THE NUTRITION OF *STAPHYLOCOCCUS AUREUS*; NITROGEN REQUIREMENTS.

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In a recent paper Fildes, Richardson, Knight and Gladstone (1936) described a simple medium suitable for the growth of a typical strain of *Staphylococcus aureus*. With the exception of one unknown component (Knight, 1935), the medium was entirely composed of known chemical substances. It has since been shown (Knight, 1937*a*, *b*) that the unknown component can be replaced by nicotinic acid (or nicotinamide) *plus* aneurin (vitamin B_1). Thus *Staphylococcus aureus* can now be grown in a medium of known chemical composition. Although 14 amino-acids were present, it was suggested (Fildes *et al.*, 1936) that not all these were required for growth. No evidence was offered, however, as to which were necessary.

In the present paper the amino-acid nutrition of *Staphylococcus aureus* has been investigated with a view to determining which amino-acids are important. The method has been to observe the effect on growth of omitting each amino-acid in turn from the complete mixture. Later, using the method of Fildes, Gladstone and Knight (1933), experiments were made in "training" the organism to grow on a more simplified medium. In this way it was possible eventually to remove all the amino-acids, and to grow the organism in a medium containing ammonia as the main source of nitrogen.

TECHNIQUE.

The technical details were similar to those of Fildes *et al.* (1936).

Medium.

The medium was a modification of that of Fildes *et al.* (1936).

Briefly, it contained in its complete form the following amino-acids: glycine, alanine, valine, leucine, phenylalanine, tyrosine, methionine, proline, oxyproline, aspartic acid, glutamic acid, lysine, arginine and histidine. With the exception of methionine, which was used in a concentration of *M*/50,000, the quantities were those used by Fildes *et al.* (1936). In addition tryptophan (*M*/20,000) and cystine (*N*/5000, *i. e.* *M*/10,000) were used. Details concerning the standards of purity of most of the amino-acids are given in the papers of Fildes *et al.* (1936) and Fildes and Richardson (1937). Where used, other amino-acids were prepared with similar care. In order that the amino-acid content could be varied, each amino-acid was made up and sterilized separately in concentrated solution, so that the addition of a small volume (0·1 ml.) of each gave the final concentration as required in the completed medium. All amino-acids were dissolved in water except cystine and tyrosine; these were made up in *M*/10 HCl, the pH being corrected after their

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addition to the medium. Sterilization was effected by autoclaving at 115° C. for 20 minutes, except in the case of methionine and cystine, which, as noted by Fildes and Richardson (1937), should always be filtered. For this purpose the glass Seitz filter of Knight and Fildes (1936) was used. Sodium dithiodiacetate* (dithiodiglycollate) was omitted from the medium. When an organic source of sulphur other than cystine was required $M/1000$ sodium mercaptoacetate* (thiolacetate) was used.

In experiments made after the discovery of the nature of Knight's staphylococcal growth factor (Knight, 1937), this factor was replaced by synthetic preparations of nicotinamide (10^{-5} M) and aneurin (vitamin B_1, 10^{-7} M). In addition to the inorganic salts used by Fildes, magnesium sulphate ($M/6000$) was added.

Inoculum.

Unless otherwise stated, the inoculum was taken from a 24-hour culture on a nutrient agar slope. A suspension was made in distilled water and its opacity standardized to a given number of organisms. The number of organisms inoculated is shown in the protocols.

Strains of Staphylococci.

Twenty-six strains of staphylococci were used as shown in Table I.

TABLE I.

