THE INHIBITORY SELECTIVE ACTION ON BACTERIA OF BODIES RELATED TO MONOCHLORACETIC ACID.

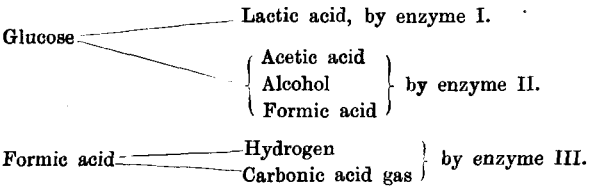
(A CONTRIBUTION TO THE THEORY OF CELL INTOXICATION.)

By W. J. PENFOLD,

Assistant Bacteriologist, Lister Institute, London.

(With Plate II.)

THE author (1911) showed that growth of *B. coli* (Escherich), on monochloracetic acid agar, led to the selection of a new strain unable to produce gas from glucose, lactose and other sugars, but still able to produce gas from alcohols in very considerable amount. Harden and Penfold (1912) compared the chemical action of this new strain with that of the original strain when grown anaerobically on glucose peptone water. The general result of the examination was to show that the new strain produced much more lactic acid, and less alcohol, acetic acid and formic acid, the gas being reckoned as formic acid, than the original strain. This pointed to the fact that the lactic acid-producing ferment was distinct from that ferment which produced alcohol, acetic acid and formic acid. Harden (1901) had previously brought forward evidence showing that the CO_2 and H were derived from the splitting up of formic acid. Since the new strain which could not produce gas from glucose, produced a normal yield from sodium formate, Harden and Penfold concluded that the splitting up of the formate was due to a third enzyme. This fermentation of glucose is shown schematically below:



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Six different strains of coliform organisms, and also *B. enteritidis* Gaertner, *B. paratyphosus* B. and *B. Grünthal*, were found constantly to give non-gas-producing strains when grown on chloracetate agar and tested in glucose. It was no accidental variation, for the new strains could be produced with certainty in ten days.

In the endeavour to understand the above selection process, various organic substances were added to agar and the evidences of variation

TABLE I.

The cultural signs of variation and selection obtained when B. coli (*Escherich*) *is grown on phenylacetic acid agar plates.*

No. of plate	Quantity of 10% solution of phenylacetic acid added to respective plates	Days of observation		
		2nd	4th	7th
1	None	Good growth. No variability in the size of the colonies. No secondary colonies present.	No secondary colonies. No variability in the size of the colonies other than that due to crowding.	As last observed.
2	None	,,	,,	,,
3	0·05 c.c.	,,	Definite naked eye papillae present on the colonies.	Many large papillae present on colonies. Marked variability in size of colonies.
4	0·3 c.c.	,,	,,	,,
5	0·5 c.c.	,,	,,	,,
6	0·7 c.c.	,,	,, Some colonies show swollen margins.	,,
7	1·0 c.c.	,,	Papillae present on some colonies. Big and little colonies are present in central crowded portions of plate.	,,
8	1·3 c.c.	,,	,,	,,
9	1·6 c.c.	,,	,,	,,
10	2·0 c.c.	,,	,,	,,
11	2·4 c.c.	Colonies inhibited in growth a little.	,,	,,
12	2·8 c.c.	Colonies inhibited in growth markedly.	As last observed.	,,

Remark. In the higher concentrations many of the colonies of large size are thickened and of a marked yellow colour.

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and selection which had been obtained with chloracetate sought for. Some of these results are detailed in previous papers by the author.

It was found *e.g.* that monochloracetate of soda was much more inhibitory than dichloracetate or trichloracetate. Neither the dichloracetate nor trichloracetate produced the papillae on the surface of the plate colonies which are so characteristic of the monochloracetate. Great variability in the size of the colonies—a feature which was strongly marked in the case of the monochloracetate plate cultures—did not occur.

TABLE II.

B. coli after growth on phenylacetic acid agar.

Agar slopes, from colonies of phenylacetic acid agar plates described in Table I, were inoculated in glucose and mannite peptone waters with the following result.

