Amino Acid Utilization by Aerobacter aerogenes and Escherichia coli

by

Rodolfo Eduardo Herrera
A.B., Paris University, 1932
B.Ph., Paris University, 1934

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Signature of Author_____

Professor in Charge of Research_____

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I Introduction

A considerable amount of work has been done on the growth of A.aerogenes and E.coli in synthetic media, but little work has been undertaken on the utilization by these organisms of amino acids as comparative sources of nitrogen. The most valuable study of this subject was made by Koser and Rettger in 1918(17), but it is incomplete due to the fact that only a few amino acids were tried and it also seems to lack in scientific precision. J. Howard Mueller more recently(24-32)worked extensively with amino acids but he was interested in pathogenic organisms(Pneumococcus, Streptococuss hemolyticus and diphtheria Bacillus)more than in the two species we are studying and, furthermore, we do not approve completely of the methods of inoculation and of growth measurement he used.(See under these headings in the following pages.)

Thus there appears to be a great opportunity for further investigation in this field leading to the acquirement of more knowledge with regard to differentiation, classification, intermediate metabolism, and essential growth factors of bacteria.

The object of this thesis is to study the relative availability of the amino acids as sole source of nitrogen in the growth of A.aerogenes and E.coli, when used individually for this purpose and in varying concentration.

II Physical and Chemical Properties of Amino Acids

Before describing the procedure entering the very field of this investigation it seems advisable to summarize some of the most important physical and chemical properties of the amino acids.

(a) Proteins can be broken down ~~by~~ with the aid of strong acids or enzymes into simpler compounds and the end products of the hydrolysis are the amino acids.

(b) Amino acids can be classified according to their structure but the number of these acids varies with the authors of the various classifications. Vickery and Schmidt(43)propose twenty one, W. C. Rose(36)twenty two, and Mitchell and Hamilton(20)believe there may be up to twenty five, while Bodansky(3)says that only nineteen are true amino acids. We prefer Rose's list to the others, but we believe with Vickery and Schmidt, with Bodansky, and with Mitchell and Hamilton that citrulline is not a true amino acid. The following classification,with twenty one amino acids, seems, therefore, the most advisable one to us:

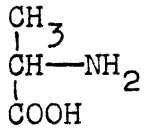
ALIPHATIC AMINO ACIDS.

Monoamino-monocarboxylic Acids.

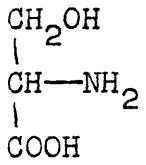
1. Glycine.

$$\begin{matrix} CH_2—NH_2 \\ COOH \end{matrix}$$

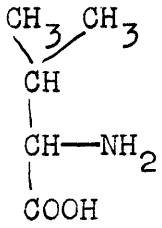
2. d-Alanine.



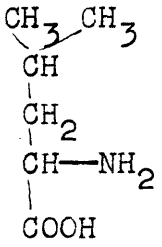
3. l-Serine.



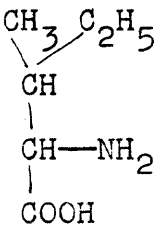
4. d-Valine.



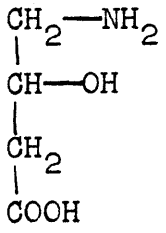
5. l-Leucine.



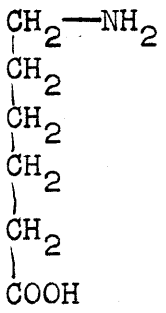
6. d-Isoleucine.



7. α-Amino-β-hydroxy-n-butyric acid.

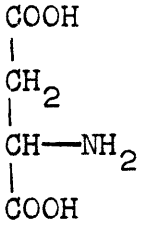


8. nor-Leucine(α-amino-n-caproic acid)

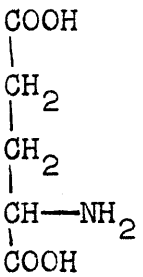


Monoamino-dicarboxylic Acids.

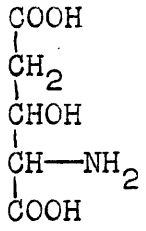
9. l-Aspartic acid.



10. d-Glutamic acid.

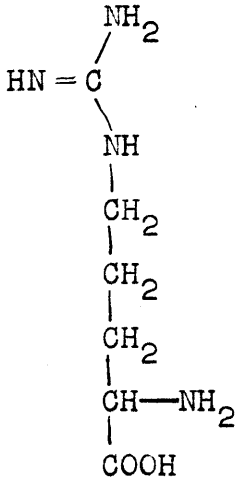


11. d-Hydroxyglutamic acid.

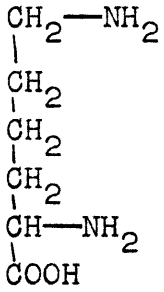


Diamino-monocarboxylic Acids.

12. d-Arginine.

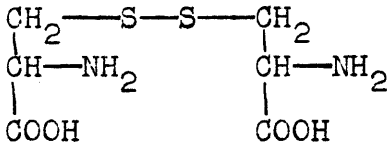


13. d-Lysine

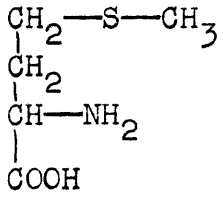


Sulfur-containing Amino Acids.

