A Critical Study of Factors Influencing the Microbiological Assay of Nicotinic Acid

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SUMMARY: The cultural characters of a strain of *Lactobacillus arabinosus* and *L. helveticus* used in the microbiological assay of riboflavin and nicotinic acid proved to be typical, excepting that neither strain fermented xylose and the strain of *L. arabinosus* fermented rhamnose but not raffinose. Neither produced catalase, so that a strongly positive catalase test in these cultures indicates probable contamination by common air-borne micro-organisms.

Modifications of the medium to achieve maximum acid production were investigated by altering, omitting or adding various constituents. A modified medium was adopted allowing a slightly higher acid production. In this medium the maximum acid production was obtained in the presence of natural substances like peptone. The maximal acid production obtainable with nicotinic acid alone was slightly lower, suggesting an additional specific factor in peptone. No confirmatory evidence, however, could be obtained of its existence. Linoleic acid in a concentration of 640 μ g./10 ml. medium depressed the acid production, and its action was antagonized by cholesterol. Other fatty acids and lipids had no effect.

A good reproducibility and a small coefficient of variation was found between tubes at various levels of nicotinic acid within any one assay, but between separate assays the variation was high (coefficient of variation = 12–19 %).

Since Snell & Wright (1941) published their original microbiological procedure for the estimation of nicotinic acid, it has been modified by a number of workers (Krehl, Strong & Elvehjem, 1943; Barton-Wright, 1944, 1945; Sarett, Pederson & Cheldelin, 1945). Experience in the assay of riboflavin with *Lactobacillus helveticus* showed that, by the use of an improved medium, a higher acid production and an increased reproducibility could be obtained (Kodicek, 1948; Pepper, 1947). An attempt was made to improve conditions likewise for the nicotinic acid assay, with *L. arabinosus* as the test organism.

EXPERIMENTAL

Cultural characteristics of Lactobacillus arabinosus and Lactobacillus helveticus

The characteristics and biochemical reactions of *L. arabinosus* were compared with those of *L. helveticus*. Snell's original strain *L. arabinosus*, 17–5 (Snell & Wright, 1941) was obtained from Dr Snell in 1944. After monthly subculture on yeast-water glucose agar slopes, and storage of the tubes at 4°, it was found in 1945 to be unsuitable for the assay of nicotinic acid. The bacteria had lost their sensitivity; their response to graded doses of nicotinic acid was very erratic, and the agreement between tubes at each level of addition was poor. A fresh culture kindly supplied by Dr Barton-Wright was investigated. It proved to be the *p*-aminobenzoic acid mutant described by Snell (see Shankman, 1943). Lewis also described this mutant strain (see Pennington, 1946) and

stated that his stock culture, used as the test organism for this particular assay, suddenly acquired the ability to dispense with *p*-aminobenzoic acid.

The strain of *L. helveticus*, obtained from Dr Barton-Wright in 1943, was derived from Snell's original strain no. 7499 A.T.C. (Snell & Strong, 1939). It was in continuous use for experimental work from the time it was obtained and never showed any sign of mutation; its response to added riboflavin was constant and satisfactory.

Both strains had the following features, characteristic of members of the genus *Lactobacillus*: Gram-positive, non-motile, non-sporing, micro-aerophilic, aciduric, fermenting carbohydrates to give lactic acid but no gas, pleomorphism and irregular beaded staining in old culture, growing well only on complex media, indole negative, methyl red positive, and catalase negative. When testing the organisms for their sugar reactions, yeast extract was added to the tubes. When this was not done, falsely negative results occurred, merely because of lack of growth.

Wilson & Miles (1946) state that some of the Lactobacillae are very slightly catalase-positive. With these two organisms there was no spontaneous evolution of gas upon adding H_2O_2 , although in the course of several hours a few bubbles of O_2 did collect. The initial lack of reaction provided a quick and useful check test for contamination of the cultures. Since the common airborne bacteria are strongly positive, and contamination from bacteria other than these is very unlikely, an immediate production of O_2 is indicative of contamination.

L. helveticus ferments litmus milk rapidly, and after 48 hr. at 37° there is an acid clot and reduction in the lower portion of the tube; *L. arabinosus*, on the other hand, takes its place in Type B of White & Avery's classification (1910), producing insufficient acid to clot the milk (even after 5 days' incubation), although here again reduction occurs slowly in the lower part of the tube.

