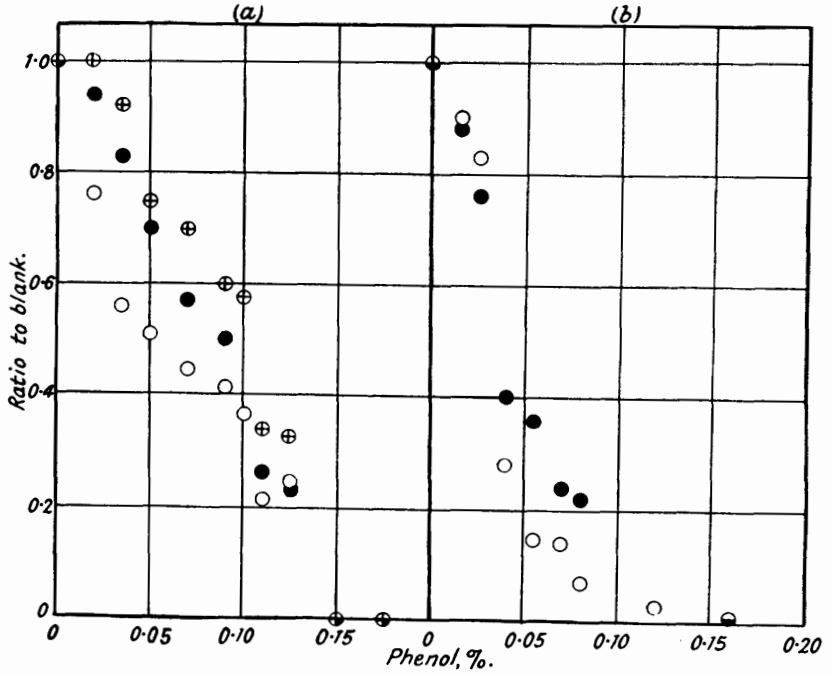
and this integral will grow very rapidly as the stationary phase is reached, so as to give the relation

$$k = k_0 - ap - b\int_0^t n \,.\, \mathrm{d}t$$

where b is a constant.

FIG. 7.

Glucose–phosphate medium, PH 7·12. *As Fig.* 6.



For the logarithmic part of the curve we shall have, from the earlier equation, $k/k_0 = 1 - (a/k_0)p$. When t has reached a value t_s, k will have fallen to zero, so that $n = n_s$, and

$$k_0 - ap - b\int_0^{t_s} n \,.\, \mathrm{d}t = 0$$

But for most of the time the equation $\mathrm{d}n/\mathrm{d}t = kn$ has in fact been followed, so that

$$b\int_0^{t_s} n \,.\, \mathrm{d}t = b\int_0^{n_s} \frac{\mathrm{d}n}{k} = \frac{bn_s}{k}$$

whence $n_s = k(k_0 - ap)/b$. When $p = 0$, let $n_s = (n_s)_0$, so that

$$n_s/(n_s)_0 = 1 - ap/k_0 = k/k_0.$$

Since $k/k_0 = T_0/T$, the required quantitative parallelism is accounted for.

Discussion.—From the results described it appears that four distinct effects of the toxic substances may be distinguished.

(I) An influence upon some lag phase reaction. It may be assumed that the lag phase is concerned with the slow building up of some substance necessary for cell division. The formation may be inhibited by the toxic substance to an extent depending upon its concentration.

(II) Some effect on the actual processes involved in cell division.

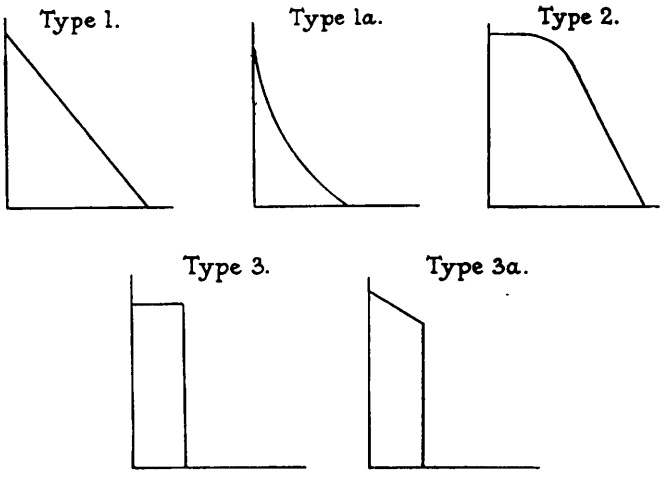
(III) An effect on the metabolic reactions which go on even when active division has ceased.

(IV) Lethal processes in which the actual death of the cell is accelerated.

If the natural assumption is made that the different processes are differently influenced by poisons, the varied types of curve shown in Fig. 8 can be explained. There is an analogy between this separation of effects and the results of Quastel and Wooldridge (*Biochem. J.*, 1927, **21**, 148, 1224) on the successive deactivations of resting *Bact. coli*.

FIG. 8.

Types of curve obtained by plotting the ratio of growth or gas evolution to that of a blank against concentration of toxic substance.



In Type 1 and 1*a* the predominant effect must be (II), *i.e.*, directly affecting the processes involved in division. Whether the curve is linear as in 1 or of the shape shown in 1*a* will presumably depend upon the shape of adsorption isotherms governing the equilibrium between the poison in solution and that taken up by the centres which it inactivates.

In Type 2 the predominant factor is again probably (II), except that there is some initial " tolerance " due to the fact that some of the poison is taken up and neutralised by the organisms or the medium, probably the latter.

In Type 3 the poison probably inhibits some lag phase reaction, with the result that the cell dies before it has elaborated substances necessary for division. Unless it dies, normal growth occurs, the division reactions being apparently somewhat less sensitive to the poison. In Type 3*a*, effect (II) predominates, as in Type 1, over a limited range, but is eventually superseded by (I). Effect (III) requires no more discussion at this stage. Effect (IV) is considered in the following paper.

In a tentative way we may now, on the basis of these results, analyse certain stages of the growth process as follows :

In the lag phase there occurs the synthesis of a necessary intermediate compound : reaction A.

After the lag phase, reaction X occurs, which is rate-determining for reaction Y, involving carbohydrate fermentation, and for reaction Z, leading to cell division. Reaction X is retarded by disinfectants, which explains why the fermentation processes and the division process itself are equally affected by phenol. Reaction Z is put out of action at some stage by the products of the cells themselves, reaction Y being distinct from it, since it can continue well after the onset of the stationary phase. Alternatively, one could assume that, at the point where growth stops, reaction A is inhibited, if A were a precursor to and involved in Z but not in X.

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