14. l-Cystine.

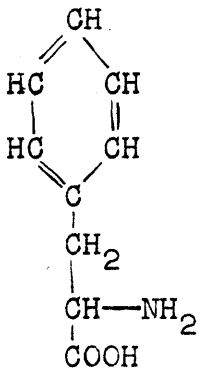


15. l-Methionine.

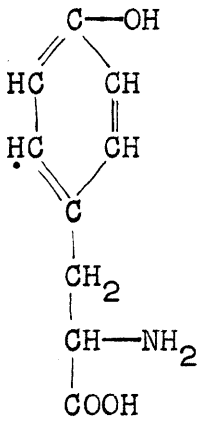


AROMATIC AMINO ACIDS

16. l-Phenylalanine.

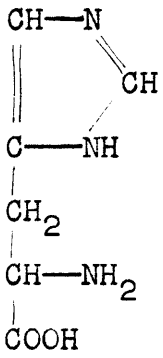


17. l-Tyrosine.

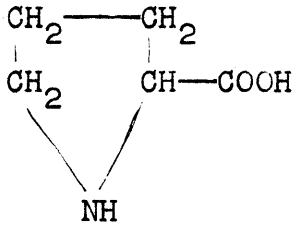


HETEROCYCLIC AMINO ACIDS.

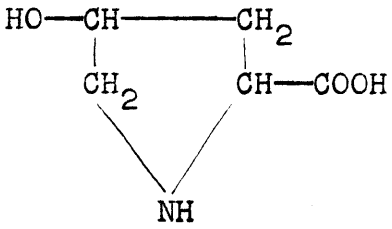
18. l-Histidine.



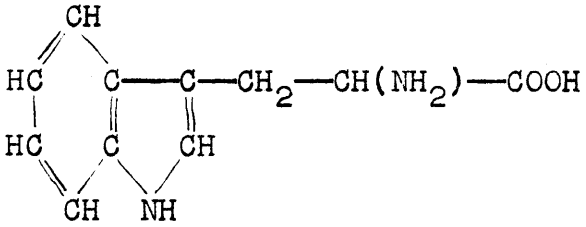
19. l-Proline



20. l-Hydroxyproline.



21. l-Tryptophane.



(c) All the amino acids with the exception of glycine are optically active.

(d) All contain an acid(carboxyl)group and a basic(trivalent nitrogen)group; consequently they all are amphoteric substances or "ampholytes." This is to say that they can form both anions and cations. In other words, if we represent the amino acids by the simple formula NH_2—R—COOH where R denotes some organic radical, such ampholytes can dissociate into$(NH_2—R—COO)^- + (H)^+$, or may react with H_2O to form $(OH)^- + (NH_3—R—COOH)^+$.

Amino acids follow the mass action law and at a given pH(which can be calculated for each one of them by a physico-chemical formula)they have no net charge; at this pH the ampholyte does not migrate to cathode nor anode when an electric current is passed through its solution, since the mobilities of anion and cation are nearly the same. Such a pH is called the "isoelectric point" of the ampholyte. At the isoelectric point some of the physical properties of ampholyte(and consequently of amino acids) are at a minimum: in particular surface tension, electrical charge, and solubility.(Gillespie-14)

(e) The amino acids are white, crystalline substances and the form of the crystals is characteristic for each one of them; they form crystalline salts with metallic bases and with mineral acids. They are usually soluble in water, dilute acids, and alkalies. Tyrosine and aspartic acid, though, are only slightly soluble(even at 25°C only 0.05 g dissolve in 100 g of the solvents mentioned)while cystine is almost insoluble(only 0.01 g dissolves in 100 g of solvent)but they are more soluble in concentrated hydrochloric acid.

III Experimental Work

A. GENERAL CONSIDERATIONS

1. Organisms used

The cultures of E.coli and A.aerogenes used were obtained from stock cultures maintained by the Department of Biology and Public Health of the Massachusetts Institute of Technology. They were kept at 37°C on agar slants and were transplanted every week to new agar slants; thus the cultures used were always young and in active growth. Before each experiment(usually once a week)the cultures were examined morphologically and biochemically so as to be sure they were always pure and typical. They showed the following characteristics throughout the experiments and consequently were considered typical strains of E.coli and A.aerogenes:

	E.coli	A.aerogenes
Morphology	short rods, motile	short rods, motile
gram stain	gram negative	gram negative
agar slant	white, thick, glistening growth	white, thick, glistening growth
Indol (in tryptophane broth)	+	-
NO_2^- (in NO_3^- broth)	+	+
lactose broth	acid & gas	acid & gas
litmus milk	acid & coagulation	acid & coagulation
methyl red	+	-
Voges-Proskauer	-	+
citrate broth	-	+
uric acid broth	-	+

2. Culture medium

a. Choice of medium

So as to have a basic culture medium of known composition and particularly in order to have only the amino acids as sources of nitrogen, a synthetic medium had to be used. Finding Burrows'(7)and Doryland's(11) definitions insufficient, we will define a synthetic medium as one which contains only compounds of known composition and structure in a known concentration.