McIntosh, James & Lazarus-Barlow (1922) and Day & Gibbs (1928), working with lactobacilli associated with dental decay, reported that the reactions of individual strains were not constant. With *L. arabinosus* and *L. helveticus*, however, we obtained the same results (Table 1) on six occasions. Some of these fermentations were atypical according to Bergey (1939), but with one exception agreed with the findings of Camien, Dunn & Salle (1947), who studied extensively the effect of various carbohydrates upon the acid production of different strains of lactobacillae; in this laboratory *L. arabinosus* has always fermented rhamnose but not raffinose.

Alterations of medium

The effect of altering single constituents of the medium for nicotinic acid assay was studied, in order to obtain an improved medium which would contain growth substances in optimal concentrations (see Leonian & Lilly, 1945); and allow for greater accuracy from a steeper response curve. Unless otherwise stated, Barton-Wright's procedure (1944) was used; the results reported are means of 3-6 tubes.

Modification of Barton-Wright's basal medium. The following alterations of Barton-Wright's basal medium (1944) were made. The quantities quoted refer to concentrations in 100 ml. of final basal medium.

- (i) The glucose concentration was increased from 2 to 3 g./100 ml.
- (ii) Potassium acetate was used instead of sodium acetate and the concentration increased from 2 to 3 g./100 ml.
- (iii) Phosphate solution (20 g./100 ml.). The amount added was increased from 0.5 to 2 ml./100 ml.

Table 1. *Sugar fermentation tests*

Sugar	<i>L. helveticus</i>	<i>L. arabinosus</i>
Arabinose	—	+
Dextrin	—	—
Glucose	+	+
Dulcitol	—	—
Galactose	+	+
Glycerol	—	—
Inositol	—	—
Inulin	—	—
Lactose	+	+
Fructose	+	+
Maltose	—	+
Mannitol	—	+
Mannose	+	+
Raffinose	—	—
Rhamnose	+	+
Salicin	+	+
Sorbitol	—	+
Sucrose	—	+
Trehalose	+	+
Xylose	—	—

(iv) A salt solution (*E*) containing Mg, Mn, Fe, Cu, Zn sulphates and NaCl (Table 6) was used in place of salt solution *B* (Snell & Strong, 1939).

These four modifications previously found optimal for the riboflavin assay (Kodicek, 1948) were adopted as being those most likely to encourage a high acid production by *L. arabinosus*.

(v) *Casein*. Spray-dried acid-hydrolyzed casein powder for microbiological assay work (Ashe Laboratories Ltd.) was tested in concentrations from 0.3 to 3 g./100 ml. As will be seen from Table 2, the blanks gave too high an acid response when concentrations of, or higher than, 1 g./100 ml. were used. The same maximum acid production was obtained both with 0.3 g. and with 0.5 g. casein, and 0.3 g./100 ml. was adopted for this particular sample of casein hydrolysate.

(vi) *B vitamins*. The concentrations of aneurin chloride hydrochloride, pyridoxin hydrochloride, *p*-aminobenzoic acid and calcium *d*-pantothenate were increased ten times from 0.01 mg./100 ml. to 0.1 mg./100 ml. each. The increased concentration of the B vitamins, however, had no significant effect on the acid production of *L. arabinosus*.

(vii) *Purines*. Adenine sulphate, guanine hydrochloride and uracil were added at different concentrations (Table 3). The concentration around

3 mg./100 ml. seemed to be about optimal and was adopted in the final medium.

(viii) *Cystine*. Increasing the concentration of L-cystine from 0.02 g. to 0.04 g./100 ml. produced no beneficial effect and, moreover, some of the cystine came out of solution when adjusting the pH of the medium to 6.8. The original concentration was therefore used throughout.

