XXX. THE EFFECT OF ACIDS ON THE GROWTH OF *BACILLUS COLI.*

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The original proposition advanced by Michaelis and Marcora [1912] stated that the final acid reaction of *B. coli* constituted a "physiological constant" of the organism, since cultures in lactose bouillon ceased activity at a hydrogen ion concentration [H·] of $1 \times 10^{-5} N$. The results of their experiments were soon challenged by Brünn [1913] who showed that an acid reaction of $P_H = 5{\cdot}0$ permitted, but that $P_H = 4{\cdot}7$ inhibited growth of *B. coli*.

More recently Clark and Lubs [1915, 1, 2 and 3] have examined the conditions affecting the growth of *B. coli* of the low-gas-rated group, and they find a certain connection between the type of colon bacillus used, and the final acid reaction it produces.

Clark [1915] determined the final [H·] attained by cultures of *B. coli* and although he agrees with the hypothesis of Michaelis and Marcora, he presents the following additional facts:

(*a*) In a medium containing 1 per cent. Witte peptone and 1 per cent. dextrose the final acid reactions attained are:

'Human' Coli $P_H = 4{\cdot}82$
'Bovine' ,, $P_H = 4{\cdot}7$–$4{\cdot}8$
'Grain' ,, $P_H = 5{\cdot}01$.

He suggests that these reactions may be 'specific' for the respective varieties of *B. coli*, and that they may be of value for diagnostic purposes.

(*b*) A higher degree of acidity is produced in dextrose than in lactose media; *e.g.*, the same strain of *B. coli* produced in 1 per cent. Witte peptone + 1 per cent. dextrose a final reaction of $P_H = 4{\cdot}26$ while in 1 per cent. Witte peptone + 1 per cent. lactose only $P_H = 4{\cdot}56$ was attained.

(*c*) In the more highly buffered media lower $H^{\cdot}$ concentrations are produced.

In one series of his experiments a very significant result is obtained, viz. that the addition of various amounts of sterile lactic acid to the Witte peptone-dextrose medium before inoculation causes considerable variations of the final acid reaction attained by the cultures of *B. coli* in the acidified media.

These facts seem to cast considerable doubt upon the validity of the 'physiological constant' hypothesis, and the recent researches of Wolf and Harris [1917] on the behaviour of *B. sporogenes* and *B. perfringens* in acid media indicate the desirability of further investigation of the final $[H^{\cdot}]$ reached by cultures of *B. coli*.

Again, Cole and Onslow [1915] claim to be able to differentiate the organisms of the *B. typhosus* group by the velocity with which they attain a certain degree of acidity. Wolf and Harris [1917] state, however, that in the case of *B. sporogenes* and *B. perfringens* this velocity is affected by the initial $[H^{\cdot}]$ of the medium.

It was thought desirable, therefore, to undertake further examination of the behaviour of *B. coli* in different acid media. Several important questions at once suggest themselves, *e.g.*

(*a*) Do various strains of *B. coli* behave similarly when subjected to similar conditions?

(*b*) Does the nature and degree of acidity of the original medium affect the final reaction of the culture of *B. coli* grown in it?

(*c*) What is the mechanism of the action of 'Grain' Coli?

The present paper embodies results obtained in working on questions (*a*) and (*b*), and it is hoped to present additional results, together with a consideration of (*c*) in a later paper.

The main points now investigated are:

(i) Do different strains of the same variety of *B. coli* (*e.g.* human or bovine) react in the same way when exposed to similar conditions?

(ii) Are there any differences between the behaviour of 'human' and 'bovine' coli, such as may be made use of for diagnostic purposes?

(iii) Is the same strain of *B. coli* differently affected by different media?

I. Material and experimental methods.

Material.

The strains of *B. coli* used in these experiments were prepared as follows. Smears of human and bovine faeces were inoculated into a medium containing 2 per cent. glucose, 2 per cent. peptone water; and at the end of 24 hours MacConkey broth was inoculated from the growths so obtained. At the end of a further 24 hours the culture produced in the MacConkey broth was used for the inoculation of fresh MacConkey broth and from this, at the end of a further 24 hours, inoculation of agar slopes was effected. The strains thus produced were subjected to microscopic examination, single colonies were transferred to new agar slopes and from the daughter colonies developed thereon the pure strains used in the experiments were obtained. In addition to a large number of strains of human and bovine *B. coli* thus prepared a pure strain of *B. coli*, referred to in the paper as *B. coli oxf.* was obtained from Dr A. D. Gardner of the Oxford University Pathological (Standards) Laboratory, and this was used in the preliminary experiment for comparison of the different strains of human coli.

The strains of *B. coli* specially prepared for the experiments were lettered H_1, H_2, H_3 etc. (human) and B_1, B_2, B_3 etc. (bovine).

Media.

Two media were used:

(*a*) 2 per cent. glucose + 2 per cent. peptone water, prepared by the tryptic digestion of caseinogen (Lait Proto A) as described by Cole and Onslow, from which the tubes of acidified media were prepared by a method similar to that of Wolf and Harris.

(*b*) Glucose-phthalate mixture.

This was prepared as follows:

8·8 g. of anhydrous disodium monohydrogen phosphate
2·0 g. of potassium acid phthalate (recrystallised)
1·0 g. of aspartic acid
4·0 g. glucose

were dissolved in distilled water, the volume of the solution being made up to 800 cc.

This solution was then brought to the required concentration by taking, for the preparation of each 100 cc. of acid medium, 50 cc. of phthalate solution; x cc. of $N/5$ acid and $(50 - x)$ cc. of distilled water.