No.	Species.	Source.
1.	*Staphylococcus aureus*	Cellulitis (Fildes & Knight, 1933).
2.	,, ,,	Septicæmia (blood).
3.	,, ,,	N.C.T.C. No. 1389 Type I (Hine).
4.	,, ,,	N.C.T.C. No. 1393 Type II (Hine).
5.	,, ,,	N.C.T.C. No. 3093, " Wood ".
6.	,, ,,	N.C.T.C. No. 3095, " Wood 46 ".
7.	,, ,,	N.C.T.C. No. 3750, " Mrs. Fox ".
8.	,, *albus*	N.C.T.C. No. 3094, " Wood 86 ".
9.	,, *aureus*	Furuncle.
10.	,, *albus*	Dissociant from strain 2.
11.	,, *aureus*	Nose.
12.	,, ,,	Granuloma of hand.
13.	,, ,,	Folliculitis of scalp.
14.	,, ,,	Osteomyelitis.
15.	,, ,,	Abscess of nasal septum.
16.	,, ,,	Conjunctivitis.
17.	,, ,,	,,
18.	,, ,,	Bronchopneumonia (lung).
19.	,, ,,	Whitlow.
20.	,, ,,	Abscess.
21.	,, ,,	Bronchopneumonia (lung).
22.	,, ,,	Aγ (Bigger, 1933).
23.	,, ,,	Sinus of leg.
24.	,, ,,	Fistula of chest.
25.	,, ,,	Mastoid abscess.
26.	,, ,,	Infected sebaceous cyst.

* For adopted nomenclature of organic sulphur compounds see Fildes and Richardson (1937).

Strains 2, 11 to 21 and 23 to 26 were supplied by Dr. L. E. H. Whitby; strain 22 was received from Dr. J. W. Bigger.

EXPERIMENTAL.

Preliminary experiments showed that all strains except Strain 3 grew well on the complete medium with the addition of cystine and tryptophan. The exception was an atypical strain, growing slowly on laboratory media and producing a viscous ropy deposit in broth.

The Effect of Tryptophan on Growth.

Fildes and Knight (1933) showed that a strain of *Staphylococcus aureus* growing on a hydrolysate of gelatin required tryptophan for growth, but was easily trained to dispense with it. The effect of tryptophan on the growth of 26 strains growing in the complete amino-acid medium is shown in Table II.

TABLE II.—*The Effect of Tryptophan on Growth of* Staph. aureus *Growing in the Complete Amino-acid Medium. Inoculum* 3 × 10^6 *Organisms per* 10 *ml. medium.*

Number of days' incubation:	Without tryptophan.					With tryptophan.				
Strain.	1.	2.	3.	4.	6.	1.	2.	3.	4.	6.
1	+++	+++	+++	++	++	+++	+++	+++	++	++
2	+++	+++	+++	++	++	+++	+++	+++	++	++
3	0	0	0	0	0	0	0	0	0	0
4	+	+++	+++	++	++	++	+++	+++	++	++
5	tr.	++	++	++	+	+–	++	++	++	+
6	+	+++	+++	++	++	++	+++	+++	++	++
7	0	0	0	0	0	+	+++	+++	++	++
8	+	+++	+++	++	++	++	+++	+++	++	++
9	++	+++	+++	++	++	++	+++	+++	++	++
10	0	tr.	+	+	+	tr.	+	+	+	+
11	0	0	0	0	0	+++	+++	+++	++	++
12	++	+++	+++	++	++	++	+++	+++	++	++
13	+	+++	+++	++	++	++	+++	+++	++	++
14	++	+++	+++	++	++	+++	+++	+++	++	++
15	+	+++	+++	++	++	++	+++	+++	++	++
16	0	++	+++	+++	++	++	+++	+++	++	++
17	0	0	0	0	0	++	+++	+++	++	++
18	0	0	0	0	0	++	+++	+++	++	++
19	++	+++	+++	++	++	+++	+++	+++	++	++
20	+	+++	+++	++	++	++	+++	+++	++	++
21	0	+++	+++	+++	++	++	+++	+++	++	++
22	++	+++	+++	++	++	+++	+++	+++	++	++
23	++	+++	+++	++	++	++	+++	+++	++	++
24	++	+++	+++	++	++	+++	+++	+++	++	++
25	++	+++	+++	++	++	++	+++	+++	++	++
26	++	+++	+++	++	++	++	+++	+++	++	++

Note.—In this and other tables plus signs refer to the degree of growth: +++ maximum growth, ++ less than maximum, + moderate, +– less than moderate, tr. trace, and f. tr. faint trace.