Table 2. *Effect of increasing the concentration of acid-hydrolyzed casein*

Material	Concentration (g./100 ml. medium)	Nicotinic acid (μ g./10 ml. medium)				
		0	0.05	0.1	0.3	0.5
		0.1 N-acid produced (ml.)				
Acid-hydrolyzed casein powder for microbiological work	0	0	1.3	1.7	2.8	3.0
	0.3	1.6	4.8	6.0	9.5	12.6
	0.5	1.7	3.4	5.1	9.0	11.5
	1.0	3.4	5.5	6.2	10.4	13.0
	2.0	5.5	7.6	8.6	12.2	14.1
	3.0	7.4	8.0	9.5	11.7	12.0

Table 3. *Effect of increasing the concentration of purines*

Material	Concentration (g./100 ml. medium)	Nicotinic acid (μ g./10 ml. medium)				
		0	0.1	0.2	0.5	1.0
		0.1 N-acid produced (ml.)				
Purine solution	0.001	0.7	4.0	6.2	10.3	12.1
	0.003	1.1	4.2	6.5	11.6	14.2
	0.005	0	3.4	5.8	11.8	11.5
	0.010	0	3.6	5.0	10.5	12.6

Purine solution contained adenine sulphate, guanine hydrochloride and uracil, 1 mg./ml. each.

(ix) *Buffers*. Various buffer solutions (Guirard, Snell & Williams, 1946) were tested instead of potassium acetate (Table 4). As they did not increase the acid production, 3 g./100 ml. of potassium acetate were retained.

Additions to the basal medium. The following sugars, which were fermented by *L. arabinosus*, were added in turn to the medium (already containing 3 g. glucose) in a concentration of 1.5 g./100 ml.: L-arabinose, galactose, mannose, mannitol, inositol and lactose. None of these compounds gave a significant increase in acid production. Acid production decreased when sucrose, but not when fructose, was substituted for glucose. Glucose was retained in the final medium.

Various other substances, including some of those reported by other workers as growth stimulants, were tested in turn. Under existing conditions no improvement was found on adding them in the following amounts per 100 ml. of final medium: Na pentose nucleotide 0.08 and 0.8 mg., dioxymaleic acid 6.4 mg., maleic acid 6.4 mg., succinic acid 6.4 mg., choline chloride 100 mg., folic acid 2.5 mg., asparagine 2000 mg., glutamine 0.1 mg., glutamic acid

(added before and after autoclaving tubes) 0.1, 0.2 and 10 mg., methionine 1.2 mg. and thioglycollic acid 100 and 700 mg.

Table 4. *Effect of alterations of buffer*

Buffer	Added (g./100 ml. medium)	Nicotinic acid (μ g./10 ml. medium)	
		0	0.5
		0.1 N acid produced (ml.)	
K acetate (own medium)	3	0	9.7
Na acetate	0.01	0.6	7.0
KH ₂ citrate	0.25		
K acetate	0.01	0.5	7.4
KH ₂ citrate	0.25		
Na acetate	0.1	1.5	9.3
KH ₂ citrate	2.2		
K acetate	0.1	1.3	9.4
NaAc	2.2		

Table 5. *The effect of omitting individual constituents from the medium*

Constituents omitted	0.1 N acid production (at 0.5 μ g. nicotinic acid level/10 ml. medium) (ml.)	% of acid production in 'complete' medium
0 (control 'complete' medium)	10.0	—
Glucose	0.4	4
Xylose	10.1	101
Potassium acetate	5.2	52
Casein, acid-hydrolyzed	0.7	7
Tryptophan	3.3	33
Cystine	6.7	67
Adenine sulphate	8.2	82
Guanine hydrochloride	8.3	83
Uracil	8.1	81
Xanthine	10.2	102
Biotin (free acid)	2.0	20
Aneurin	9.1	91
Riboflavin	10.1	101
Pyridoxin hydrochloride	8.6	86
Calcium <i>d</i> -pantothenate	5.3	53
<i>p</i> -Aminobenzoic acid	9.9	99
Salt solution A (phosphate)	5.8	58
Salt solution E	7.0	70
Ammonium sulphate	11.2	112

Omissions from the basal medium. The substances listed in Table 5 were omitted in turn from basal medium to which 5 μ g./100 ml. of nicotinic acid had been added. The table gives the average results from three separate experiments. Since omission of xylose had no effect, and it was not fermented by *L. arabinosus*, it was not included in the final medium. Riboflavin was found to be non-essential, confirming the work of other authors (Snell & Strong, 1938; Krehl *et al.* 1943; Snell, 1945; Barton-Wright, 1945) and was also omitted, as were ammonium sulphate and xanthine. The maintained acid production of our strain of *L. arabinosus*, in absence of *p*-aminobenzoic acid, showed that we

have the *p*-aminobenzoic acid mutant mentioned by Snell (Shankman, 1943; Shankman, Camien, Block, Merrifield & Dunn, 1947). The vitamin was, nevertheless, included in the final medium in case the mutant strain reverted to the normal type. Omission of the other constituents lowered the acid production, and they were retained in the final medium.