This glucose-phthalate medium was selected for use in these experiments because it had been found by Clark and Lubs to be a medium in which

B. coli can be grown with considerable facility, and it was also known to have a considerable 'buffer' effect: this being more marked than is the case with the glucose-peptone medium. *It was found that the gas production due to the activity of* B. coli *was much less in glucose phthalate than in glucose peptone.*

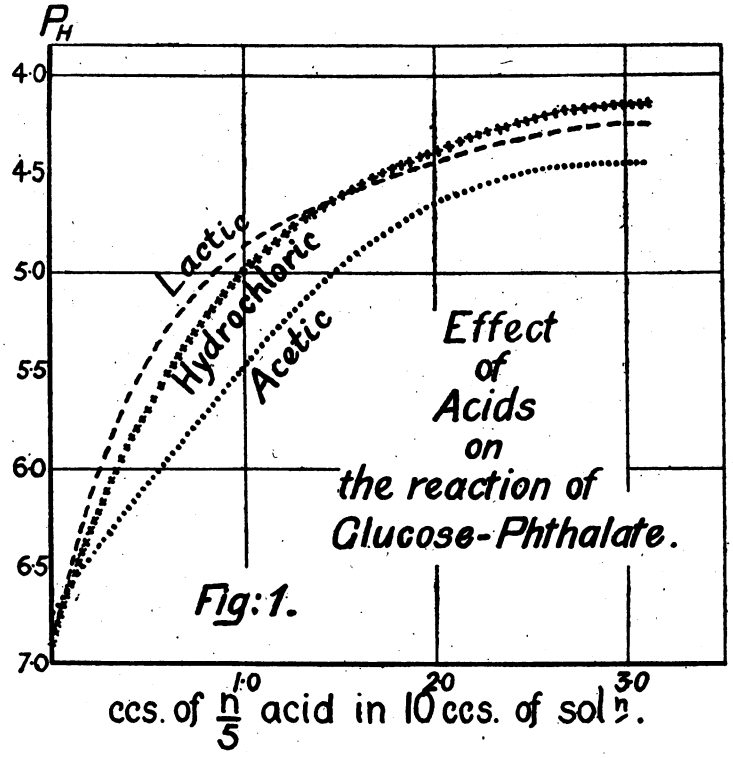
Experimental Methods.

For each series of experiments A, B, C, D, etc., a number of sets of tubes, 1, 2, 3, 4, etc., were prepared, each tube containing 10 cc. of a solution containing medium, acid, and water. The set of tubes containing the least amount of acid was numbered 1, the next more acid set 2,—the most acid set of tubes in each series receiving the highest number. In general, each set consisted of eight tubes of the same mixture, and each series comprised eight or ten sets of tubes. Into each tube of medium a sterilised Durham tube, for the collection of evolved gas, was introduced. The tubed media were sterilised at 100° for one hour per diem on each of three successive days. One tube of each set was used for the determination of the initial reaction of the sterilised medium. The reaction was determined at about 20°, and corrected to 20° when necessary. The H-electrode was used, and the P_H of the tubed medium was calculated from the E.M.F. so determined. The values of P_H were used in constructing the titration curves for the known media and acids employed.

The amounts of acid added to the media were so regulated as to produce a number of mixtures having P_H ranging from 4·0 to 7·0.

For each acid and medium a titration curve was constructed, in which the number of cc. of $N/5$ acid present in 10 cc. of the solution was plotted against the absolute acid reaction (P_H) produced. These curves showed that in order to produce a given acid reaction (P_H) in a medium, it was necessary to add to a given volume of it, a smaller mass of a highly dissociated acid (*e.g.* hydrochloric acid) than of a less dissociated acid (*e.g.* acetic acid).

The addition of the less dissociated acids soon produces an acid reaction (P_H) at which subsequent considerable additions of acid result in little change of reaction. In the more highly buffered media this point is more quickly attained. There does not appear to be any marked 'specificity' of action due to the *anion* groups of the various acids upon the media. Titration curves showing the effect of different acids upon 2 per cent. glucose peptone have been given by Wolf and Harris and as similar curves were obtained in the present experiments it was thought unnecessary to reproduce them. As the curve for glucose phthalate is not known it is shown in Fig. 1.



RESULTS OF INOCULATION EXPERIMENTS.

In the data given below strains H_1, H_2, etc. are *B. coli* obtained from human faeces; B_1, B_2, etc. prepared from bovine faeces, and "final P_H" is the lowest value recorded during a period (unless otherwise stated) of 168 hours.

+ indicates that fermentation occurred in the tube so marked; − means failure to ferment.

TABLE I. *Medium, 2 per cent. glucose peptone. Acid, hydrochloric.*

Series Z_1 Inoculated with H_4 18 hrs. old				*Series Z_2* Inoculated with B_4 18 hrs. old			
Tube	Result	Initial P_H reaction	Final P_H reaction	Tube	Result	Initial P_H reaction	Final P_H reaction
1	+	6·66	4·65	1	+	6·66	4·64
2	+	6·23	4·54	2	+	6·23	4·50
3	+	5·73	4·45	3	+	5·73	4·46
4	+	5·42	4·37	4	+	5·42	4·43
5	+	4·95	4·33	5	+	4·95	4·42
6	+	4·79	4·30	6	+	4·79	4·35
7	+	4·64	4·29	7	+	4·64	4·30
8	+	4·45	4·29	8	+	4·45	4·31

(In both of these series all the tubes fermented, the most acid reactions obtained being: Series Z_1, Tubes 7 and 8 P_H=4·29, and Series Z_2, Tube 7 P_H=4·30.)

Series Y_1 — Inoculated with H_1 18 hrs. old

Tube	Initial P_H reaction	Result	Final P_H reaction
1	5·89	+	4·50
2	5·24	+	4·40
3	4·91	+	4·38
4	4·62	+	4·30
5	4·02	–	—
6	3·96	–	—
7	3·53	–	—

Series Y_2 — Inoculated with B_2 18 hrs. old

Result	Final P_H reaction
+	4·53
+	4·41
+	4·39
+	4·30
–	—
–	—
–	—

The tubes of Series Y_1 and Y_2 were examined 72 hours after inoculation.

The most acid tubes to ferment were those numbered 4 in each series, both of which had an initial reaction of $P_H = 4·62$ and reached a final reaction of $P_H = 4·30$. The next tube, No. 5 in each series, with initial reaction of $P_H = 4·02$ failed to ferment.

Series X_1 — Inoculated with H_1 16 hrs. old

Tube	Initial P_H reaction	Result	Final P_H reaction
1	5·87	+	4·47
2	5·27	+	4·35
3	4·65	+	4·30
4	4·34	–	—
5	4·16	–	—
6	4·03	–	—
7	3·77	–	—
8	3·70	–	—

Series X_2 — Inoculated with B_2 16 hrs. old

Result	Final P_H reaction
+	4·50
+	4·34
+	4·295
–	—
–	—
–	—
–	—
–	—

The tubes were examined 90 hours after inoculation.