Of the 25 strains able to grow in the amino-acid medium, only 4 were unable to do so in the absence of tryptophan. Another 13 grew slowly, but eventually reached maximum growth. With the remaining 8 no effect was apparent. When strains giving late growth in the absence of tryptophan were subcultured on to the same medium, growth occurred without delay as if tryptophan were present. From the work of Fildes and Knight (1933), it is justifiable to assume that all strains require tryptophan for growth. The initial differences between them were differences in synthetic ability. Strains in which the effect of tryptophan was not apparent were able to synthesize it as rapidly as they could make use of it. When once growth had occurred and they had become adapted to carry out the synthesis easily, subsequent subcultures grew without delay. It is of interest that the strain described by Fildes and Knight (1933) as requiring tryptophan (Strain 1) now grew equally well in its absence. Since it was used by them, it had been subcultured daily on a nutrient agar slope. During this time it may be assumed that it had become adapted to synthesize tryptophan for itself.

Determination of Essential Amino-acids.

In experiments designed to determine the amino-acid requirements of bacteria, Fildes and Richardson (1935) have stressed the importance of using amino-acids of synthetic origin wherever possible. In the complete medium, all amino-acids were synthetic except proline, oxyproline, glutamic acid, tyrosine, arginine, histidine, tryptophan and cystine. Two of these, oxyproline and glutamic acid, were found to have no effect on growth when cystine was present in the medium. They were therefore omitted. Alanine was also excluded as being unimportant. When strains which grew well in the absence of tryptophan were used, this amino-acid was also left out.

Table III summarizes a series of experiments designed to determine which amino-acids were necessary for growth. Each amino-acid was omitted in turn from a mixture containing 12 amino-acids and the effect on growth observed.

The amino-acids that appeared to be most necessary for rapid growth were cystine, leucine, valine, proline, glycine, aspartic acid, phenylalanine and arginine. Histidine and methionine were less important, and tyrosine (except for Strain 9) and lysine were not required. In the absence of any one of the more important acids, with the exception of cystine, most strains were able to grow eventually and even produced maximum growth. No continuous growth of any strain was obtained in the absence of cystine ; the faint trace of growth apparent in some cultures after prolonged incubation was probably due to cystine carried over in the inoculum. Fildes and Richardson (1937) have shown that extremely small quantities of cystine are required by the organism for growth. They have also shown that cystine can be replaced by methionine, but that much larger quantities are required. In the above experiments the concentration of methionine was too small to have this effect.

Certain amino-acids are more important for some strains than for others. For example, leucine was primarily required by Strain 1, phenylalanine by Strain 9, valine by Strains 13, 14, and to a lesser extent by 15, aspartic acid

TABLE III.—*Determination of Essential Amino-acids.*

Inoculum	Strain 1. 30,000 organisms.						Strain 6. 3 × 10^6 organisms.					
Time of growth (hours)	18.	24.	50.	72.	96.	144.	16.	24.	36.	72.	96.	144.
All 12 amino-acids	+−	+	+++	+++	++	++	tr.	++	+++	+++	+++	+++
Lacking glycine	0	0	tr.	+−	++	+++	0	tr.	++	+++	+++	+++
valine	0	0	+	++	+++	+++	0	0	0	+	++	++
leucine	0	0	0	0	0	0	0	0	++	+++	+++	+++
proline	0	0	0	+	+++	+++	0	0	0	+	++	+++
phenylalanine	0	0	+	+++	+++	++	0	0	++	+++	+++	+++
tyrosine	tr.	+	++	+++	+++	++	f. tr.	+	+++	+++	+++	+++
methionine	0	+−	++	+++	+++	++	f. tr.	+−	++	+++	+++	+++
aspartic	0	0	tr.	tr.	+	++	0	0	tr.	++	+++	+++
lysine	+−	+	+++	+++	++	++	tr.	++	+++	+++	+++	+++
arginine	0	0	+−	+	++	++	0	tr.	+	++	+++	+++
histidine	0	tr.	++	+++	+++	++	0	tr.	+	++	+++	+++
arginine + histidine	0	0	0	+−	++	++	0	0	0	tr.	+	++
cystine	0	0	0	0	f. tr.	f. tr.	0	0	0	0	0	f. tr.