Table 6. *Table of composition of own final medium and of other authors*

Constituents*	Own medium (mg.)	Barton- Wright (1945) (mg.)	Krehl <i>et al.</i> (1943) (mg.)	Sarett <i>et al.</i> (1945) (mg.)	Woolley & Sebrell (1945) (mg.)
Glucose	3000	2000	2000	2000	2500
Potassium acetate	3000	2000 (NaAc)	2000 (NaAc)	1800 (NaAc)	2000 (NaAc)
Casein powder, acid-hydro- lyzed vitamin-free	300	600	500	500	1000
L-Tryptophan	10	10 (DL)	10 (DL)	—	10 (DL)
L-Cystine	20	20	20	20	10
Adenine sulphate	3	1	1	—	1
Guanine hydrochloride	3	1	1	—	1
Uracil	3	1	1	—	1
Xanthine	—	1	—	—	—
KH ₂ PO ₄	200	50	50	50	50
K ₂ HPO ₄	200	50	50	50	50
MgSO ₄ ·7H ₂ O	120	20	20	20	20
MnSO ₄ ·4H ₂ O	3	1	1	1	1
NaCl	1	500	1	1	1
Xylose	—	100	—	—	—
Ammonium sulphate	—	300	—	—	—
Lloyd's reagent treated:					
Peptone	—	—	—	250	—
Liver extract	—	—	—	100	—
Yeast extract	—	—	—	100	—
Norite treated yeast extract	—	—	—	100	—
	(μg.)	(μg.)	(μg.)	(μg.)	(μg.)
FeSO ₄ ·7H ₂ O	8	1000	1000	1000	1000
CuSO ₄ ·5H ₂ O	8	—	—	—	—
ZnSO ₄ ·7H ₂ O	8	—	—	—	—
Biotin (free acid)	0·04	0·04	0·02	—	0·1
Aneurin chloride hydro- chloride	100	10	10	—	20
Pyridoxin hydrochloride	100	12	10	—	20
Calcium <i>d</i> -pantothenate	100	10	10	10	40
<i>p</i> -Aminobenzoic acid	100	10	10	—	20
Riboflavin	—	20	20	—	40

* Amounts stated per 100 ml. final medium (50 ml. of double-strength medium finally diluted to 100 ml.).

Results with the alterations in the medium

Table 6 shows the composition of our medium as finally modified and of the different basal media reported by other workers.

Our best response curve (Fig. 1) showed a greater acid production than that cited by Barton-Wright (1945), but taking the average response at different levels for 71 standard reference curves obtained in the course of over one year, the values for the improved medium were only slightly higher. The variation from experiment to experiment was considerable; for 71 experiments the

coefficient of variation calculated for different levels of nicotinic acid ranged from 12 to 19 % (Table 7). On the other hand, in one single experiment the agreement between tubes at various levels of nicotinic acid was found to be very close and the variation small (Table 8).

Fig. 1. Dose response curve of *L. arabinosus* in different media.

It was difficult to compare our average values for the standard reference curve with figures reported by other workers, as they do not indicate whether their curves referred to average values nor do they give any idea of their variation.

Because of the great variation in the acid productions from one experiment to another, the effect of altering the various constituents of the medium were tested within one experiment, and a control standard curve was always determined at the same time.

A similar erratic response was found when using *L. helveticus* with a certain riboflavin-free medium. When this was modified to give an acid production several times greater than the original, the results were then far more reproducible (coefficient of variation 3–7 %; Kodicek, 1948). Some deficiency of the medium may also be the reason for the erratic responses with *L. arabinosus*. As will be seen below, this organism is also capable of producing large amounts of acid of the order of that produced by *L. helveticus*.