In series X_1 the most acid tube to ferment was No. 3 with reaction $P_H = 4·65$, the acidity of which rose to $P_H = 4·30$. In series X_2 a similar result was obtained and a final value $P_H = 4·295$ was reached. It is noteworthy that in both series Tube No. 4 with an initial $P_H = 4·34$ failed to ferment.

Series O

Inoculated with *B. coli oxf.* 16 hrs. old

Tube	Initial P_H reaction	Result	Final P_H reaction
1	6·63	+	4·79
2	6·06	+	4·70
3	5·80	+	4·66
4	5·18	+	4·54
5	4·93	+	4·50
6	4·62	+	4·30
7	4·34	–	—
8	4·21	–	—

The most acid tube to ferment was No. 6 with initial reaction of $P_H = 4{\cdot}62$ and final reaction of $P_H = 4{\cdot}30$. It must be noted, however, that the next tube, No. 7, which failed to ferment had an initial reaction of $P_H = 4{\cdot}34$ which is less acid than the final value reached by the fermented No. 6.

The existence of this phenomenon in the case of *B. sporogenes* and *B. perfringens* has already been recorded by Wolf and Harris.

	Series S_1			*Series* S_2	
	Inoculated with H_8 18 hrs. old			Inoculated with B_8 18 hrs. old	
Tube	Initial P_H reaction	Result	Final P_H reaction	Result	Final P_H reaction
1	7·14	+	4·53	+	4·53
2	5·17	+	4·39	+	4·45
3	4·52	+	4·30	+	4·34
4	4·29	+	4·26?	–	—
5	4·00	–	—	–	—
6	3·695	–	—	–	—
7	3·525	–	—	–	—
8	3·37	–	—	–	—

In Series S_1 the most acid tube certainly to ferment was No. 3, with initial $P_H = 4{\cdot}52$ which rose to $P_H = 4{\cdot}30$. Tube No. 4 with initial acid reaction $P_H = 4{\cdot}29$ rose to $P_H = 4{\cdot}26$ although the usual signs of fermentation were absent. Tube No. 5 with $P_H = 4{\cdot}00$ failed to ferment.

In Series S_2 the most acid tube to ferment was No. 3 with an initial acid reaction $P_H = 4{\cdot}52$ which rose to $P_H = 4{\cdot}34$. Tube No. 4 with $P_H = 4{\cdot}29$ failed to ferment.

TABLE II. *Medium, 2 per cent. glucose peptone. Acid, lactic.*

	Series W_1			*Series* W_2	
	Inoculated with H_3 16 hrs. old			Inoculated with B_4 16 hrs. old	
Tube	Initial P_H reaction	Result	Final P_H reaction	Result	Final P_H reaction
1	6·46	+	4·60	+	4·61
2	5·46	+	4·48	+	4·47
3	4·53	+	4·47	+	4·46
4	4·39	–	—	–	—
5	4·15	–	—	–	—
6	3·98	–	—	–	—
7	3·83	–	—	–	—
8	3·73	–	—	–	—

In Series W_1 the most acid tube to ferment was No. 3 with acid reaction $P_H = 4{\cdot}53$ which rose to $P_H = 4{\cdot}47$. The next tube, No. 4, with $P_H = 4{\cdot}39$ failed to ferment.

In Series W_2 the most acid tube to ferment was No. 3, with acid reaction $P_H = 4{\cdot}53$, which rose to $P_H = 4{\cdot}46$. The next tube, No. 4, with $P_H = 4{\cdot}39$ failed to ferment.

	Series T_1			*Series* T_2	
	Inoculated with H_2 18 hrs. old			Inoculated with B_2 18 hrs. old	
Tube	Initial P_H reaction	Result	Final P_H reaction	Result	Final P_H reaction
1	6·90	+	4·69	+	4·70
2	4·88	+	4·61	+	4·60
3	4·62	+	4·57	+	4·56
4	4·44	–	—	–	—
5	4·275	–	—	–	—
6	4·08	–	—	–	—
7	3·93	–	—	–	—
8	3·70	–	—	–	—

In Series T_1 the most acid tube to ferment was No. 3, with acid reaction $P_H = 4{\cdot}62$ which rose to $P_H = 4{\cdot}57$. The next tube, No. 4, with $P_H = 4{\cdot}44$ failed to ferment.

In Series T_2 the most acid tube to ferment was No. 3, with acid reaction $P_H = 4{\cdot}62$ which rose to $P_H = 4{\cdot}56$. The next tube, No. 4, with $P_H = 4{\cdot}44$ failed to ferment.

TABLE III. *Medium, 2 per cent. glucose peptone. Acid, acetic.*

	Series V_1			*Series* V_2	
	Inoculated with H_2 16 hrs. old			Inoculated with B_2 16 hrs. old	
Tube	Initial P_H reaction	Result	Final P_H reaction	Result	Final P_H reaction
1	7·14	+	4·52	+	4·54
2	5·39	+	4·60	+	4·61
3	4·83	+	4·77	+	4·79
4	4·60	–	—	–	—
5	4·46	–	—	–	—
6	4·41	–	—	–	—
7	4·35	–	—	–	—
8	4·28	–	—	–	—

In Series V_1 the most acid tube to ferment was No. 3, with acid reaction $P_H = 4{\cdot}83$ which rose to $P_H = 4{\cdot}77$. The next tube, No. 4, with $P_H = 4{\cdot}60$ failed to ferment except in one doubtful case in which the P_H rose to 4·58.

In Series V_2 the most acid tube to ferment was No. 3, with acid reaction $P_H = 4{\cdot}83$ which rose to $P_H = 4{\cdot}79$. The next tube, No. 4, with $P_H = 4{\cdot}60$ failed to ferment.

TABLE IV. *Medium, glucose phthalate. Acid, hydrochloric.*

	Series A_1			*Series* A_2	
	Inoculated with H_4 18 hrs. old			Inoculated with B_5 18 hrs. old	
Tube	Initial P_H reaction	Result	Final P_H reaction	Result	Final P_H reaction
1	6·90	+	5·73	+	5·76
2	6·71	+	5·35	+	5·38
3	6·45	+	5·06	+	5·06
4	6·25	+	4·93	+	4·94
5	5·87	+	4·83	+	4·83
6	5·66	+	4·77	+	4·76
7	5·10	+	4·72	+	4·71
8	4·40	−	—	−	—

In Series A_1 the most acid tube to ferment was No. 7 with acid reaction $P_H = 5{\cdot}10$ which rose to $P_H = 4{\cdot}72$. The next tube, No. 8, with $P_H = 4{\cdot}40$ failed to ferment.