The medium used by Koser and Rettger(17)seemed advisable since it contained all the ingredients favorable to bacterial growth with the exception of nitrogen(19) (7); its composition is:

NaCl	0.5%
$MgSO_4$	0.02%
$CaCl_2$	0.01%
KH_2PO_4	0.1%
K_2HPO_4	0.1%
Glycerol	3%

in distilled water 1000 cc.

After dissolving the ingredients in 1000 cc distilled water, the pH was adjusted with NaOH to pH = 6.8. To make sure this basic medium offered no growth possibility without the addition of a source of nitrogen, we decided to inoculate some tubes of the basic medium. After tubing we proceeded to sterilize the tubes; but after autoclaving at 15 lbs. pressure for twenty minutes the medium was no longer clear; each tube was so turbid that growth would have been impossible to estimate accurately. The precipitate was soluble in hydrochloric acid and the medium became clear when HCl was added but the pH at which the precipitate was soluble was too low for favorable growth conditions(pH below 6).

To determine which salt was the one causing the turbidity, the following solutions were autoclaved after pH adjustment to pH = 6.8, and turbidity was obtained as indicated below:

Soln. 1	Soln. 2	Soln. 3	Soln. 4	Soln. 5
3% glycerol	glycerol	glycerol	glycerol	glycerol
0.5% NaCl	NaCl	NaCl	NaCl	NaCl
0.01% $CaCl_2$	-	$CaCl_2$	$CaCl_2$	-
0.1% K_2HPO_4	K_2HPO_4	K_2HPO_4	-	K_2HPO_4
0.1% KH_2PO_4	KH_2PO_4	KH_2PO_4	-	KH_2PO_4
0.02% $MgSO_4$	$MgSO_4$	-	$MgSO_4$	-
turbid	turbid	turbid	clear	clear

The turbidity consequently was due to the formation of any or all of the following insoluble phosphates: $Ca_3Mg_3(PO_4)_4$, $Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$. (Treadwell-41). Koser(16)reports that after autoclaving "a cloudiness was present which disappeared upon standing," and this statement is not understandable since we reproduced exactly the same conditions he mentions and still the cloudiness did not disappear upon standing. Mueller(24)who used this same medium in some cases says that "a good deal(of Ca and Mg)is probably lost in the precipitate which usually forms on adjusting the reaction and boiling." As far as we are concerned practically all of

the Ca and Mg is lost in the precipitate. There were two solutions to the problem offered by Koser's medium: either filter through Berkefeld instead of autoclaving, or else do without the Ca and Mg. The latter solution was preferred and consequently the following medium was chosen for our work after careful experimentation:

NaCl	0.5%
K_2HPO_4	0.25%
KH_2PO_4	0.25%
Glycerol	3%

In distilled water 1000 cc.

It proved to be favorable to growth when a source of nitrogen(NH_4NO_3)was added, and the concentration of the salts (which was chosen after experimentation)provided not only an adequate buffering action but also a pH which needed no adjustment since it was of 6.8.

b. Preparation of the medium

(1) Two thousand cc were prepared at a time as follows:

10g of NaCl (C. P.)
5g of K_2HPO_4 (C. P.)
5g of KH_2PO_4 (C. P.)
60g of Glycerol(C. P.)

were dissolved by shaking in 2000 cc of distilled water. The solution was tested for ammonia with Nessler reagent- (50g of KI in 35cc of cold ammonia-free distilled water; 415cc saturated solution of $HgCl_2$ - until slight persistent precipitate; 400cc of 9N, NaOH; dilute to 1000cc, let stand a few days, and filter.) This testing was repeated before each experiment throughout this work and NH_3 was never found to be present in the basic uninoculated medium either before or after incubation. The basic medium thus prepared was then weighed in its flask and before being used it was always made up to the recorded weight with ammonia-free water so as to remedy the evaporation and consequent concentration.

(2) Seventy five cc portions of the basic medium were used to dissolve the various amino acids; and the correct amounts of these were weighed so as to obtain the following five different concentrations: 0.2% - 0.1% - 0.05% - 0.01% and 0.005%. After working with the first three amino acids it was found that the three highest concentrations were the most favorable ones for growth, and since the two lowest concentrations could not be very reliable due to the smallness of the amounts to weigh, three different concentrations were used subsequently: 0.2% - 0.1% and 0.05%.

(3) The 75cc solution of amino acid was titrated colorimetrically and found to have a pH = 6.8 in almost every case. If the pH was lower or higher than 6.8 it was adjusted to the desired value with N, NaOH or N, HCl.