Acid-producing potentialities of Lactobacillus arabinosus

In order to determine whether *L. arabinosus* was capable of such high acid production as *L. helveticus* in the riboflavin assay, *L. arabinosus* was seeded into a modified riboflavin-free medium containing 0.5 µg./10 ml. of added nicotinic acid. The acid production was between 22 and 27 ml. equivalents of

Table 7. *Average acid production in modified medium and variation between experiments*

Medium	No. of expts.	Nicotinic acid (µg./10 ml. medium)							
		0	0.05	0.1	0.15	0.2	0.25	0.3	0.5
		0.1 N acid produced (ml.)							
Barton-Wright (1945)	—	0.9	2.3	3.4	4.5	5.5	6.4	—	—
Own medium	71	0.9	—	4.2	—	6.5	—	8.5	11.0
Standard deviation		± 0.864		± 0.802		± 0.967		± 1.025	
Coefficient of variation (%)		96		19		15		12	

Table 8. *Variation between tubes*

Concentration of nicotinic acid (10 ml. medium)	No. of tubes	0.1 N acid produced (ml.)	Standard deviation	Coefficient of variation (%)
0.1 µg.	22	4.3	± 0.258	6.0
0.2 µg.	22	7.1	± 0.213	3.0

0.1 N-NaOH. When, however, peptone was omitted from the medium it no longer supported such high acid production. When photolyzed Bacto-peptone (Snell & Strong, 1939) was added in a comparable concentration, i.e. 100 mg./10 ml., to the modified medium used in the nicotinic acid assay, the acid production at 0.5 µg. nicotinic acid/10 ml. was increased from about 11 to 22 ml. equivalents. Untreated Bacto-peptone had a similar effect; in 13 experiments the average acid production was 21.3 ml. ± 0.698 equivalents. Similar high values, however, were also obtained when no nicotinic acid was added (Table 9).

The nicotinic acid content of Bacto-peptone was found by chemical tests to be 37 µg./g. The addition of 100 mg. of peptone/10 ml. would add 3.7 µg. of nicotinic acid to the nicotinic acid-free basal medium. To decide whether the high acid production could be attributed solely to nicotinic acid, the vitamin was added in comparable amounts to the basal medium (Table 9). Addition of peptone resulted in a slightly higher acid production than that found upon addition of 5 µg. of nicotinic acid. When the 't' test was applied to test for the significance of the differences, the probability that these results were due to chance could not be excluded.

Further experiments were carried out to distinguish the peptone factor from nicotinic acid. The factor which seems to increase the acid production above

that obtained with a corresponding level of nicotinic acid was stable to alkali, insensitive to light, not precipitated by 90 % ethanol, not extractable by CHCl_3 at acid pH, adsorbed completely on activated charcoal at acid pH, and partially at neutral pH. These properties are similar to those of nicotinic acid and no conclusion could be drawn from these experiments. Further work on this matter is being carried out.

It is clear that *L. arabinosus* is capable of producing as much acid as *L. helveticus* (Fig. 2). The shape of the response curve is such that the increased acid production does not affect the lower portion of the response curve (between 0.1 and 0.2 μg . of nicotinic acid) which is used for assay work.

Table 9. *Comparison between acid productions in response to peptone and high amounts of nicotinic acid*

Addition per 10 ml. medium	No. of exps.	Average 0.1 N acid production (ml.)	Standard error of the mean	Remarks
Nicotinic acid:				
0.5 μg .	71	11.0	± 0.154	—
5 μg .	14	18.6	± 0.473	—
10 μg .	5	19.4	—	—
200 μg .	1	18.6	—	—
Bacto-peptone:				
100 mg.	9	20.1	± 0.828	Equivalent to 3.7 μg . nic. acid
100 mg. + 0.5 μg . nic. acid	13	21.3	± 0.698	Equivalent to 4.2 μg . nic. acid
100 mg. + 5 μg . nic. acid	2	27.8	—	—
100 mg. + 10 μg . nic. acid	1	23.5	—	—

'*t*' for difference of means between peptone and 5 μg . nicotinic acid = 1.76; '*t*' = 1.72 corresponds to $P=0.10$.