In Series A_2 the most acid tube to ferment was No. 7 with acid reaction $P_H = 5{\cdot}10$ which rose to $P_H = 4{\cdot}71$. The next tube, No. 8, with $P_H = 4{\cdot}40$ failed to ferment.

Series N

	Inoculated with *B. coli cxf.* 16 hrs. old		
Tube	Initial P_H reaction	Result	Final P_H reaction
1	6·69	+	5·21
2	6·56	+	5·20
3	6·44	+	5·18
4	6·15	+	5·12
5	5·89	+	5·05
6	5·70	+	4·94
7	5·37	+	4·91
8	4·85	+	4·78
9	4·31	−	—
10	3·70	−	—
11	3·31	−	—

The most acid tube to ferment was No. 8, with acid reaction $P_H = 4{\cdot}85$ which rose to $P_H = 4{\cdot}78$. The next tube, No. 9, with $P_H = 4{\cdot}31$ failed to ferment.

	Series C_1			*Series* C_2	
	Inoculated with H_4 18 hrs. old			Inoculated with B_4 18 hrs. old	
Tube	Initial P_H reaction	Result	Final P_H reaction	Result	Final P_H reaction
1	7·11	+	5·60	+	5·43
2	6·80	+	5·11?	+	4·99?
3	6·69	+	4·99?	+	4·96?
4	6·52	+	5·05	+	5·04
5	6·27	+	5·01	+	5·01
6	6·13	+	4·93	+	4·96
7	5·95	+	4·94	+	4·88
8	5·77	+	4·89	+	4·91
9	5·01	+	4·75	+	4·76
10	4·96	+	4·68	+	4·75
11	4·41	–	—	–	—

In Series C_1 the most acid tube to ferment was No. 10, with acid reaction $P_H = 4·96$ which rose to 4·68. The next tube, No. 11, with $P_H = 4·41$ failed to ferment.

In Series C_2 the most acid tube to ferment was No. 10, with initial reaction $P_H = 4·96$ which rose to 4·75. The next tube, No. 11, with $P_H = 4·41$ failed to ferment.

TABLE V. *Medium, glucose phthalate. Acid, lactic.*

	Series K_1			*Series* K_2	
	Inoculated with H_7 16 hrs. old			Inoculated with B_7 16 hrs. old	
Tube	Initial P_H reaction	Result	Final P_H reaction	Result	Final P_H reaction
1	6·90	+	5·75	+	5·75
2	6·00	+	5·02	+	5·05
3	5·55	+	4·94	+	4·95
4	5·20	+	4·82	+	4·85
5	5·10	+	4·78	+	4·76
6	4·99	+	4·78	+	4·79
7	4·85	+	4·76	+	4·75
8	4·71	–	—	–	—
9	4·55	–	—	–	—
10	4·44	–	—	–	—
11	4·26	–	—	–	—

In Series K_1 the most acid tube to ferment was No. 7, with an acid reaction of $P_H = 4·85$ which rose to $P_H = 4·76$. The next tube, No. 8, with $P_H = 4·71$ did not ferment.

In Series K_2 the most acid tube to ferment was No. 7, with an acid reaction of $P_H = 4·85$ which rose to $P_H = 4·75$. The next tube, No. 8, with $P_H = 4·71$ failed to ferment.

TABLE VI. *Medium, glucose phthalate. Acid, acetic.*

	Series D_1			*Series D_2*	
	Inoculated with H_8 16 hrs. old			Inoculated with B_8 16 hrs. old	
Tube	Initial P_H reaction	Result	Final P_H reaction	Result	Final P_H reaction
1	6·85	+	5·13	+	
2	6·06	+	5·16	+	5·18
3	5·56	+	5·20	+	5·25
4	4·98	+	4·93	+	4·90
5	4·78	–	—	–	—
6	4·60	–	—	–	—
7	4·50	–	—	–	—

In Series D_1 the most acid tube to ferment was No. 4, with initial acid reaction $P_H = 4·98$ which rose to 4·93. In one tube of No. 5 (initial $P_H = 4·78$) the acid reaction rose to $P_H = 4·74$ but other signs of fermentation were absent. The next tube, No. 6, with $P_H = 4·60$ failed to ferment.

In Series D_2 the most acid tube to ferment was No. 4, with acid reaction $P_H = 4·98$ which rose to $P_H = 4·90$. The next tube, No. 5, with $P_H = 4·78$ failed to ferment.

TABLE VII. *Values of final acid reaction* (P_H) *of different strains of* B. coli *in different media and in the presence of different acids.*

		"Human" Coli			"Bovine" Coli		
Medium	Acid	Series	Strain	Final P_H	Series	Strain	Final P_H
2 % glucose peptone	Hydrochloric	O	Oxf.	4·30	—	—	—
		Z_1	H_4	4·29	Z_2	B_4	4·30
		Y_1	H_1	4·30	Y_2	B_2	4·30
		X_1	H_1	4·30	X_2	B_2	4·29
		S_1	H_8	4·30 (4·26?)	S_2	B_8	4·34
		Average value P_H = 4·30			Average value P_H = 4·31		
2 % glucose peptone	Lactic ...	W_1	H_3	4·47	W_2	B_4	4·46
		T_1	H_8	4·57	T_2	B_8	4·56
		Average value P_H = 4·52			Average value P_H = 4·51		
2 % glucose peptone	Acetic ...	V_1	H_8	4·77	V_2	B_8	4·79
Glucose phthalate	Hydrochloric	A_1	H_4	4·72	A_2	B_5	4·71
		A_2	H_4	4·72	—	—	—
		N	Oxf.	4·78	—	—	—
		C_1	H_4	4·68	C_2	B_4	4·75
		Average value P_H = 4·71			Average value P_H = 4·73		
Glucose phthalate	Lactic ...	K_1	H_7	4·76	K_2	B_7	4·75
Glucose phthalate	Acetic ...	D_1	H_8	4·88 (4·74?)	D_2	B_8	4·90 (4·82?)

Reaction resultants.