(4) Fifty 10 x 1.2 cm test tubes containing 5cc of water were plugged and autoclaved at 15 pounds pressure for twenty minutes, and by measuring the loss of weight of the tubes it was found that on the average about 0.1cc of water evaporated during sterilization; consequently, before tubing, $\frac{0.1 \times 70}{5} = 1.4$cc of ammonia-free distilled water were added to the 70cc amino acid solution(70cc because 5cc were used for pH estimation). And thus the concentration of the ingredients in the tubes after sterilization was not appreciably different from that in the basic medium prepared originally.

(5) Five cc of the solution were introduced into twelve tubes with a pipette. The tubes were plugged with non-absorbent cotton and sterilized at 15 pounds pressure for twenty minutes. The twelve tubes are used as follows: 5 for A.aerogenes, 5 for E.coli, and 2 as controls (not to be inoculated). Five cc amounts are used in 10 x 1.2 cm tubes, instead of 10cc amounts in the usual 20cm tubes, for reasons of economy mainly, due to the high cost ~~prices~~ of some of the amino acids.

The amino acids were obtained from the Research Laboratory of the Eastman Kodak Company or Hoffmann-La Roche & Company and were guaranteed "in regard to identity and quality." They were kept in tightly stoppered brown glass bottles, away from heat and reagents. Glycine, dl-valine, l-tryptophane, dl-methionine, dl-β-phenylalanine, l-leucine, dl-isoleucine, dl-alanine, dl-serine, l-proline, glutamic acid, l-hydroxyproline, l-tyrosine, aspartic acid, l-cystine, and nor-leucine were used as such; while d-arginine, and l-hystidine were used as monohydrochlorides, and d-lysine as dihydrochloride; α-amino-β-hydroxy-n-butyric acid, d-hydroxyglutamic acid, were the only amino acids not experimented with since they could be procured from none of the leading Chemical Companies. Tyrosine, aspartic acid, and cystine had been obtained as such but due to their insolubility in water had to be dissolved in concentrated HCl (sp. grav. = 1.19)and since their solution was brought back to pH = 6.8, they were considered to be in solution partly as pure amino acids and partly as hydrochlorides. In the case of these last three amino acids and due again to their low solubility even in HCl, only the two lowest concentrations, instead of the three usual ones(0.2%, 0.1% and 0.05%), were used.

3. Technique

In addition to the special technique used for the preparation of the medium and already described, the following methods were also used:

a. Before each experiment the glassware(test tubes, flasks, and pipettes)was soaked for one-half hour in cleaning solution(10g of potassium dichromate are dissolved in 25cc of distilled water with the aid of heat; then, after cooling, 325cc of concentrated sulphuric acid are added slowly while stirring)then rinsed thoroughly(five times) with tap water and(three times)with ammonia-free distilled water. Thus we were sure that no organic matter(and especially no nitrogen)was available for the organisms.

b. It has been reported(16&5)that the ammonia given off by some bacteria in the incubator can be utilized by others(among which E.coli)which have none in their medium; so for greater security, we inoculated and incubated two tubes with basic medium alone(no source of nitrogen)together with the other tubes.

c. It has been reported also that the cleaning solution may be absorbed by the glass test tubes, and subsequently have a toxic effect on bacteria; hence, in addition, we also inoculated and incubated two tubes containing the basic medium + 0.05% NH_4NO_3(as source of nitrogen).

Consequently, each time we experimented with a given concentration of a given amino acid, we had fourteen tubes to incubate:

Group 1: 5 tubes with basic medium + amino acid, inoculated with E.coli;

Group 2: 5 tubes with basic medium + amino acid, inoculated with A.aerogenes;

Group 3: 2 tubes with basic medium + amino acid, not inoculated;

Group 4: 2 tubes with basic medium alone inoculated one with E.coli and one with A.aerogenes;

Group 5: 2 tubes with basic medium + NH_4NO_3 inoculated one with E.coli and one with A.aerogenes.

And before considering any experiment of any value, we had to fulfill the following requirements with the controls: No growth at all in the 2 "Group 3" tubes; no growth at all in the 2 "Group 4" tubes; and abundant growth in the 2 "Group 5" tubes.

4. Method of inoculation and of growth measurement

To make this work as quantitatively and scientifically accurate as possible, a great deal of attention was payed to the choice of the method for estimation of growth. We realized that the appreciation of growth by the turbidity if done with the naked eye would allow for great inaccuracy, but we also realized that direct counting and plate counting

would require too much time. The method which appeared best was that involving the use of a photoelectric turbidimeter or nephelometer. The nephelometer, constructed by Jennison of the Department of Biology and Public Health of the Massachusetts Institute of Technology and described by Breed (6) was used throughout the work. But it has been shown(15 & 39)that, contrary to what Richards and Jahn(35) report, the light absorbed is not "directly proportional to the number of cells": changes in size and cytological content must be taken into consideration, and due to a physical phenomenon involving probably relations between absorption and reflection of light by the cells, "there is no straight relationship between direct counts of bacteria and readings in microamperes of the nephelometer, unless the mean cell size is the same." Since this condition does not exist, even among organisms of a single strain, it would have been necessary to standardize the nephelometer for each organism in each different amino acid solution. Therefore, it was decided not to have recourse to such a tedious and time-consuming method; and the nephelometer was used in a partly quantitative manner. We did not transcribe the microampere readings into the number of cells

but the growth was expressed as the difference between the reading of the microammeter when a sterile tube of the given medium was placed in the nephelometer; and the reading of the microammeter when an inoculated tube of the same medium was placed in the nephelometer; these two tubes had been sterilized, incubated, etc., under exactly the same conditions.