Variety of colony from which the agar slope was taken	Day of observation	Glucose	Mannite
From plate 10 of the series.			
(1) Large yellow colony	1	Af. G. 1/6	Af. G. 4/10
	2	Af. G. 1/5	Af. G. 5/6
(2) ,,	1	Af. G. 1/3	Af. G. 4/10
	2	Af. G. 5/12	Af. G. 2/3
(3) ,,	1	Af. G. 1/5	Af. G. 1/4
	2	Af. G. 5/12	Af. G. 3/4
(4) ,,	1	Af. G. 1/3	Af. G. 1/2
	2	Af. G. 5/12	Af. G. 5/6
(5) ,,	1	Af. G. 1/6	Af. G. 1/3
	2	Af. G. 1/3	Af. G. 2/3
(6) Little colony	1	Af. G. 1/6	Af. G. 1/2
	2	Af. G. 1/3	Af. G. 2/3
(7) ,,	1	Af. G. 1/4	Af. G. 7/16
	2	Af. G. 1/3	Af. G. 2/3
(8) ,,	1	Af. G. 1/4	Af. G. 7/12
	2	Af. G. 1/3	Af. G. 2/3
From plate 11 of the series.			
(A) Large colony	1	Af. G. 1/3	Af. G. 7/12
	2	Af. G. 5/12	Af. G. 7/12
(B) ,,	1	Af. G. 1/5	A. 3/4 G. 7/16
	2	Af. G. 1/3	Af. G. 5/6
(C) ,,	1	Af. G. 1/4	Af. G. 1/3
	2	Af. G. 1/3	Af. G. 2/3
(D) ,,	1	Af. G. 1/4	Af. G. 1/5
	2	Af. G. 1/3	Af. G. 1/2
(E) ,,	1	Af. G. 3/8	Af. G. 1/3
	2	Af. G. 1/2	Af. G. 3/4
From plate 12 of the series.			
(F) Large yellow colony	1	Af. G. 1/3	Af. G. 1/2
	2	Af. G. 5/12	Af. G. 2/3
(G) ,,	1	Af. G. 3/8	Af. G. 1/2
	2	Af. G. 5/12	Af. G. 3/4
(H) ,,	1	Af. G. 1/2	Af. G. 7/16
	2	Af. G. 7/12	Af. G. 3/4

Monobromacetate of soda added to agar gave the same papillae as the monochloracetate but big colonies did not appear in high concentrations as in the case of monochloracetate plates, and from none of the plates with lower concentrations, was it possible to obtain non-gas-producing strains.

Phenylacetic acid. Phenylacetic acid was dissolved in saturated sodium carbonate solution until the solution was just faintly alkaline to neutral litmus paper. Distilled water was then added to render the solution of 10% strength. The solution was sterilized by filtration through a Doulton filter. Ascending quantities of the solution were put into Petri dishes and 15 c.c. of melted agar were added to each plate, the whole being then well mixed.

The plates were inoculated with *B. coli* (Escherich) and grown at 37° C. Their appearances are indicated in Table I.

The results recorded in Table I are similar to those obtained when *B. coli* (Escherich) was grown on chloracetic acid agar.

From plates 10, 11 and 12 of the series, after eight days' growth at 37° C., various colonies were inoculated on to agar slopes and subsequently tested on mannite and glucose peptone waters. The results are recorded in Table II[1].

It will be noticed that the average yield of gas of the strains from big colonies of plate 10 was the same as that from the little colonies, so that the phenylacetic acid does not appear to differentiate between the colonies in respect of gas-forming power. The gas-producing function in all the colonies is, however, slightly depressed.

Other substances which were tried, were found ineffectual in producing marked selections on the plates or non-gas-producing variants, but details of the experiments are omitted here because they are elsewhere dealt with and because of the negative results. The substances in question were cyanoacetic acid, α-bromproprionic acid, dibromsuccinic acid, chlormalonic acid, hippuric and benzoic acid, all used as alkaline salts. On the other hand monochlorhydrin, a monochlor substitution product of glycerine, was found to give rise to a similar variant in the case of the *B. coli,* as monochloracetate of soda. The appearances indicating selection as they occurred in the colonies of the monochlorhydrin agar plates were somewhat different from those observed on monochloracetate of soda plates.

[1] In Table II Af. = full acid reaction. G. = gas. The fraction after G. = the amount of the gas tube occupied with gas. The gas tests were performed in Durham's tubes. The fractions following A. indicate varying degrees of partial acidity, of the litmus solutions.