From the values shown in Tables I to VI curves giving the reaction resultants of the various strains of *B. coli* in 2 per cent. glucose peptone, and the glucose phthalate mixture were drawn by the method of Wolf and Harris [1917]. These show the behaviour of the organisms in these media in the presence of hydrochloric, lactic, and acetic acids, the initial acid reactions (P_H) being plotted against those finally produced (Figs. 2 ff.).

CONSIDERATION OF EXPERIMENTAL DATA.

It is now possible to suggest some conclusions relative to the questions originally proposed for investigation.

1. A comparison of the values of the final acid reactions shown in Tables I to VI indicates no specificity of reaction of the different strains of *B. coli*. This may be illustrated by the following examples:

(*a*) H_4 caused the acid reaction of glucose peptone and hydrochloric acid to rise from $P_H = 4{\cdot}64$ to 4·29, while H_1 caused a rise of P_H from 4·64 to 4·30, and *Oxf.* produced a rise of P_H from 4·62 to 4·30.

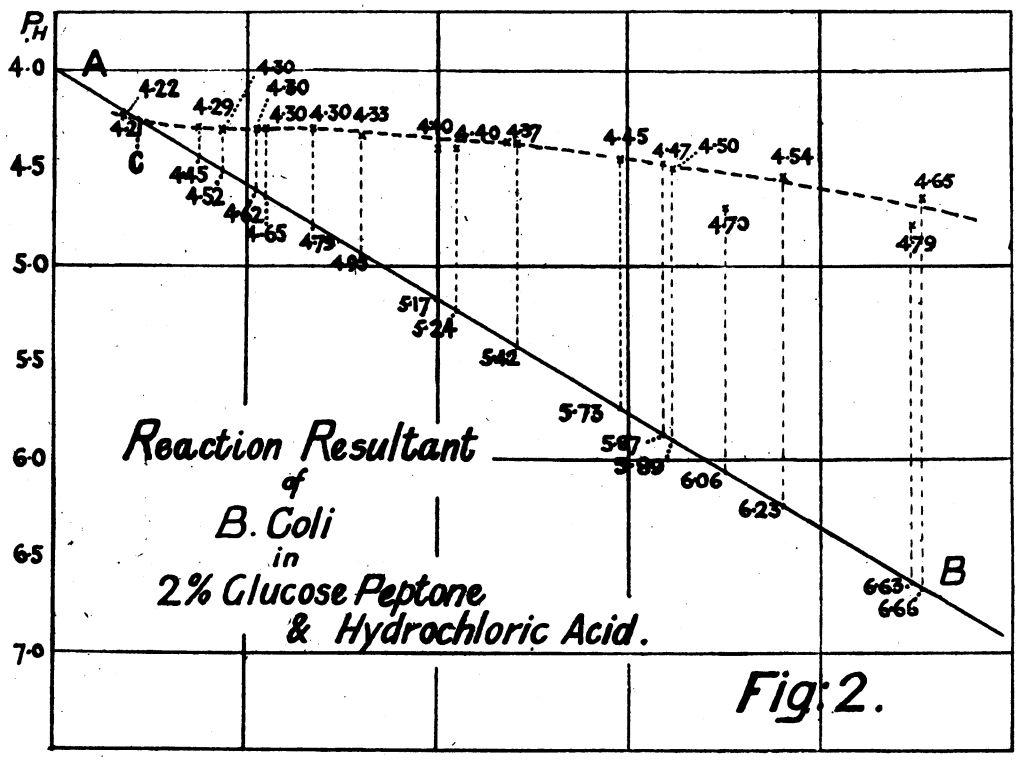
When glucose phthalate was the medium used, the results support the same conclusion, for example, *Oxf.* caused the reaction of the medium to rise from $P_H = 5{\cdot}95$ to 4·94, while H_4 caused a rise from $P_H = 5{\cdot}87$ to 4·83.

It may be concluded, therefore, that the different strains of the same variety of *B. coli* react in the same way when exposed to similar conditions.

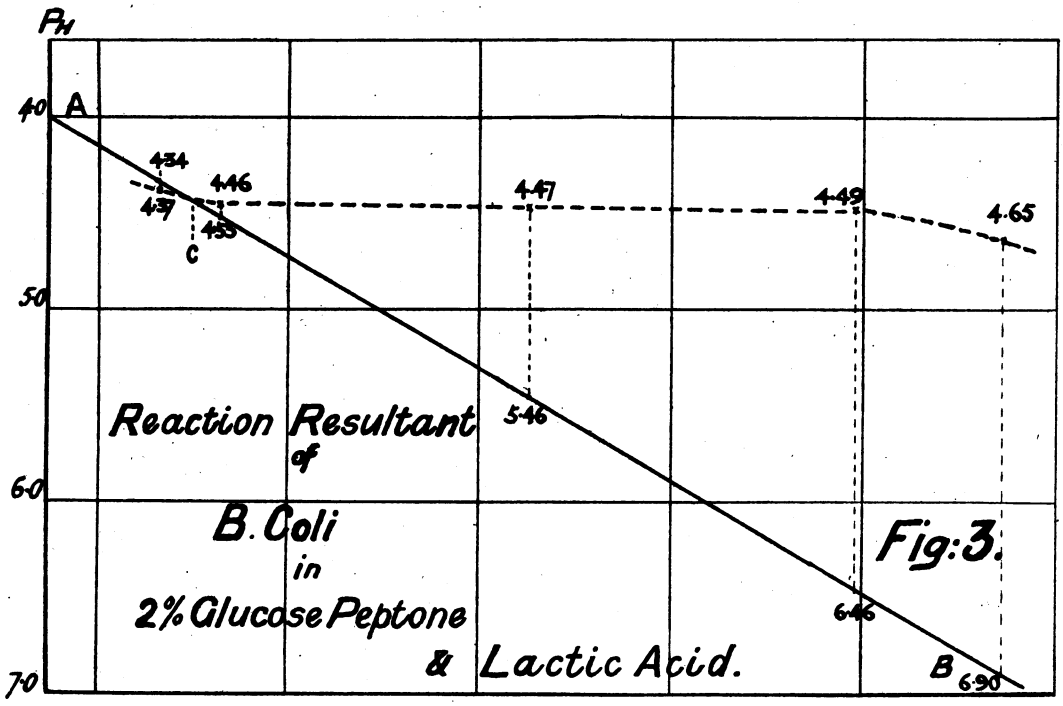
2. In no single case during the whole series of experiments, did any appreciable difference develop between the behaviour of "human" and "bovine" strains of *B. coli* (cf. Table VII).