'*t*' for difference of means between peptone with added nicotinic acid and 5 μg . nicotinic acid = 3.24; '*t*' = 2.79 corresponds to $P=0.01$.

Inhibitory substances

Iron and calcium. In view of the findings of Chattaway, Happold & Sandford (1943), regarding the toxicity of certain cations, the effect of both iron and calcium was investigated in the modified final medium (Table 10) containing 0.1 and 0.5 μg . nicotinic acid/10 ml. respectively.

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was added to a final maximum concentration of 1 mg./100 ml. of medium. Calcium was added as CaCl_2 in concentrations of 10, 70 and 140 mg./100 ml. These amounts were chosen as being considerably in excess of concentrations likely to occur in extracts from most iron- and calcium-rich foods.

As will be seen from Table 10, there was no significant decrease in acid production in the presence of the iron salts or of CaCl_2 . With extremely high concentrations of CaCl_2 (70 and 140 mg./100 ml.) there was a certain decrease in acid production only at the level of 0.5 μg . nicotinic acid.

Linoleic acid and other fatty substances. Unlike *L. helveticus*, *L. arabinosus* has

been reported to be insensitive to fatty acids (Krehl *et al.* 1948). Their effect was tested on the organism used in this laboratory. Similar precautions were taken as in experiments with *L. helveticus* (Kodicek & Worden, 1945), and the tests were repeated with CHCl_3 -extracted medium to prevent any interference

Fig. 2. Maximum acid production of *L. arabinosus* and *L. helveticus* on addition of essential vitamin.

Table 10. Effect of increasing the concentration of iron and calcium salts

Material	Added (mg./100 ml.)	Nicotinic acid (µg./10 ml.)	
		0.1	0.5
Control	—	4.0	9.7
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.1	—	10.9
	0.5	4.4	9.3
	0.10	4.5	9.8
	10.0	—	9.9
CaCl_2 (dried)	70.0	4.5	7.3
	140.0	5.3	7.7

from fat-soluble substances. The results are shown in Table 11. The acid production in CHCl_3 -extracted medium was practically the same as that in the ordinary medium. There was almost complete inhibition of growth upon addition of $640 \mu\text{g.}/10 \text{ ml.}$ of potassium linoleate (prepared from pure methyl linoleate, iodine value 173). In presence of cholesterol ($640 \mu\text{g.}/10 \text{ ml.}$) this inhibition with linoleate did not take place. The inhibition by linoleic acid occurred, irrespective of whether CHCl_3 -extracted or ordinary medium was used.

At a level of 160 μg . linoleic acid there was proportionally less inhibition. The depressant effect was more marked at higher levels of nicotinic acid (Fig. 3).

Fig. 3. Effect of linoleate on acid production of *L. arabinosus*.

Table 11. *Effect of fatty acids and lipoids*

Medium	Additions (640 μg ./10 ml. medium)	Nicotinic acid (μg ./10 ml.)				
		0	0.1	0.3	0.5	1.0
		0.1 N acid produced (ml.)				
Unextracted	—	0	4.0	8.7	11.5	13.1
CHCl ₃ -extracted	—	0	4.1	8.8	12.6	—
Unextracted	Linoleate	0	0	—	2.3	1.4
CHCl ₃ -extracted	Linoleate	0	1.1	—	0.5	2.0
CHCl ₃ -extracted	Linoleate and cholesterol	0	3.7	—	7.8	9.1
CHCl ₃ -extracted	Palmitic acid	2.0	4.7	—	12.0	—
CHCl ₃ -extracted	Stearic acid	0.4	3.8	—	10.2	—
CHCl ₃ -extracted	Lecithin	0.2	3.3	—	10.3	—
CHCl ₃ -extracted	Cholesterol	0.3	3.3	—	11.5	—

Palmitic and stearic acid, lecithin and cholesterol were each tested at a level of 640 μg ./10 ml. (Table 11); none of these substances had a marked effect on the acid production.

Kodicek & Worden (1945, 1946) suggested that the inhibitory effect of linoleic acid might be due to the formation of a molecular monolayer on the surface of the bacteria. Accordingly, it was decided to investigate the effect

of surface-active Aerosols. These compounds are long chain di-esters of sodium sulpho-succinate, e.g.