Inoculum	Strain 8. 3 × 10^6 organisms.						Strain 9. 3 × 10^6 organisms.					
Time of growth (hours)	16.	24	36.	72.	96.	144.	16.	24.	36.	72.	96.	144.
All 12 amino-acids	tr.	+	++	++	+	+	+−	++	+++	+++	+++	++
Lacking glycine	0	0	0	tr.	+	++	0	0	0	tr.	tr.	tr.
valine	0	0	+	++	++	+	0	0	0	++	++	++
leucine	0	+	++	++	++	+	0	+	+++	+++	+++	++
proline	0	0	tr.	++	++	++	0	0	0	tr.	++	++
phenylalanine	0	0	+	++	++	+	0	0	0	0	0	0
tyrosine	f. tr.	+−	++	++	+	+	0	0	tr.	++	++	++
methionine	f. tr.	+−	++	++	+	+	tr.	++	+++	+++	+++	++
aspartic	0	0	tr.	++	++	++	tr.	++	+++	+++	+++	++
lysine	tr.	+	++	++	+	+	+−	++	+++	+++	+++	++
arginine	0	0	+	++	++	+	0	0	tr.	++	++	+
histidine	f. tr.	tr.	+−	++	++	+	tr.	++	+++	+++	+++	++
arginine + histidine	0	0	0	0	tr.	+	0	0	+−	+	+	+
cystine	0	0	f. tr.	f. tr.	f. tr.	f. tr.	0	0	f. tr.	f. tr.	f. tr.	f. tr.

Inoculum	Strain 13. 30,000 organisms.						Strain 14. 30,000 organisms.					
Time of growth (hours)	18.	24.	50.	72.	96.	144.	18.	24.	50.	72.	96.	144.
All 12 amino-acids	0	tr.	+++	+++	++	++	+	+	+++	+++	++	++
Lacking glycine	0	0	0	+−	++	++	0	0	+−	+−	+	++
valine	0	0	0	0	0	0	0	0	0	0	0	0
leucine	0	0	+	+++	+++	++	0	f. tr.	++	+++	+++	++
proline	0	0	0	+	++	++	0	f. tr.	++	+++	+++	++
phenylalanine	0	0	0	+	++	++	0	0	+−	+++	+++	++
tyrosine	0	0	++	+++	+++	++	tr.	+−	+++	+++	++	++
methionine	0	0	++	+++	+++	++	tr.	+−	+++	+++	++	++
aspartic	0	0	0	0	0	0	0	tr.	++	++	+++	+++
lysine	0	tr.	+++	+++	+++	++	+	+	+++	+++	++	++
arginine	0	0	0	tr.	++	++	0	0	0	0	+	+
histidine	0	0	++	+++	+++	++	+−	+	++	+++	+++	++
arginine + histidine	0	0	0	0	tr.	+	0	0	0	0	0	0
cystine	0	0	0	0	0	0	0	0	0	0	f. tr.	f. tr.

Inoculum	Strain 15. 30,000 organisms.						Strain 22. 30,000 organisms.					
Time of growth (hours)	18.	24.	50.	72.	96.	144.	18.	24.	50.	72.	96.	144.
All 12 amino-acids	+−	+−	++	++	++	+	+−	+	+++	+++	+++	++
Lacking glycine	0	0	tr.	+−	+−	+−	0	0	tr.	tr.	tr.	+−
valine	0	0	0	0	tr.	++	0	0	0	+	++	++
leucine	0	0	+	++	++	+	0	0	+++	+++	+++	++
proline	0	0	0	+	++	++	0	0	0	0	0	f. tr.
phenylalanine	0	0	+−	++	++	+	0	0	0	+−	+++	+++
tyrosine	+−	+	++	++	++	+	tr.	+−	+++	+++	+++	++
methionine	0	tr.	++	++	++	+	0	tr.	++	+++	+++	++
aspartic	0	0	0	0	++	++	0	0	0	0	+−	+
lysine	tr.	tr.	++	++	++	+	+−	+	+++	+++	+++	++
arginine	0	0	0	tr.	++	++	0	0	tr.	++	++	+
histidine	tr.	tr.	++	++	++	+	0	tr.	++	+++	+++	++
arginine + histidine	0	0	0	0	0	0	0	0	tr.	++	++	+
cystine	0	0	0	0	0	0	0	0	0	0	0	0

by Strain 13, arginine by Strain 14, proline by Strain 22, and glycine by Strains 9 and 22. As in the case of tryptophan, the assumption is that all strains require all these amino-acids for growth. Differences between strains are not differences in the indispensability of particular amino-acids, but differences in the ability of strains to synthesize these acids for themselves. In the second part of this paper it will be seen that, by a process of training, *i. e.* subculturing into a still more simplified medium, such differences tend to become lost.