The method of growth measurement used by Mueller(24) involves determining the bacterial cell nitrogen with Pregl's micro-kjeldahl method(34)after sterilizing the incubated tubes, centrifuging them and washing the cells. This method is longer than the photoelectric one but it seems more quantitative. The difficulty it involves, though, is that of washing the cells thoroughly enough to eliminate all the nitrogen-containing culture medium which may have stayed between the cells; but such a thorough washing will probably allow some of the nitrogenous cellular content to be extracted from the cell and lost(by osmosis.). This difficulty, it seems to us, will outbalance the quantitative superiority of Mueller's method and consequently we think that the nephelometric method will give us as reliable results in a shorter and (more easier easy) way.

The Mueller method presents another disadvantage in our opinion: that of introducing a technique which allows for a large "personal factor," to a much greater extent than the nephelometric method does.

In addition we do not approve completely of the method Mueller used for inoculation of the tubes(24): he inoculated with a loopful of a 48 hour culture of the orgamism in nutrient broth. The loop will most likely introduce the same volume of culture each time but, due to uncontrollable factors, it seems very probable that the number of organisms in that constant volume will not be constant itself; and furthermore, with the organisms, some broth will be introduced into the synthetic medium.

For the present work we chose a method which seems preferable to us: we put into suspension in some sterile ammonia-free water the bacterial growth of the organism after incubation at 37°C for 48 hours on agar slants. This suspension was diluted with ammonia-free water until, when introduced in the nephelometer, it caused a deviation of exactly 220 microamperes. In other words, a tube with distilled water gave a reading of 340 microamperes and the tube with the bacterial suspension was diluted so as to give a reading of 120 microamperes: 340 - 120 = 220 microamperes deviation. The tubes of synthetic medium were then inoculated with one drop of the prepared standardized suspension of the organisms as delivered by a standard one cc

volumetric pipette which was used throughout the experiments(held at as much the same angle as possible each time). This pipette delivered the one cc in 12 drops; in other words, one pipette drop = $\frac{1}{12}$ cc.

A voltage of 110 volts(as read by the voltmeter on the nephelometer)was used throughout this work. Also, all the tubes were as similar as possible: same diameter, same thickness, and same color of glass; they gave very approximately the same reading - within 4 microamperes - in the nephelometer when filled with distilled water, and they were always cleaned externally and shaken vigorously (stoppered with sterile stopper)before being introduced into the apparatus. In addition, care was always taken to let the nephelometer "warm up" before using it.

5. Summarized procedure used

To summarize, this is the procedure followed for this experimentation.

a. Each amino acid was dissolved in the basic medium in the desired concentration(page 16).

b. Twelve thoroughly washed test tubes were filled with 5cc amounts of the prepared amino acid solution(page 17) and 4 controls were prepared in similar tubes and similar amounts(page 19).

All tubes prepared were sterilized by autoclaving them at 15 pounds pressure for twenty minutes.

c. A standard suspension of each of the two organisms was prepared(page 23).

d. Ten amino acid tubes were inoculated with one standard drop of the bacterial suspensions - five with the A.aerogenes suspension and five with the E.coli suspension. And similar inoculation was done to four of the controls - one tube of basic medium alone and one of basic medium + NH_4NO_3 with A.aerogenes, one tube of basic medium alone and one of basic medium + NH_4NO_3 with E.coli(page 20).

e. The fourteen inoculated tubes, together with the two non-inoculated amino acid solution controls, were incubated for 48 hours at $37^{\circ}C$.

f. After incubation the tubes were introduced (properly cleaned and shaken)into the nephelometer. All readings were recorded and the controls examined particularly for reliability of results(page 20).

g. The growth of the organisms in the amino acid tubes was reported in terms of the microampere deviation it caused in the nephelometer(page 20). If we call the reading of the inoculated tube(of amino acid solution)R_1, and the

reading of the non-inoculated control tube(of the same amino acid solution)R_c, the growth G will be expressed by

$$G = R_c - R_i$$

Since R_c was 235 throughout the experiments, the value of G was always obtained from the expression

$$G = 235 - R_i$$

The standardized bacterial suspensions had been chosen so that when used to inoculate tubes of nutrient broth, the mean value of G was close to 100. Hence, G = 100 will be considered the maximum growth, and the values of G may be interpreted as percentages.

B. RESULTS

For greater clarity the results will be reported in tabular form - one table for each amino acid studied - followed by a short interpretation. The nephelometer readings will be recorded so that the similarity of these readings(and consequently their reliability)will be seen. Since the nephelometer gave different readings when the tubes were rotated, the highest reading was always the one recorded. Differences in growth below 5% were never considered significant.