It may be concluded therefore that the existence of differences between the behaviour of "human" and "bovine" coli such as may be of diagnostic value has not been demonstrated. In many of the experiments the two types of *B. coli* examined although fermenting under identical conditions behave identically at every stage of the experiment.

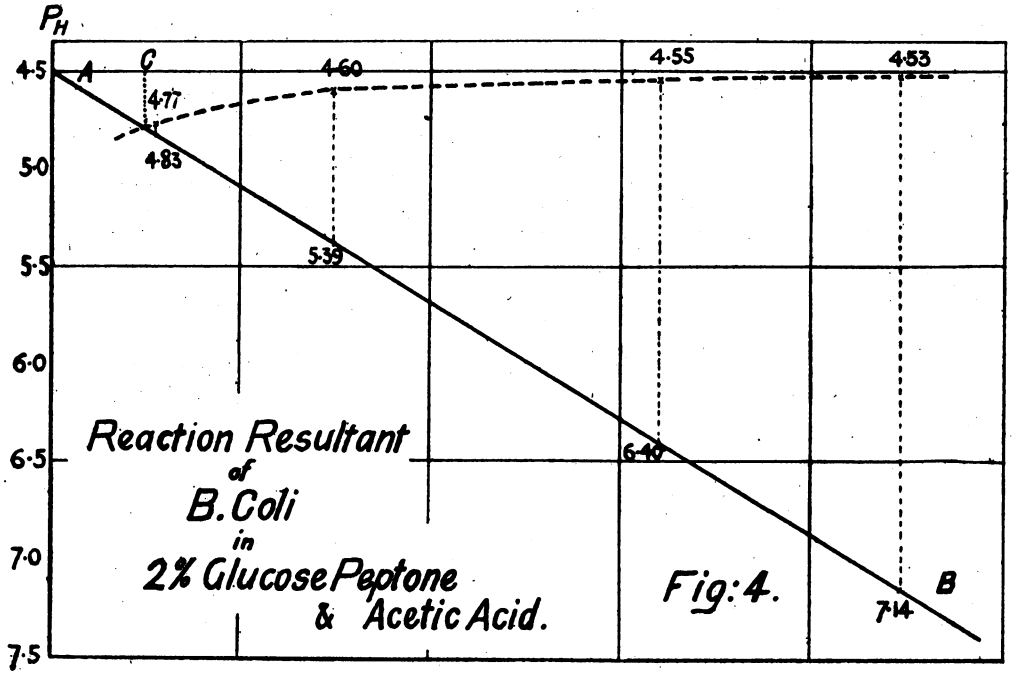
3. The experimental data lead to the conclusion that the same strain of *B. coli* is differently affected when allowed to ferment in different media. This is true both as to the final acid reaction attained in the presence of a given acid, and as to the rapidity with which this final reaction is reached. In Table I, Series Z_1, and Table IV, Series A_1, may be seen the results obtained by the fermentation of strain H_4 in glucose peptone and hydrochloric acid, and glucose phthalate and hydrochloric acid, respectively. When media to



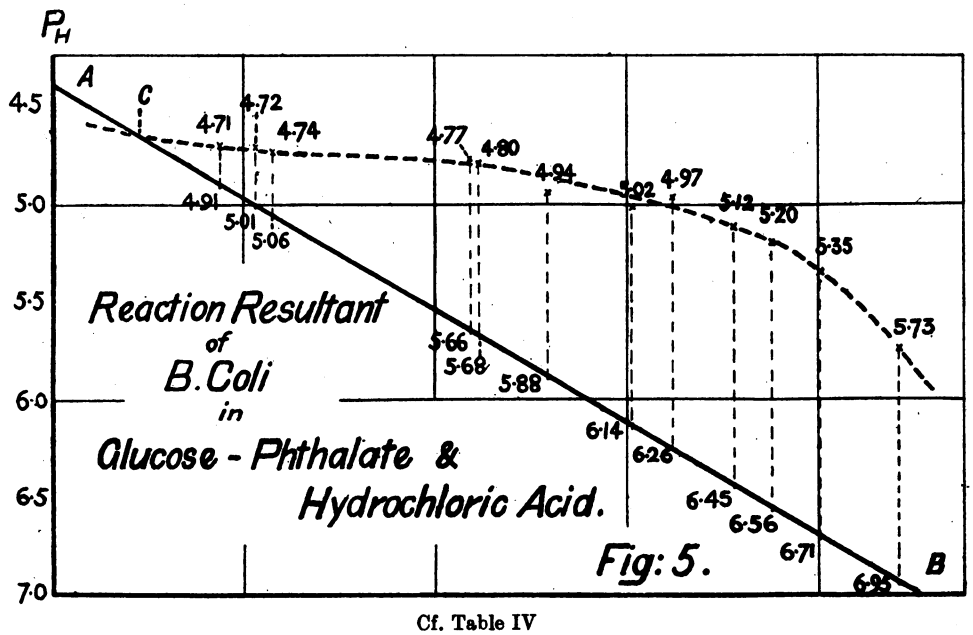
Cf. Table I.