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The *monochlorhydrin* medium is an agar to which a known quantity of 20% filtered solution of monochlorhydrin is added. Ascending quantities of the solution were placed in Petri dishes and 15 c.c. of melted agar were added to each plate and thoroughly mixed. The plates were then inoculated with *B. coli* and grown at 37° C. The appearances obtained are given in Table III.

Table III shows, therefore, that at suitable concentrations marked variability in the size of similarly situated colonies may occur, and papillae may be present, which however are more inclined to be situated towards the cortex of the colonies than in the case of colonies of *B. coli* when grown on monochloracetate agar. The most peculiar feature of the plates is the large tree-like outgrowths from the periphery of the colonies. These seem to have some similarity to the appearances described by R. Müller (1909) in colonies of *B. paratyphosus* B. when grown on gelatine. (Pl. II, figs. 1, 2, 3.)

Subcultures on to agar slopes were made from different colonies of the series described in Table III and were then tested on carbohydrate media as recorded in Table IV.

The results of the examination of the different colonies and portions thereof show that the dense colonies and the papillae are good gas producers, whereas on chloracetate of soda plates the big dense colonies were either poor gas producers or failed to produce gas at all.

The lateral tree-like expansions of the colonies on the 0·2 c.c. chlorhydrin plates had, however, lost the power of producing gas from lactose. Two of these were tested on the whole series of carbohydrates with results given in Table V.

Table V shows that the variant *B. coli* obtained by growth on chlorhydrin agar is similar to the monochloracetate variety of the same organism. It gives either little or no gas from sugars and a fair yield from alcohols and it takes a longer time than normal to clot milk. On the other hand it appears almost entirely unable to produce gas from sodium formate while the normal strain gives 6/12 to 7/12 of a tubeful as also did the chloracetate variety of *B. coli* which I first described. It would be very interesting to know if an accumulation of formic acid is taking place in the glucose tubes on which this new variety is grown, but that point has not yet been decided.

TABLE III.

Record of plates of B. coli (*Escherich*) *on monochlorhydrin agar. Each plate contained* 15 *c.c. of agar and the quantity of* 20 % *chlorhydrin solution indicated.*

Amount of chlorhydrin solution	Day of observation			
	1st	2nd	5th	10th
None	Good growth.	Edges of colonies somewhat spreading in character.	As last observed.	No lateral expansions, no papillae, no variability in size of the colonies in centre of plate.
·1 c.c.	Slight inhibition of growth.	Edges of colonies sharper, but the colonies are well grown.	Lateral expansions present on all large colonies. Cortical papillae also present on colonies.	A few small opaque dense colonies outgrowing the rest in the crowded centre of plate. (See Pl. II, fig. 1.)
·2 c.c.			,,	The lateral expansions of these colonies have a tree-like appearance and are better developed than in any other concentration. Many papillae are present on the colonies, and dense colonies are present on the centre of the crowded plate. (See Pl. II, fig. 2.)
·35 c.c.			,, The expansions are however smaller and not so tree-like.	
·5 c.c.			Marginal colonies of large size show swollen edges. Big and little colonies are present in the centre of the plate.	Many papillae present on the colonies.
1·0 c.c.		The colonies on this plate show marked inhibition but are of uniform size.	All colonies small. With low magnification they show a wrinkled surface and a few lateral papillae.	As observed 5th day. (See Pl. II, fig. 3.)
1·5 c.c.	Marked inhibition.	As observed 1st day.	A few of the colonies in crowded part of plate are of a larger size than the rest.	
2·0 c.c.	Sterile.	Sterile.	Sterile.	

All observations are naked eye unless otherwise stated.

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TABLE IV.