Table 1. Utilization of Glycine by A.aerogenes and E.coli

Glycine Concentrations		0.2%	0.1%	0.05%	0.01%	0.005%
A.aerogenes	Nephelometer Readings	185	205	180	215	210
		210	205	185	215	215
		210	210	215	220	225
		210	205	215	215	225
		210	190	220	225	215
	Average Growth	30	33	32	17	17
E.coli	Nephelometer Readings	215	175	205	215	215
		205	185	215	220	220
		215	180	210	220	225
		215	175	210	225	225
		215	175	215	230	225
	Average Growth	23	57	22	13	15

Glycine is readily utilized by the two organisms; it is a somewhat better source of nitrogen for A.aerogenes than for E.coli in all but one of the concentrations used. For both organisms the three highest concentrations of amino acid were the most propitious ones, but the 0.1% concentration besides being appreciably the most favorable one for E.coli, also allowed a growth of this organism about 25% more abundant than the corresponding aerogenes one.

Table 2. Utilization of dl-alanine by A.aerogenes and E.coli

dl-Alanine Concentrations		0.2%	0.1%	0.05%	0.01%	0.005%
A.aerogenes	Nephelometer Readings	210	212	215	205	225
		210	210	210	210	220
		200	210	210	210	225
		210	210	205	215	225
		200	202	215	215	215
	Average Growth	29	26.2	24	24	15
E.coli	Nephelometer Readings	210	215	210	210	225
		205	210	210	205	225
		205	210	205	210	215
		205	210	210	210	225
		210	205	210	215	215
	Average Growth	28	25	26	25	14

dl-Alanine was as available to E.coli as to A.aerogenes. The highest concentrations of the amino acid were more favorable to growth and the concentration had no differential value.

Table 3. Utilization of dl-serine by A.aerogenes and E.coli

dl-Serine Concentrations		0.2%	0.1%	0.05%	0.01%	0.005%
A.aerogenes	Nephelometer Readings	200	205	205	215	215
		190	200	200	215	210
		200	200	195	210	215
		200	195	215	210	215
		210	200	210	210	215
	Average Growth	35	35	30	23	21
E.coli	Nephelometer Readings	210	210	210	210	215
		200	215	205	215	210
		215	215	215	215	215
		180	215	210	220	215
		185	215	205	220	220
	Average Growth	37	21	26	19	20

dl-Serine was utilized as easily by the two organisms. The amino acid concentration had no appreciable value for differential availability but, as in the case of glycine and of dl-alanine, the three highest concentrations were the most favorable ones for growth. This, in addition to the fact that considerable inaccuracy was apt to exist in the two lowest concentrations(see page 16 (2)), made us decide to experiment only with the 0.2, 0.1, and 0.05% concentrations in the future.

Table 4. Utilization of dl-valine by A.aerogenes and E.coli

dl-Valine Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	225	235	220
		220	225	225
		220	220	225
		225	220	225
		225	215	225
	Average Growth	12	13	11
E.coli	Nephelometer Readings	222	223	230
		225	227	230
		227	230	225
		225	220	220
		225	222	225
	Average Growth	10.2	10.6	9

dl-Valine was not a good source of nitrogen for either A.aerogenes or E.coli. The concentration of this amino acid was unimportant(at least in the range of concentrations we experimented with).

Table 5. Utilization of l-leucine by A.aerogenes and E.coli

l-Leucine Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	200	210	200
		205	185	205
		205	202	205
		195	215	205
		205	195	205
	Average Growth	33	33.6	31
E.coli	Nephelometer Readings	210	212	210
		210	210	215
		212	210	215
		212	207	215
		210	210	215
	Average Growth	24.2	25.2	21

l-Leucine was about a 9% better source of nitrogen for A.aerogenes than for E.coli. The concentration of the amino acid did not appear to be of differential value.

Table 6. Utilization of dl-isoleucine by A.aerogenes and E.coli

dl-Isoleucine Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	225	220	225
		225	215	225
		220	215	225
		225	210	225
		225	215	225
	Average Growth	11	20	10
E.coli	Nephelometer Readings	225	230	230
		225	220	232
		230	230	232
		230	230	232
		225	230	232
	Average Growth	8	5	3.4

dl-Isoleucine was a rather unpropitious source of Nitrogen for the two organisms and especially so for E.coli. The 0.1% concentration was not only the most favorable one for A. aerogenes, but also 15% more so for this organism than for E.coli.

Table 7. Utilization of nor-leucine by A.aerogenes and E.coli

nor-Leucine Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	215	215	212
		215	210	215
		210	225	210
		215	220	210
		210	210	210
	Average Growth	22	19	23.6
E.coli	Nephelometer Readings	230	225	230
		230	239	230
		227	230	227
		222	225	232
		225	227	225
	Average Growth	8.2	7.2	6.2

nor-Leucine like isoleucine was utilized with difficulty by E.coli which grew 15% less abundantly than A.aerogenes. No concentration of amino acid seemed to be optimum for growth or of differential value.