Two samples, Aerosol AY ($n=8$) and IB ($n=5$), were kindly supplied by the Colloid Science Department, Cambridge. As will be seen from Table 12, the Aerosols in concentrations of $640\text{ }\mu\text{g./10 ml.}$ showed no such inhibitory effect as was observed with linoleic acid. It seems that the effect of linoleic acid, if it is a physico-chemical one, is different from that of detergents like Aerosols.

Table 12. *Effect of linoleic acid and Aerosols added to CHCl_3 -extracted medium*

Addition ($640\text{ }\mu\text{g./10 ml. medium}$)	Nicotinic acid ($\mu\text{g./10 ml.}$)	
	0	0.5
	0.1 N acid produced	
None	0.5	11.5
Linoleic acid	0	0
Aerosol 1 B	0	12.3
Aerosol AY	1.7	11.5

DISCUSSION

The strain of *L. arabinosus* used in this laboratory proved to be the *p*-amino-benzoic acid mutant mentioned by American workers. Its cultural characteristics agreed well with published findings with the exception of its fermenting properties for raffinose and rhamnose. The former, contrary to findings of Camien *et al.* (1947), was fermented by the organism, while raffinose was not.

We can confirm the growth-promoting effect of peptone (Pollack & Lindner, 1943; Sarett *et al.* 1945; Camien *et al.* 1947), but we were unable to decide whether it was due to a specific factor or to the high nicotinic acid content of this substance.

We have omitted several constituents which were found to be non-essential for *L. arabinosus*, namely xylose, xanthine, ammonium sulphate and riboflavin, and, on the other hand, increased the concentration of glucose, potassium acetate, phosphates, magnesium, manganese salts and B-vitamins. Knight (1945) reported in his review that purines had definite growth-promoting effects on *L. arabinosus*. Sarett *et al.* (1945), however, found high amounts to be deleterious. We did not find this to be the case and increased the concentration of adenine, guanine and uracil. The amount of tryptophan was not altered, since no beneficial effect was found when increasing its concentration ten times above that used in the original medium.

Contrary to reports that iron salts exerted a marked depressing effect on certain bacteria, they did not interfere in our tests in concentrations which were in excess of those usually encountered when assaying iron-rich extracts. Calcium salts, only in a very high concentration, 70–140 mg./100 ml., decreased the acid production slightly, but such a high concentration of calcium is unlikely to occur in extracts of foodstuffs.

Linoleic acid inhibited the acid production of *L. arabinosus*. Higher concentrations were needed than those that were bacteriostatic for *L. helveticus*. This relative insensitivity may explain the negative findings of previous workers. The inhibition could be counteracted by cholesterol, as was the case with *L. helveticus*. The mode of action, if it is a physico-chemical one, would be different from that of such detergents as Aerosols which are ineffective in comparable concentrations. We can confirm that stearic and palmitic acids, cholesterol and lecithin have no enhancing effect on the acid production even at high concentrations. For assay work, the removal of lipids is advisable because of possible effects by unsaturated fatty acids, although the relative insensitivity of *L. arabinosus* makes this less essential than when working with *L. helveticus*.

The modified final medium fell short of our expectations of obtaining the high acid production experienced in assays with *L. helveticus*. Within any one experiment the results between tubes agreed closely, but a rather high variation between experiments was observed. This somewhat erratic response from one experiment to another will be referred to in another paper (Kodicek & Pepper, 1948) which deals with actual results on foodstuffs. There the reproducibility of the nicotinic acid values in repeats and the good agreement between chemical and microbiological values contrast pleasantly with our experience of the response of *L. arabinosus* towards nicotinic acid alone. Nevertheless, we feel that *L. arabinosus* is not the most appropriate organism for nicotinic acid assays. Its effective range for assay of nicotinic acid is far below its optimal requirements for this vitamin; under certain conditions it may, on the other hand, dispense with nicotinic acid (Shankman *et al.* 1947).

We wish to thank Drs A. E. Alexander and A. I. McMullen for kindly supplying us with samples of Aerosols. Our thanks are due to Dr L. J. Harris for his continued interest in this work and Dr C. G. McGaughey for the facilities kindly granted by the Institute of Animal Pathology, Cambridge.

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(Received 2 February 1948)