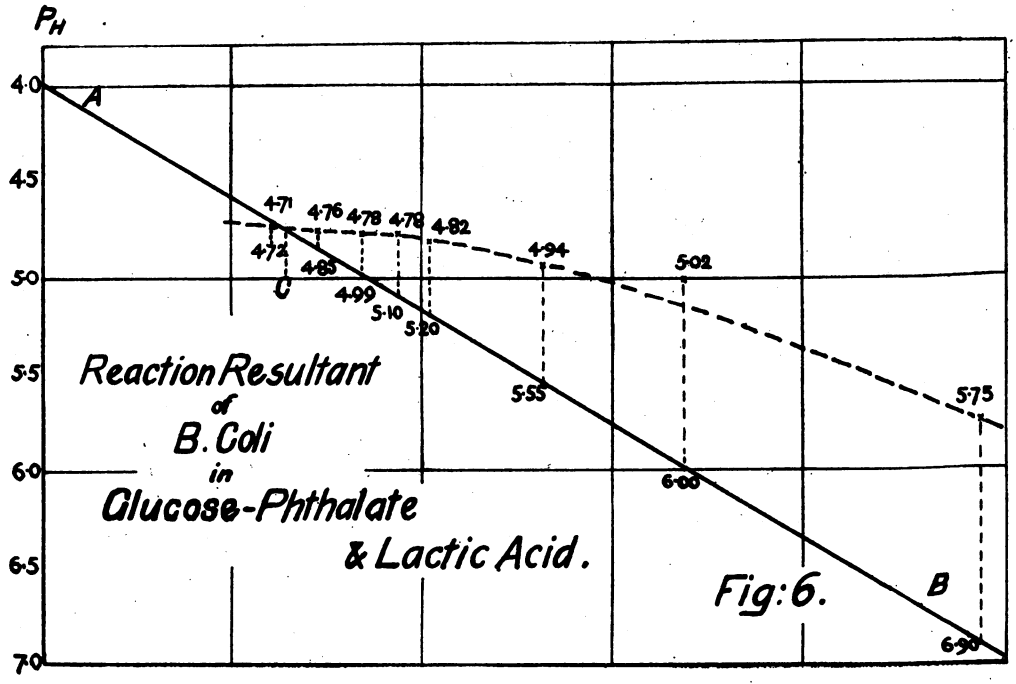
Cf. Table II.



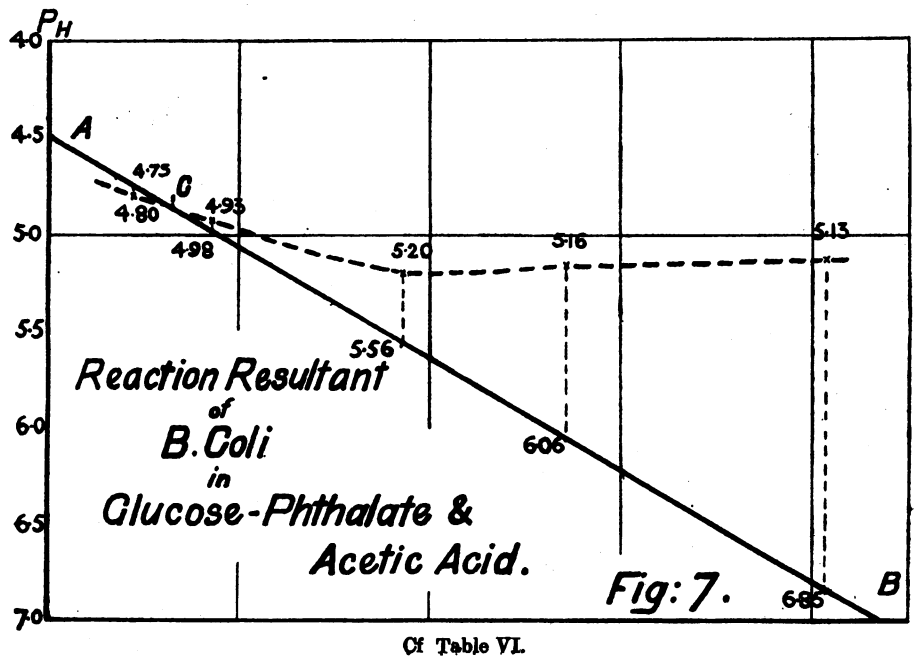
Cf. Table III.



Cf. Table IV



Cf. Table V.



Cf Table VI.

which no acid has been added are considered, it is evident that any given strain of *B. coli* causes a higher final acid reaction in peptone than in phthalate of similar initial reaction: *e.g.* H_4 fermenting in peptone to which no acid has been added, causes a rise of P_H from 6·66 to 4·65, while it produces in phthalate of initial $P_H = 6·91$ a final reaction of $P_H = 5·73$.

All the available evidence therefore leads to the supposition that *B. coli* ferments more strongly in glucose peptone than in glucose phthalate, whether the media be devoid of acid, or whether equivalent amounts of the same acid have been added to each.

4. An examination of Tables I to VI and of the curves in Figs. 2 to 7 (which represent the reaction resultants in the different media, and in the presence of various acids), proves conclusively that the final acid reaction produced by the fermentation of *B. coli* is by no means a "physiological constant." It cannot be doubted that the final reaction reached in any given experiment is dependent upon the initial reaction of the medium in which the organism is allowed to ferment. In Figs. 2 to 7 the forms of the curves obtained are evidently dependent upon the nature of the acid added to the medium, and also to a less extent upon the nature of the medium itself. The exact value of the acid reaction at which fermentation is completely inhibited is more conveniently discussed in connection with the next question.

5. It is evident that for any given medium there is a certain definite acid reaction at which the growth of *B. coli* is completely inhibited. It is possible to define this "critical point" within very narrow limits. In the figures of the reaction curves this point is represented by C, the point at which the curve representing the final reactions obtained in the medium cuts the axis along which the initial concentrations are plotted. The critical value may also be approximately taken in any given case as the mean of the values obtained for the final acid reactions and the lowest acid reaction of the medium which completely inhibits fermentation. These values are shown in the following table:

TABLE VIII.

Medium	Acid	Most acid reaction permitting growth	Least acid reaction inhibiting growth	Mean critical point (Calc.)	Critical point (Curve)
Glucose peptone	Hydrochloric	4·30	4·25	4·27	4·25
	Lactic	4·52	4·42	4·47	4·43
	Acetic	4·77	4·60	4·68	4·78
Glucose phthalate	Hydrochloric	4·71	4·38	4·55	4·67
	Lactic	4·76	4·71	4·73	4·74
	Acetic	4·88	4·78	4·83	4·85

It will be seen that columns 5 and 6 of the above Table define, within very narrow limits, the critical point for *B. coli* fermenting in the given media and acids.

Although all strains of a particular variety, and both the "human" and "bovine" varieties of *B. coli* appear to react similarly in the presence of a given acid and medium it is evident that, quite apart from the influence upon the acid reaction exerted by the particular medium, there is a small but definite result due to the specificity of action of the added acid. The titration curves of the different acids in any given medium reveal, as has already been noted, but little specificity of action; the final reactions obtained appear to be a measure of the true acidity produced by the acid. When the reaction resultants are examined a much greater specific action of the acid is visible.