Table 8. Utilization of l-aspartic acid by A.aerogenes and E.coli

l-Aspartic acid Concentrations		0.1%	0.05%
A.aerogenes	Nephelometer Readings	180	185
		185	195
		185	195
		190	180
		185	190
	Average Growth	50	46
E.coli	Nephelometer Readings	205	205
		200	205
		195	205
		200	205
		205	210
	Average Growth	34	29

This amino acid had to be dissolved in HCl(see page 18). The 0.2% concentration was not considered reliable since some of the amino acid precipitated out during adjustment of pH and consequently was not inoculated. Aspartic acid was utilized readily by the two organisms, and it was about 15% more favorable to A.aerogenes than to E.coli.

Table 9. Utilization of glutamic acid by A.aerogenes and E.coli

Glutamic acid Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	195	200	190
		195	195	195
		185	195	195
		190	195	195
		190	195	190
	Average Growth	44	39	42
E.coli	Nephelometer Readings	190	200	200
		195	195	205
		195	195	205
		200	195	200
		195	210	200
	Average Growth	40	36	35

Glutamic acid was as favorable to A.aerogenes as to E.coli at all concentrations.

Table 10. Utilization of dl-arginine monohydrochloride by A.aerogenes and E.coli

dl-Arginine Monohydrochloride Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	195	200	200
		200	202	200
		195	200	200
		195	200	205
		190	195	205
	Average Growth	40	35.6	35
E.coli	Nephelometer Readings	210	220	215
		210	210	210
		210	210	210
		210	210	210
		210	210	207
	Average Growth	27	23	24.6

dl-Arginine monohydrochloride was utilized about 12% more easily by A.aerogenes than by E.coli; the concentrations of the amino acid had no appreciable effects on growth.

Table 11. Utilization of d-lysine dihydrochloride by A.aerogenes and E.coli

d-Lysine Dihydrochloride Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	220	210	220
		210	215	215
		215	215	210
		215	215	215
		220	220	215
	Average Growth	19	20	20
E.coli	Nephelometer Readings	220	220	220
		215	225	225
		215	220	220
		215	210	220
		220	215	225
	Average Growth	18	17	15

d-Lysine dihydrochloride was as moderately propitious to A.aerogenes as to E.coli. There was no optimum concentration.

Table 12. Utilization of l-cystine by A.aerogenes and E.coli

l-Cystine Concentrations		0.1%	0.05%
A.aerogenes	Nephelometer Readings	205	195
		205	205
		205	205
		205	200
		205	205
	Average Growth	30	33
E.coli	Nephelometer Readings	150	165
		160	150
		150	160
		150	150
		140	150
	Average Growth	85	80

l-Cystine had to be dissolved in HCl and as in the case of aspartic acid only two concentrations could be tried. This amino acid is readily utilized by the two organisms, but it is considerably more favorable(about 50%)to E.coli than to A.aerogenes. ~~In the case of~~ with no other amino acid is the difference so greatly marked.

Table 13. Utilization of dl-methionine by A.aerogenes and E.coli

dl-Methionine Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	210	210	215
		210	210	210
		205	205	205
		205	205	210
		210	205	210
	Average Growth	27	28	25
E.coli	Nephelometer Readings	210	210	220
		215	210	220
		220	215	210
		220	215	210
		210	215	220
	Average Growth	20	22	17

dl-Methionine is not appreciably more favorable to A.aerogenes than to E.coli(only about 7% more). This amino acid is a moderately good source of nitrogen for the organisms and its concentration was not of differential value.

Table 14. Utilization of dl-β-phenylalanine by A.aerogenes and E.coli

dl-β-Phenylalanine Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	205	205	210
		210	205	205
		205	205	210
		210	200	200
		205	200	200
	Average Growth	28	32	27
E.coli	Nephelometer Readings	225	220	220
		225	210	225
		215	215	220
		220	215	210
		225	220	220
	Average Growth	15	19	16

dl-β-Phenylalanine was about 12% more favorable to A.aerogenes than to E.coli at all concentrations.

Table 15. Utilization of tyrosine by A.aerogenes and E.coli

Tyrosine Concentrations		0.1%	0.05%
A.aerogenes	Nephelometer Readings	200	205
		210	210
		200	205
		200	210
		200	210
	Average Growth	30	27
E.coli	Nephelometer Readings	215	215
		215	215
		215	210
		220	210
		202	215
	Average Growth	21.5	22

As in the case of aspartic acid and of cystine, since it also had to be dissolved in HCl, only two concentrations of tyrosine were tried. This amino acid is readily used by the two organisms, - slightly more so(about 6%)by A.aerogenes.

Table 16. Utilization of l-histidine monohydrochloride by A.aerogenes and E.coli

l-Histidine monohydrochloride Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	170	175	175
		165	180	175
		175	180	175
		167	175	175
		170	185	175
	Average Growth	65.6	56	60
E.coli	Nephelometer Readings	175	182	180
		175	185	185
		180	195	187
		175	175	180
		175	180	180
	Average Growth	59	51.6	52.6

l-Histidine monohydrochloride is a very good source of nitrogen for the organisms. No other amino acid is more readily utilized by A.aerogenes; and it is second only to cystine for E.coli. A.aerogenes did not grow appreciably (only about 7%)better than E.coli at any concentration of this amino acid.