Changes in Nitrogen Utilization : " Training " to Dispense with Amino-acids.

That the nutritional requirements of bacteria can be changed by an alteration in the substrate on which they are grown has long been known. The literature has been reviewed by Knight (1936). Knight defines the term " training " as " the derivation of cultures having simpler nutrient requirements from cultures with a more complex nutrition ", and in this sense it is used in this paper.

By successive subcultures into a medium from which the amino-acids were progressively eliminated, Fildes, Gladstone and Knight (1933) were able to " train " *Bact. typhosum* to grow on a medium whose sole source of nitrogen was ammonia. This method has been used in the present paper. The steps in the training process are illustrated in Table IV.

It will be seen from Table IV that an organism which had become trained to grow in the absence of a particular amino-acid, *i. e.* to synthesize it for itself, had also become less susceptible to the effects of omitting other and unrelated amino-acids ; an increase in synthetic ability for one amino-acid resulted in an increase in synthetic ability for others. Thus a culture of Strain 22 which had become trained to dispense with alanine grew without delay when subcultured, not only in the absence of this acid, but also in the absence of valine, leucine or histidine, which had previously been important. The prolonged lag phase brought about by the absence of proline, glycine, aspartic acid or arginine was also shortened and finally abolished. In the same way, in a single subculture from a tube lacking proline, growth was obtained of Strain 1 in the absence of leucine, of Strains 13 and 14 in the absence of valine, and of Strain 13 in the absence of aspartic acid ; without proline, each of these strains grew without delay in the course of a single subculture.

Five strains, Strains 6, 13, 14, 15 and 22, were adapted to grow on an ammonia-cystine medium by the method shown in Table IV. With another strain (Strain 1), adaptation to grow on this medium was not so easy. The training process proceeded without difficulty until the organism was growing on a medium containing ammonia, leucine and cystine. Although earlier in the training, when several amino-acids were present, leucine could be omitted, the removal of leucine from the *simple* medium resulted in complete absence of growth. In connection with the nutritional adaptation of *Bact. typhosum*, previous work showed that the final removal of tryptophan could be effected more rapidly and constantly if, in the penultimate stage of training, a variety of nitrogenous substances were provided from which tryptophan could be synthesized (Gladstone, 1937). This method was used in the present instance.

TABLE IV.—*Simplification in Nitrogen Requirements: Training to Dispense with Amino-acids.* (*Strain* 22.)

Agar →	Time of growth (hours)	18.	24.	50.	72.	96.	144.
	All 12 amino-acids	+−	+	+++	+++	+++	++
	Lacking glycine	0	0	tr.	tr.	tr.	+−
	valine	0	0	0	+	++	++
	leucine	0	0	+++	+++	+++	++
	proline	0	0	0	0	0	f. tr.
	phenylalanine	0	0	0	+−	+++	+++
	tyrosine	tr.	+−	+++	+++	+++	++
	methionine	0	tr.	++	+++	+++	++
	aspartic acid	0	0	0	0	+−	+
	lysine	+−	+	+++	+++	+++	++
	arginine	0	0	tr.	++	++	+
	histidine	0	tr.	++	+++	+++	++
	cystine	0	0	0	0	0	0

* →

	19.	36.	72.	120.
All 9 amino-acids	+−	+++	+++	++
Lacking glycine	0	+	+++	++ →
valine	tr.	++	++	++
leucine	+−	+++	+++	++
proline	0	0	+	++
phenylalanine	+−	+++	+++	++
aspartic acid	0	0	0	++
arginine	0	++	++	++
histidine	+−	+++	+++	++
cystine	0	0	0	0