The plate in the series from which the subculture was made	Type of colony or portion of colony from which the subculture was made	Days of observation	Lactose	Glycerine	Saccharose	Broth	Indol	Motility and staining
0·1 c.c. plate	Lateral expansion of colony.	1	Af. G. 1/2	An. Gn.	An. Gn.	Cloud	+ +	+
		2	Af. G. 7/12	,,	,,			Gram
		6	Af. G. 1/2	A. 1/2 G. 1/6	,,			negative
,,	,,	1	Af. G. 1/2	An. Gn.	,,	Cloud	+ +	+
		2	Af. G. 3/5	,,	,,			Gram
		6	Af. G. 7/12	A. 1/2 G. 1/12	,,			negative
,,	Dense colony of crowded portion of the plate.	1	Af. G. 1/8	An. Gn.	,,	Cloud	+ +	+
		2	Af. G. 5/12	,,	,,			Gram
		6	Af. G. 5/12	Af. G. 1/2	,,			negative
,,	,,	1	Af. G. 1/3	An. Gn.	,,	Cloud	+ +	+
		2	Af. G. 5/12	,,	,,			Gram
		6	Af. G. 7/12	A. 1/2 G. 1/6	,,			negative
0·2 c.c. plate	Lateral expansion of colony.	1	Af. Gn.	An. Gn.	,,	Cloud	+ +	+
		2	,,	,,	,,			Gram
		6	,,	A. 1/2 G. 1/6	,,			negative
,,	,,	1	,,	An. Gn.	,,	Cloud	+ +	+
		2	,,	As. Gn.	,,			Gram
		6	,,	A. 1/2 G. 1/8	,,			negative
,,	,,	1	Af. G. 7/12	An. Gn.	,,	Cloud	+ +	+
		2	Af. G. 7/12	,,	,,			Gram
		6	Af. G. 1/12	,,	,,			negative
)·5 c.c. plate	Papillae.	1	Af. G. 5/12	,,	,,	Cloud	+ +	+
		2	Af. G. 1/2	,,	,,			Gram
		6	Af. G. 1/2	An. G. 1/6	,,			negative
,,	,,	1	Af. G. 5/12	An. Gn.	,,	Cloud	+ +	+
		2	Af. G. 1/2	,,	,,			Gram
		6	Af. G. 1/2	A. 1/2 G. 1/12	,,			negative
,,	Dense colony of crowded portion of the plate.	1	Af. G. 5/12	An. Gn.	,,	Cloud	+ +	+
		2	Af. G. 1/2	,,	,,			Gram
		6	Af. G. 5/12	A. 1/2 G. 1/10	,,			negative
,,	,,	1	Af. G. 1/2	An. Gn.	,,	Cloud	+ +	+
		2	Af. G. 7/12	,,	,,			Gram
		6	Af. G. 1/2	Af. G. 1/2	,,			negative
,,	,,	1	Af. G. 1/2	An. Gn.	,,	Cloud	+ +	+
		2	Af. G. 1/2	,,	,,			Gram
		6	Af. G. 1/2	A. 1/2 G. 5/12	,,			negative

An. = no acid. Gn. = no gas. As. = slight acid reaction.

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TABLE V.

Strain	Day of observation	Arabinose	Xylose	Isodulcite	Glucose	Laevulose	Mannose	Galactose	Maltose	Lactose
(1) Lateral expansion	1	Af. Gn.	Af. Gn.	Af. G. bub.	Af. Gn.	Af. Gn.	Af. G. bub.	Af. Gn.	Af. Gn.	Af. Gn.
of a colony of a 0·2 c.c.	2	,,	Af. G. bub.	,,	,,	,,	,,	,,	Af. G. bub.	,,
chlorhydrin plate.	6	Af. G. bub.	,,	,,	,,	,,	,,	,,	Af. G. 1/4	,,
(2) Second lateral expansion of a colony of the same plate.	1	Af. Gn.	Af. Gn.	Af. Gn.	,,	,,	,,	,,	Af. Gn.	An. Gn.
	2	,,	Af. G. bub.	Af. G. bub.	,,	,,	,,	,,	,,	,,
	6	Af. G. bub.	,,	,,	,,	,,	,,	,,	,,	,,
(3) Control of normal *B. coli* (Escherich).	1	Af. G. 5/12	Af. G. 5/12	Af. Gn.	Af. G.?	Af. G. 5/12	Af. G. 5/12	Af. G. 1/3	Af. G. 5/12	Af. G. 7/12
	2	Af. G. 1/2	Af. G. 7/12	,,	Af. G. 1/2	Af. G. 1/2	Af. G. 1/2	Af. G. 1/2	Af. G. 7/12	Af. G. 3/4