The results obtained in the foregoing experiments suggest that in any given medium "a strong" or highly dissociated acid (*e.g.* hydrochloric or lactic) permits a higher final acid reaction before complete inhibition of fermentation takes place. When however the mass of acid introduced is considered, it is found that the strong acids are more strongly inhibitory than is the "weaker" or less dissociated acetic acid, *e.g.* in glucose peptone the addition of an amount of hydrochloric acid sufficient to produce an acidity equivalent to 0·0180 *N* completely inhibits growth, the final acid reaction reached being $P_H = 4{\cdot}30$ while the acid constitutes 0·0657 per cent. of the mass of the solution. With lactic acid growth ceases at $P_H = 4{\cdot}47$ when the acidity is 0·0180 *N* and the acid constitutes 0·1637 per cent. of the mass of the solution. With acetic acid although the final acid reaction at which fermentation is inhibited is only $P_H = 4{\cdot}77$, the acid added to the medium is equivalent to an acidity of 0·0240 *N* and is 0·1440 per cent. of the mass of the solution. It is obvious that the mixture of medium and hydrochloric acid contains the least, that of medium and acetic acid the greatest, and that of medium and lactic acid an intermediate mass of undissociated acid. Pending an examination of the results obtained with other acids and media it would be premature to make a definite statement, but the results already obtained certainly suggest, that, when equivalent quantities are considered, highly dissociated acids are more effective in inhibiting the growth of *B. coli* than are those which are less dissociated. Further the final reaction reached by the culture depends rather upon the true acidity produced than upon the mass per cent. of acid present in the medium.

Examination of the forms of the curves expressing the reaction resultants of the various mixtures of media and acids reveals further signs of specificity.

While for hydrochloric and lactic acids the final reactions of the media become more acid, as the initial acid reaction of the medium increases, with acetic acid the reverse is obtained. For the former acids, it is evident that the amount of acid produced in the mixture is not rapidly diminishing in proportion to the rise of initial acid reaction, while with acetic acid a rise of initial acidity is accompanied by a marked diminution of acid formation.

It must be concluded that no attempts at diagnosis of the varieties of *B. coli* by means of their final reactions in a given medium can safely be made, since all strains and both varieties of the organism appear to react in the same manner when exposed to similar conditions. Also the final reaction attained is dependent upon the medium used, and the nature and amount of acid added to it.

In nearly every experiment it was found that at or near the expiration of a period of 168 hours from inoculation the P_H of the cultures showed a slight decrease of acidity. Possibly this is caused by the accumulation of metabolites produced during fermentation, and the phenomenon is graphically expressed in those figures in which the reaction resultant curve crosses, and passes to the left of, the initial reaction axis *AB*.

TABLE IX. *Glucose phthalate + hydrochloric acid, inoculated with* H_4 18 *hours old.*

Tube	*N*/5 acid per 10 cc. solution	Result	Time in hrs.	P_H Initial	4	8	24	48	72	168
1	0	+		7·11	6·89	5·60	5·60	5·6	5·65	5·70
2	0·3	+		6·80	6·73	5·43	5·41	5·27	5·11	5·11
3	0·4	+		6·69	6·64	5·36	5·20	5·16	5·07	5·09
4	0·5	+		6·52	6·50	5·29	5·21	5·13	5·05	5·06
5	0·7	+		6·27	6·27	5·75	5·34	5·27	5·04	5·01
6	0·8	+		6·13	6·13	5·30	5·01	5·13	4·97	4·97
7	0·9	+		5·95	5·95	5·04	5·03	5·01	4·94	4·97
8	1·0	+		5·77	5·77	5·38	4·94	5·04	4·99	4·94
9	1·5	+		5·01	5·01	5·01	4·90	5·85	4·75	4·75
10	1·8	+		4·96	4·96	4·90	4·82	4·68	4·68	4·68
11	2·4	–		4·41	4·41	4·41	4·41	4·41	4·42	4·42

6. A number of "retardation" experiments were performed, and it was conclusively proved that in addition to the total inhibition of growth produced by the addition of an acid to a medium, smaller quantities of acid caused a well-marked retardation of the fermentation of *B. coli*. For any given acid and medium an increase in the amount of acid added resulted in a longer period of retardation before the solution reached its final acid

reaction. With either glucose peptone, or glucose phthalate, to which no acid had been added, signs of fermentation were always visible within four hours of inoculation, but when acid insufficient to produce total inhibition of growth was added the period of retardation was sometimes prolonged to nearly 48 hours. Table IX shows the results obtained in a typical "retardation" experiment.

Summary.

1. All strains of *B. coli*, whether of human or of bovine origin behave similarly when exposed to similar conditions.

2. The degree of acidity of the final reaction produced by a culture of *B. coli* cannot be used for diagnostic purposes. This value is not a "physiological constant," but depends upon:

(*a*) The initial H-ion concentration of the medium in which fermentation occurs.

(*b*) The composition of the medium—especially the degree to which it is "buffered."

(*c*) The nature of the acid used to produce the initial reaction of the medium.

3. When the amount of acid added to a medium is insufficient completely to inhibit the fermentation of *B. coli* therein, a definite latency of growth results. For any given medium, a greater initial addition of acid causes a longer latent period.

4. Each acid appears to have a specific effect in inhibiting the growth of *B. coli* in a given medium, highly dissociated acids being more strongly inhibitory than those undergoing a less degree of dissociation.

5. For a mixture of any given medium and acid there appears to be a definite "critical point," beyond which the slightest rise in the degree of acidity results in a complete inhibition of the growth of *B. coli*. The critical points approximately determined as a result of the foregoing experiments are:

2 per cent. glucose peptone +			hydrochloric acid	$P_H = 4{\cdot}27$
,,	,,	,,	lactic ,,	$P_H = 4{\cdot}47$
,,	,,	,,	acetic ,,	$P_H = 4{\cdot}68$
Glucose + phthalate +			hydrochloric acid	$P_H = 4{\cdot}55$
,,	,,		lactic ,,	$P_H = 4{\cdot}73$
,,	,,		acetic ,,	$P_H = 4{\cdot}83$

The specificity of action of the acids in these mixtures is also illustrated by the form of the reaction-resultant curve obtained in any given case.

In conclusion I wish gratefully to acknowledge my indebtedness to Captain G. G. L. Wolf, R.A.M.C., at whose suggestion this research was undertaken, for much valuable advice and guidance during the prosecution of the work.

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