Time of growth (hours)	17½.	23.	42.	72.	120.
All 6 amino-acids	tr.	+	+++	+++	++
Lacking glycine	tr.	+−	+++	+++	++
valine	tr.	+−	+++	+++	++
proline	f. tr.	tr.	+	+++	++
aspartic acid	0	0	0	+	++
arginine	tr.	+−	++	++	++
cystine	0	0	0	0	0

↗

	23.	57.	72.	96.
3 amino-acids	tr.	++	++	++
Lacking aspartic acid	f. tr.	+	+	++ →
arginine	tr.	++	++	+
cystine	0	0	0	0

Time of growth (hours)	17.	24.	32.	45.	72.	84.	96.	120.
2 amino-acids	0	tr.	+	++	++	++	+	+
Lacking aspartic acid	0	0	0	tr.	+	+	+	+ →
cystine	0	0	0	0	0	0	0	0

	16.	23.	41.
1 amino-acid (cystine) (main source of nitrogen = ammonia)	0	tr.	+

* The arrows represent subcultures; in each series the inoculum was taken from the culture directly in front of the arrow. It was centrifuged and the organisms resuspended in distilled water. About 3×10^6 organisms were inoculated. Throughout the process of training, the nitrogen content of the medium was made equal to that of the complete amino-acid medium by the addition of ammonium sulphate.

The concentration of leucine in the ammonia-leucine-cystine medium was reduced to give minimum growth, and to this was added a number of other amino-acids (glycine, valine, proline, phenylalanine, aspartic acid and histidine). Growth occurred in 4 days. All the amino-acids other than cystine were then omitted; after a week's delay growth was obtained on the ammonia-cystine medium, and this could be subcultured indefinitely (Table V).

After 7 daily subcultures on nutrient agar, the trained organism was still capable of growing in the ammonia-cystine medium. After 12 subcultures on agar, however, the acquired property was lost.

The Removal of Cystine.

Fildes and Richardson (1937) have studied the requirements of *Staphylococcus aureus* with respect to sources of sulphur. When the organism was growing in a medium containing several amino-acids, cystine could be effectively replaced by other organic sulphur compounds, *e. g.* mercaptoacetic acid. This, however, was not the case under the present conditions, in which no amino-acids were present except cystine itself.

Training to dispense with the addition of cystine.—Strain 6, adapted to grow in an ammonia-cystine medium, was subcultured 7 times in the same medium, containing a reduced quantity of cystine (N/16,000), together with sodium mercaptoacetate (M/1000). It was then tested for growth in the absence of cystine (Table VI).

Table VI shows that *Staphylococcus aureus*, growing in the ammonia medium, can be trained to dispense with cystine. It was thus growing on a medium from which all amino-acids were excluded. The only sources of nitrogen other than ammonia present were the minute quantities of aneurin (vitamin B_1) and nicotinamide. These were synthetic preparations. The actual constituents of the medium were as follows:

KH_2PO_4	M/30
NaOH	M/37
$FeSO_4.(NH_4)_2SO_4.6H_2O$	M/20,000
$MgSO_4.7H_2O$	M/6000
$(NH_4)_2SO_4$	M/250
Glucose	M/80
Sodium mercaptoacetate	M/1000
Aneurin (vitamin B_1)	$M/1\times10^{-7}$
Nicotinamide	$M/1\times10^{-5}$

Morphological, Cultural and Biochemical Characteristics of Strains Growing on Synthetic Media.

No permanent alteration in morphological, cultural and biochemical characteristics of strains, after growing in these simple controlled media, was

TABLE V.—*Scheme showing the Training of Strain* 1 *to Dispense with Leucine and Strain* 6 *to Dispense with Cystine.*

STRAIN 1.

Medium : Full amino-acid medium	Fewer amino-acids	Ammonia + cystine	Ammonia + cystine + 6 amino-acids	Ammonia + cystine
Leucine required.	*Leucine not required.*	*Leucine required.*	*Leucine not required.*	*Leucine not required.*

* Dotted arrows represent training processes.

STRAIN 6.

Medium : Full amino-acid medium + SH †	Ammonia + SH	Ammonia + SH
Cystine not required (Fildes and Richardson, 1937).	*Cystine required.*	*Cystine not required.*

† SH added as mercaptoacetic acid.