Strain	Day of observation	Cane sugar	Raffinose	Dextrin	Inulin	Salicin	Amygdalin	Glycerine	Erythrite	Adonite
(1) Lateral expansion	1	An. Gn.	An. Gn.	As. Gn.	An. Gn.	An. Gn.	Green, no gas	An. Gn.	An. Gn.	–
of a colony of a 0·2 c.c.	2	,,	,,	A. 1/2 Gn.	,,	,,	,,	As. Gn.	,,	–
chlorhydrin plate.	6	,,	,,	,,	,,	,,	,,	A. 3/4 G. 1/2	,,	–
(2) Second lateral expansion of a colony of the same plate.	1	,,	,,	As. Gn.	,,	,,	,,	An. Gn.	,,	–
	2	,,	,,	A. 1/2 Gn.	,,	,,	,,	,,	,,	–
	6	,,	,,	,,	,,	Af. Gn.	,,	Af. G. 1/4	,,	–
(3) Control of normal *B. coli* (Escherich).	1	–	–	Gp. An.	–	–	–	–	–	–
	2	–	–	Gp. A.	–	–	–	A.? G. 1/12	–	–

Strain	Day of observation	Sorbite	Mannite	Dulcite	Sodium formate	Milk	Broth	Motility and staining	Indol
(1) Lateral expansion	1	Af. Gn.	Af. G. 5/12	An. Gn.	An. Gn.	A. No clot	Cloud	+ Gram negative	
of a colony of a 0·2 c.c.	2	Af. G. 1/3	Af. G. 5/12	Af. G. 1/8	An. G. bub.	,,	,,		
chlorhydrin plate.	6	Af. G. 5/12	Af. G. 5/12	Af. G. 5/12	An. G. bub.	A. Clot	,,		+
(2) Second lateral expansion of a colony of the same plate.	1	Af. Gn.	Af. G. 5/12	An. Gn.	An. Gn.	A. No clot	,,	+	
	2	Af. G. 1/3	Af. G. 5/12	Af. G. 1/4	An. G. bub.	,,	,,		
	6	Af. G. 5/12	Af. G. 5/12	Af. G. 5/12	An. G. bub.	A. Clot	,,	Gram negative	+
(3) Control of normal *B. coli* (Escherich).	1	Af. G. 5/12	Af. G. 7/12	A. 1/2 G. bub.	G. 1/2	A.	,,	+	
	2	Af. G. 7/12	Af. G. 3/4	Af. G. 7/12	G. 7/12	A. Clot	,,	Gram negative	+

B. lactis aerogenes.

We will now consider the response of *B. lactis aerogenes* to certain selecting agents.

Several experiments made with the object of selecting out non-gas-producing varieties of *B. lactis aerogenes* on monochloracetate agar proved unsuccessful and it was considered probable that the non-success was due to the very different fermentation of sugar effected by this organism as compared with *B. coli* (Escherich).

Attempts were however made with chlorhydrin agar to obtain variants of *B. lactis aerogenes* and I would like to submit here a preliminary notice of the results.

Appearance of B. lactis aerogenes *when grown at* 37° C. *on chlorhydrin agar plates.*

Plates containing 1 c.c. of 20 % chlorhydrin solution to 15 c.c. of agar gave fairly good growth by the second day though not so good as controls without chlorhydrin, and from the second day onward, in the crowded portions of the plates, a few colonies could be seen which were denser and more opaque than their neighbours. The difference however was slight, and not at all so marked as that shown by colonies of *B. coli* (Escherich) on plates of the same strength. After ten days' growth two colonies which projected slightly from the centre of the plate were inoculated on to agar slopes. These slopes grew slowly at 37° C.—probably because some of the chlorhydrin may have been carried over with the organisms. On the third day, however, the agar slopes were well covered with a thick growth. These cultures were then tested on a small series of carbohydrate media when one of them (Strain "6") was found to have lost the power to ferment glycerine.

The other strain retained that power in normal amount. Strain "6" was then plated out and two separate colonies tested again. They each gave the same results as before in respect of glycerine-fermentation. The loss of fermentation power was confined to glycerine, while mannite and glucose gave good yields of gas.

Strain "6" was finally tested on a large series of carbohydrate media along with a control of the normal strain and gave the results shown in Table VI.

A consideration of Table VI shows us that the chlorhydrin variety of *B. lactis aerogenes* gave acid and gas in the same sugars and alochols

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TABLE VI.