Table 17. Utilization of l-proline by A.aerogenes and E.coli

l-Proline Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	195	192	205
		195	190	205
		200	200	205
		195	190	200
		195	190	200
	Average Growth	39	40.6	34
E.coli	Nephelometer Readings	185	185	180
		185	195	180
		190	190	190
		190	170	190
		190	180	190
	Average Growth	47	53	49

l-Proline is very easily utilized by the two organisms and about 12% more so by E.coli than by A.aerogenes. The concentration of the amino acid had no effect on the availability of the latter.

Table 18. Utilization of l-hydroxyproline by A.aerogenes and E.coli

l-Hydroxyproline Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	185	175	185
		190	200	187
		190	185	185
		195	180	190
		190	185	190
	Average Growth	45	50	47.6
E.coli	Nephelometer Readings	210	210	205
		215	217	215
		215	210	215
		215	215	220
		210	200	215
	Average Growth	22	24.6	21

l-Hydroxyproline is an appreciably better source of nitrogen for A.aerogenes than for E.coli. With no other amino acid is the difference(about 25%)in favor of aerogenes so greatly marked.

Table 19. Utilization of l-tryptophane by A.aerogenes and E.coli

l-Tryptophane Concentrations		0.2%	0.1%	0.05%
A.aerogenes	Nephelometer Readings	190	200	210
		195	200	215
		190	200	215
		200	190	210
		205	200	215
	Average Growth	39	37	22
E.coli	Nephelometer Readings	190	215	215
		200	205	215
		195	205	215
		205	205	215
		205	185	220
	Average Growth	36	32	17

l-Tryptophane was as readily utilized by A.aerogenes as by E.coli; the highest concentrations were more favorable for growth. The concentration of amino acid had no effect on the production of indol by E.coli.

IV Conclusions

1. Although we cannot say, as for man, that there are "essential amino acids" for A.aerogenes and E.coli since all those studied allowed these organisms to grow, we may say, though, that some amino acids are a "first class" source of nitrogen, while others may be classified as "second class," and others as "third class." The same amino acids are not in the same class for the two organisms, or, in other words, a "first class" amino acid for A.aerogenes is not necessarily a "first class" one for E.coli and vice versa.

The first class amino acids are:

For A.aerogenes	For E.coli
l-histidine	cystine
l-hydroxyproline	glycine(in 0.1% concentration)
l-aspartic acid	l-histidine
	l-proline

The second class amino acids are:

For A.aerogenes	For E.coli
glutamic acid	glutamic acid
l-proline	l-tryptophane
l-tryptophane	l-aspartic acid
dl-arginine	dl-arginine
l-leucine	dl-serine
glycine	dl-alanine

The second class amino acids are: (Continued)

For A.aerogenes	For E.coli
dl-serine	l-leucine
l-cystine	l-hydroxyproline
tyrosine	tyrosine
dl-β-phenylalanine	dl-methionine
dl-methionine	
dl-alanine	
nor-leucine	
d-lysine	

The third class amino acids are:

For A.aerogenes	For E.coli
dl-isoleucine	d-lysine
dl-valine	dl-β-phenylalanine
	dl-valine
	nor-leucine
	dl-isoleucine

This is naturally an arbitrary classification due to the arbitrary choice of borderlines for the classes, but it does give, we think, an idea of the readiness with which the amino acids can be utilized by A.aerogenes and by E.coli.

2. Although the 0.1% concentration of amino acid was usually the most favorable one, we may say that in most of the cases the concentration had no appreciable effect on the availability of the amino acid. There was one exception to this rule in the case of glycine which was at least 30% more readily utilized by E.coli in the 0.1% concentration than in any other of the concentrations tried.

3. Of the 19 amino acids and at the concentrations of these experimented with, 15 were utilized more readily by A.aerogenes than by E.coli. Among these, l-hydroxyproline allowed aerogenes to grow 25% better than coli; and this was the greatest differential value ~~showed~~ demonstrated by any amino acid in favor of A.aerogenes. Of the three amino acids which were more available to coli than to aerogenes, glycine(in the 0.1% concentration)favored coli 25% and cystine 50%. The difference ~~showed~~ by cystine was a striking one.

4. We only worked with a single strain of E.coli and a single strain of A.aerogenes and it would be interesting to perform similar experiments with various strains of these organisms. The solutions we used contained a single amino acid and a large field remains open for further investigation by combining the acids and using them in mixtures.

Another possibility for promising research is offered by varying more extensively the concentration of the amino acids which have proved to favor one of the organisms. In the case of cystine particularly we are inclined to believe that, by using lower concentrations of it, a point may be reached where the growth of E.coli will be even more than 50% greater than that of A.aerogenes.

In conclusion let us say that the field of this research is an almost infinite one, but the fact that it is not only interesting but promising too, makes it a tempting subject for further extensive, intelligent investigation.

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