TABLE VI.—*The Elimination of Cystine from the Glucose-Ammonia-Cystine Medium.*

Time growth (hours)	. . .	20.	24.	43.	96.	120.		24.	48.	96.		22.	47.	72.
Strain 6, adapted to utilize NH_3 and grown for 7 subcultures on a medium* containing NH_3, mercaptoacetic acid (M/1000) and cystine (N/16,000) →	NH_3 + mercaptoacetic acid (M/1000) .	0	0	+−	+	++	→ NH_3 + mercaptoacetic acid (M/5000) .	0	0	tr.†	→ NH_3 + mercaptoacetic acid (M/1000)	0	+−	++
	Ditto (M/5000) .	0	0	tr.	+−	+−†								
	Ditto (M/25,000) .	0	0	0	0	0								

Arrows represent subcultures.

* Inoculum centrifuged, washed twice with distilled water and resuspended; about 3×10^6 organisms inoculated.

† Inoculum 2 drops inoculated direct.

found. Gelatin liquefaction, fermentation, coagulase and hæmolysin tests were all positive, when the organism was transferred to the usual laboratory media, even after it had been adapted to grow on the ammonia-cystine medium. During growth in the amino-acid medium a small quantity of hæmolysin was produced and, under certain conditions, this could be considerably increased. These conditions are being investigated and will be reported in a subsequent paper. In the ammonia-cystine medium the organism produced no hæmolysin, although, as previously mentioned, it had not lost the ability to do so when transferred to a more complex medium. No coagulase was produced in any of these media. In the ammonia-cystine medium the growth of some strains tended to be somewhat granular.

DISCUSSION.

These results suggest that differences in nutritional requirements between strains of staphylococci are largely dependent on their previous nutritional conditions. Amino-acids, which initially appear to be essential, on further adaptation are no longer so. Owing to this ease of adaptation, the term " indispensable ", as applied to the amino-acid nutrition of staphylococcus, is therefore not justified. Assuming that the organism requires all these amino-acids in its metabolic processes, the removal of each apparently essential amino-acid in the training process acts as a stimulus for the production of new, or reactivation of latent, synthesizing enzymes.

As Fildes and Richardson (1937) suggest, for an organism to grow in the absence of an essential food substance it must not only possess enzymes capable of synthesizing it, but the constituents of the medium must be capable of being used by it for synthesis. To ensure success in the training process, therefore, both these requirements must be taken into consideration. With Strain 1, for example, although synthesizing enzymes for leucine could be formed in a relatively complex medium, as shown by growth in its absence, the constituents of a simpler medium were not adequate for synthesis and growth without leucine could not occur. Whether the simplified substrate could not be used by existing enzymes or whether enzymes able to synthesize leucine could not initially be formed in the simplified medium cannot yet be determined. By the addition of amino-acids which were not required for growth, conditions were provided which allowed leucine to be synthesized, and this ability persisted after the amino-acids were withdrawn (Table V).

The apparent initial " indispensability " of cystine was not due to its being required as an amino-acid, which could not be synthesized, but to its being, with the exception of an amount of methionine too small to permit growth alone, the only organic form of sulphur present. Fildes and Richardson (1937) showed that, when other adequate forms of organic sulphur together with an adequate supply of amino-acids were present, cystine could be omitted. They concluded that cystine was synthesized from amino-nitrogen and organic sulphur. With the removal of the supply of other amino-acids, however, cystine became important not only as a source of organic sulphur, but also as a source of amino nitrogen ; no growth occurred when it was replaced by mercaptoacetic acid.

With a further training to dispense with amino nitrogen, however, cystine thereupon became wholly dispensable (Table VI).

SUMMARY.

(1) Twenty-five out of 26 strains of staphylococci grew well on a medium of known chemical composition which included 16 amino-acids.

(2) Initial differences in amino-acid requirements were found to exist among these strains. The organisms were easily adapted to utilize fewer amino-acids, when such differences tended to disappear.

(3) Finally, by a process of training, strains were produced which could grow on a medium from which all amino-acids were excluded, and whose main source of nitrogen was ammonia.

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