Strain	Day of observation	Arabinose	Isodulcite	Glucose	Laevulose	Mannose	Galactose	Maltose	Lactose	Cane sugar
B. lactis aerogenes	2	As. G. 1/12	Af. G. 1/10	Af. G. 7/12	Af. G. 1/2	Af. G. 5/12	Af. G. 1/4	Af. G. 1/7	Af. G. 7/16	Af. G. 5/8
Strain 6.	6	As. G. 1/12	Af. G. 1/10	Af. G. 1/2	Af. G. 1/2	Af. G. 5/12	Af. G. 1/4	Af. G. 1/7	Af. G. 1/2	Af. G. 1/2
Normal *B. lactis*	2	As. G. 1/8	Af. G. 1/8	Af. G. 2/3	Af. G. 7/10	Af. G. 1/2	Af. G. 1/2	Af. G. 4/5	Af. G. 9/10	Af. G. 2/3
aerogenes.	6	As. G. 1/8	Af. G. 1/12	Af. G. 1/2	A. 1/2 G. 1/3	Af. G. 1/3	Af. G. 1/3	Af. G. 5/8	Af. G. 1/2	Af. G. 1/2

		Raffinose	Dextrin	Inulin	Salicin	Amygdalin	Glycerine	Erythrite	Adonite	Sorbite
B. lactis aerogenes	2	Af. G. 3/4	Af. G. 1/8	–	Af. G. 1/3		–	–	Af. G. 3/4	Af. G. 1/12
Strain 6.	6	Af. G. 1/2	Af. G. 1/6	–	Af. G. 1/4		–	–	Af. G. 2/3	Af. G. 1/2
Normal *B. lactis*	2	Af. G. 5/8	Af. G. 5/8	–	Af. G. 3/8		An. G. 5/8	–	Af. G. 7/16	Af. G. 1/2
aerogenes.	6	A. 1/2 G. 1/3	Af. G. 1/8	–	Af. G. 1/4		A. 1/2 G. 1/3	–	Af. G. 5/16	Af. G. 1/2

		Mannite	Dulcite	Sodium formate	Milk	Broth	Motility	Indol	V. & P.
B. lactis aerogenes	2	Af. G. 11/12	–	–	Af.	Cloud	Non-motile	–	+
Strain 6.	6	Af. G. 4/5	–	–	A. Clot	–			
Normal *B. lactis*	2	Af. Gf.	–	–	,,	Cloud	Non-motile		+
aerogenes.	6	Af. G. 3/4	–	–	,,	–		–	

as the normal strains except in the case of glycerine. The new variety appeared to have lost a single ferment and that particular ferment was probably closely related chemically to the toxic agent used in the selection process.

The whole process was repeated and a strain (No. 13) obtained which gave the same result.

The process was again repeated with the results given in Table VII from which we see that strain "F" gave only slight fermentation of glycerine by the sixth day, and that strain "G" gave no acid or gas by the fourth day but on the sixth day showed acid without gas.

All these altered strains of *B. lactis aerogenes* reacted with Voges and Proskauer's test and were non-motile and did not liquefy gelatine.

TABLE VII.

Strain	Day of observation	Glucose	Mannite	Adonite	Glycerine
0·5 c.c. *B. lactis aerogenes* (large) A.	2	Af. G. 12/12	Af. G. 7/8	Af. G. 1/20	Af. G. 1/2
	4	A. 1/2 G. 7/12	Af. G. 9/10	Af. G. 1/10	Avs. G. 1/2
	6	A. 1/4 G. 7/12	Af. G. 4/5	Af. G. 1/9	Avs. G. 4/10
0·5 c.c. (large) B.	2	Af. G. 1/6	Af. G. 11/12	Af. G. 1/4	Af. G. 1/2
	4	A. 1/2 G. 1/2	Af. G. 7/8	Af. G. 3/10	Avs. G. 7/16
	6	A. 3/4 G. 5/12	Af. G. 4/5	Af. G. 1/4	Avs. G. 4/10
0·5 c.c. (large) C.	2	Af. G. 9/10	Af. G. 11/12	Af. G. 5/12	A. 3/4 G. 7/12
	4	Af. G. 9/16	Af. G. 9/10	Af. G. 5/12	Avs. G. 1/2
	6	A. 1/2 G. 1/2	Af. G. 5/6	Af. G. 2/5	Avs. G. 4/10
0·5 c.c. Lat. Exp. D.	2	Af. G. 12/12	Af. G. 12/12	Af. G. 1/6	Af. G. 5/12
	4	A. 3/4 G. 5/8	Af. G. 12/12	Af. G. 1/5	A. 1/2 G. 1/2
	6	A. 1/2 G. 5/8	A. 3/4 G. 11/12	Af. G. 1/6	Avs. G. 4/10
0·5 c.c. Lat. Exp. E.	2	Af. G. 7/8	Af. G. 12/12	Af. G. 1/5	A. 1/4 G. 7/12
	4	A. 1/2 G. 5/12	Af. G. 9/10	A. 1/4 G. 1/5	A. 1/4 G. 1/2
	6	A. 1/2 G. 7/12	Af. G. 5/6	Af. G. 1/5	Avs. G. 5/12
0·5 c.c. Lat. Exp. F.	2	Af. G. 3/4	Af. G. 11/12	Af. G. 5/12	–
	4	A. 3/4 G. 1/2	Af. G. 11/12	Af. G. 5/12	–
	6	A. 3/4 G. 1/2	Af. G. 7/8	Af. G. 4/10	A. 1/2 G. 1/16
1·5 c.c. Big col. G.	2	Af. G. 1/4	Af. G. 4/5	Af. G. 1/3	–
	4	Af. G. 3/8	Af. G. 11/12	Af. G. 4/10	–
	6	Af. G. 1/3	Af. G. 5/6	Af. G. 3/8	Af. Gn.
1·5 c.c. Big col. H.	2	Af. G. 1/2	Af. G. 4/5	Af. G. 4/10	A. 1/2 G. 7/16
	4	A. 3/4 G. 2/3	Af. G. 12/14	Af. G. 3/8	A. 1/4 G. 1/2
	6	A. 3/4 G. 1/2	Af. G. 7/8	Af. G. 1/2	A. 1/16 G. 1/2

Avs. = very slight reaction.

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After being grown on ordinary agar for two months, strains "13" and "G" were tested again on glycerine peptone water. "13" gave 1/8 of a tubeful of gas by the sixth day; the other strain gave no gas whatever after six days' growth, though it gave a late acid reaction.

I have tested on glycerine peptone water over 50 colonies of the strain of *B. lactis aerogenes* used, and have not yet met one which failed to give a good yield of gas in one day.

Theoretical considerations.

The loss of the glycerine-fermenting power of *B. lactis aerogenes* when grown in the presence of a toxic substitution product of glycerine suggests that the selection might be due to intoxication of the glycerine-fermenting bacteria by the agency of the glycerine ferment. It seems on the face of it more than a coincidence that chlorhydrin permits the selection of a non-glycerine-fermenting strain. The loss of the gas-forming power of *B. coli* when grown on monochloracetate agar was thought possibly to be due to intoxication, by the agency of the formic acid-producing enzyme, of those bacteria having a relative excess of this enzyme. This enzyme produces also acetic acid and probably therefore has a chemical affinity with acetic acid and hence with chloracetic acid. Harden has shown that the formic acid is the source of the gas, so that any toxic agent removing the formic acid-producing ferment would deprive the organism of the source of gas[1]. This explanation appears, however, doubtful, in face of the fact that chlorhydrin produces a very similar though not identical variety of *B. coli*. The intoxication and selection may be due to the CH_2Cl group common to each compound, and the acetate structure, as such, may have no specific effect.

The portion of the cell reacting with this group is obscure as there seems no reason to believe that the formic acid-producing ferment has a special affinity with it. Chloracetic acid has not been able in my experience to remove the glycerine-fermenting power of *B. lactis aerogenes*, so that we appear to have some more specific intoxication in the case of this organism with this substance.

[1] Harden and Walpole (1905) showed that the percentage yield of acetic acid in the decomposition of glucose by *B. lactis aerogenes* was only about one-third of that obtained when *B. coli* (Escherich) fermented this sugar. Now if the acetic acid producing enzyme were the agent of intoxication one would expect chloracetate to be much less effective in the case of *B. lactis aerogenes* than in that of *B. coli* (Escherich). This appears to be the case.

The possibility of ferment intoxication led me to try lactose phenylhydrazone as a selecting agent hoping therewith to obtain a non-lactose-fermenting variant. It was soon evident that the intoxication of the bacteria was conditioned by the NH group in this substance. The hydrogen was therefore replaced by a methyl group when the toxicity of the substance was found to be greatly reduced. This methyl derivative is being used at present but the work is not sufficiently advanced to permit of any statement of its effects as a selecting agent.

Ehrlich has held for some time that the nutriceptors play a part in cell intoxication. To prove this, one ought to be able to show that the surviving cells, after a selective intoxication with the given agent, are not able to use a definite food which the original cells used, or at least do not use it to the same extent. This view does not appear as yet to rest on any experimental basis[1].

SUMMARY.

1. *B. coli* (Escherich) produces papillated colonies and shows marked variability in the size of its colonies when it is grown on agar to which phenylacetic acid has been added in the form of the sodium salt.

The big and little colonies produce about the same amount of gas when tested on glucose.

2. When *B. coli* (Escherich) is grown on agar containing monochlorhydrin it throws off variants similar to those produced when grown on monochloracetate of soda media. Speaking broadly they ferment alcohols with gas formation, and sugars without gas formation.

3. *B. lactis aerogenes* grown on monochlorhydrin agar gives rise to variants unable to ferment glycerine.

4. In cases of inhibitory bacterial selection by chemical agents a careful comparison of the surviving cells with the original strain from which they were derived is calculated to indicate that portion or function of the cell which is implicated in the cell's intoxication. This question does not seem to have been attacked hitherto from this standpoint.

5. The cell ferments by virtue of their specific chemical affinities may play a part in cell intoxication.

[1] It appears probable that the cell enzymes frequently play a secondary part in cell intoxication. We know for example that carbolic acid is rendered much more germicidal by the addition of acids. Now in many media the cell enzymes will produce acids, hence it is probable that carbolic acid selections of bacteria commonly result in the development of new strains with impaired fermenting power.

I desire to express my indebtedness to the authorities of the Lister Institute and to the "Constance Research Fund" administered by Dr E. C. Hort for all the experimental facilities which made the work of this paper possible.

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Note: For description of Plate II, see Table III, p. 40.

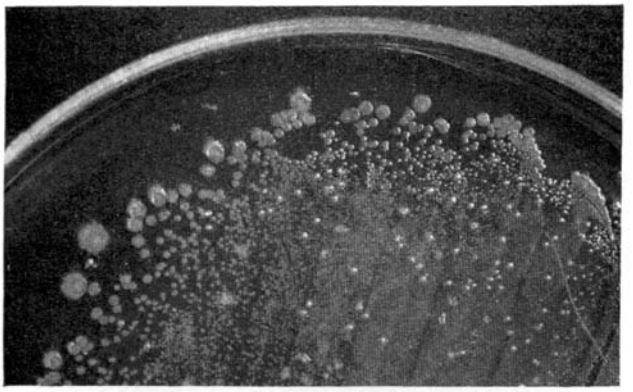

Fig. 1. For description see Table III.

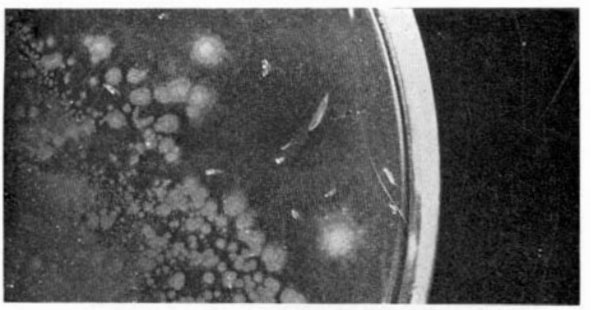

Fig. 2. For description see Table III.

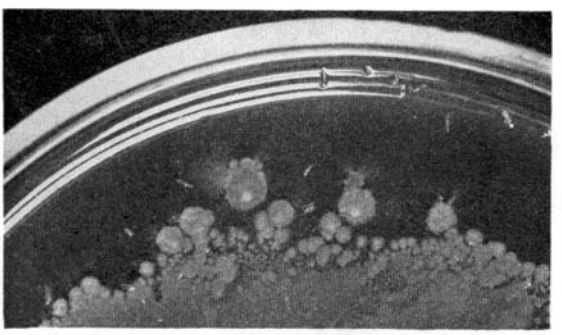

Fig. 3. For description see Table III.

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