SOME AMINO ACID REQUIREMENTS OF SALMONELLA

by

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TABLE OF CONTENTS

INTRODUCTION

In the past, a number of investigations have been made to determine the growth requirements of many different organisms. This has been done in an effort to gain more exact information relative to sources of carbon which may be used by the bacterial cell for energy, and sources of nitrogen which may be used for reproduction and maintenance. In addition to the sources of carbon and nitrogen, the role played by other accessory factors in growth, such as vitamins and minerals, also has received much attention.

With different organisms, there is a wide variance in the nutritional requirements for optimum growth, particularly with respect to sources of carbon and nitrogen. It can be generally stated, however, that organic sources have proved of special importance to most microorganisms and are most widely used.

The problem of nutritional factors has been of increasing importance to the bacteriologist. Knowledge gained in this field has enabled him to place more reliance in his work, since the more exact control of cultural factors, which frequently vary widely from laboratory to laboratory, is possible. It has also simplified his work by making possible the substitution of simple media for the more complex peptones, tissue extracts, animal extracts, and other materials.

Knowledge of the exact nutritional conditions which control the growth and multiplication of different bacteria may be vitally important in the identification of organisms and in regulating the production of toxins, antigenic properties, antibiotics, and numer-

ous products that are of value from an economic and industrial point of view. Such knowledge also may be of extreme value in the bioassay field.

Koser and Saunders (1938) have stated:

> Our knowledge of the requirements of a good basal media, though constantly being revised, is still far from satisfactory.....Many organisms still have many unknown factors to determine, including their actual needs in regard to their amino acids and other nitrogenous ingredients. The inorganic salts and their proper physiological balance and other factors as osmotic, surface and interfacial tension, gaseous environment, redox potential, and kindred conditions still lie in doubt.

Since the above was written, much work along the suggested lines has been undertaken and many factors previously unknown have been discovered. The following work was undertaken with a desire to gain a better understanding of how some of these factors are related to the growth of certain members of Salmonella.

REVIEW OF LITERATURE

The first member of this large group of bacteria was described by Gartner (1888), who had isolated the organism from diseased beef responsible for an outbreak of gastroenteritis. He named the organism Bacillus enteritidis. The name Salmonella was given this genus by Lignieries in 1900.

Uschinsky (1893) was probably the first to report on the nutritional requirements of Salmonella. This work was incidental in itself as Uschinsky was working primarily to develop an artificial medium upon which Clostridium tetani could be grown. From this work was developed a basal medium from which numerous modifications have been made and used by many workers to determine the growth requirements of organisms. His basal medium consisted of distilled

water, glycerine, sodium chloride, calcium chloride, magnesium sulfate, dipotassium hydrogen phosphate, ammonium lactate, and sodium asparaginate. With this he was able to grow a strain of S. typhosa.

Zunz and Gyorgy (1916) found, however, that the typhoid bacillus had rather exacting nitrogenous requirements and that certain peptides used in combination with each other proved to be acceptable. Their best medium for growth included sodium chloride, glycyltryptophane, and d-histidine. With this medium they found indole to be a metabolic product of the organism. There is some disagreement over this point in the literature. Bergy's Manual Sixth Edition (1948), states that no indole is produced by the genus Salmonella. Burrows (1938, 1939), using the Rawlings, Hopkins, Sommersby, and Bormvic strains of S. typhosa, found that both tryptophane and indole were produced in a medium which included ammonium salts, with tryptophane as a starter. Burrows stated that insufficient indole is produced to be detected by the standard Ehrlich's reagent. (From 0.003 to 0.004 mg of indole per ml was found and this is slightly below the sensitivity of the test.)

Koser and Rettger (1919), in a survey of literature prior to 1919, noted a tendency to avoid the more complex organic compound and to center attention on the simpler inorganic nitrogen compounds. Using a modification of Uschinsky's basal medium, they found that dextrose differed very slightly from glycerol in providing a source of carbon. With S. paratyphi and S. schottmuelleri, they found glycocoll, aspartic acid, lysine, phenylalanine, and histidine, added singly to the basal medium supported growth. With urea, very scanty growth could be found. Similar results were reported by

Kisch (1918). No growth could be found with S. typhosa and S. pullorum. However, a combination of the previously mentioned materials with valine, glutamic acid, tryptophane, taurine, creatine, and allantoin provided support for these organisms. Tryptophane proved to be essential for growth of the typhoid bacillus. Taurine and creatine failed to supply the necessary nitrogen sources.

Kisch (1918), using strains of S. paratyphi, S. schottmuelleri, S. typhosa, S. typhi-murium, and S. enteritidis, investigated various inorganic and organic sources of nitrogen. He found that only S. enteritidis grew in calcium nitrate and calcium nitrite. With ammonium salts (including the phosphate, chloride, sulfate, and carbonate) almost identical results were obtained; i.e., poor growth with S. typhosa and S. typhi-murium, none with S. paratyphi, and growth with S. schottmuelleri, and S. enteritidis. With asparagine, doubtful growth was accorded S. paratyphi while excellent growth was obtained with the other organisms studied. These observations agree with those of Uschinsky (1893) and Robertson (1924). Kisch concluded that S. paratyphi is a "peptone" bacillus and thought that some amino acids might prove favorable to growth.

Brawn and Cahn-Bronner (1922) found non-ammonia assimilating strains of S. typhosa and S. enteritidis could be grown with either tyrosine, alanine, or leucine.

Brawn and Guggenheim (1933), investigating cyanide as a possible source of nitrogen, found that it completely inhibited growth, even when used in conjunction with different peptones.

Vasarhelyi (1934), studying strains of S. typhosa, found that certain strains required organic nitrogen while others could uti-

lize inorganic nitrogen. He assigned the term "pretentious strains" to those which required organic nitrogen. These strains grew best in a medium composed of tryptophane, sodium lactate, sodium asparaginate, tyrosine, and glutamic acid.

Fildes et al. (1933), using a mixture of amino acids which included alanine, glycine, leucine, valine, glutamic acid, asparagine, tyrosine, phenylalanine, proline, histidine, arginine, lysine, cystine and tryptophane, found that fine growth with the Rawling's strain of S. typhosa could be achieved. They further demonstrated that they could, by diminishing the concentration of the essential nitrogen, tryptophane, induce growth with ammonia as the sole source of nitrogen. They concluded that this process of acclimation was due to some physiological development or to the reactivation of certain enzymes. Furthermore, they found that the omission of cystine in particular, and also glycine, valine, glutamic acid, and histidine, delayed growth more or less. Their strains acclimated to utilize ammonia no longer required either lysine or leucine, but the absence of these compounds caused a delay in growth.

Further work by Fildes (1940) revealed that the failure of certain strains of S. typhosa to grow with ammonia as the source of nitrogen lay in the inability of the organism to synthesize indole. Gradual adaptation of the organism must in some way bring about regeneration of the enzyme necessary for the utilization of ammonia. This condition was proved by the introduction of indole into the basal medium.

Beckwith and Geary (1940) found that indole 3-acetic acid was stimulatory to the growth of S. typhosa in high dilutions, especially

at 1-3,000,000. They found further that if a concentration of 1-1,000 was present, an inhibitory effect could be observed.

Brawn and Silberstein (1943) noted that certain strains of S. typhosa unable to utilize ammonia could be made to do so by the simple addition of hydrogen sulfide.

Burrows found that threonine was essential for his strains of S. typhosa. The minimum concentration of tryptophane lay between 0.0005 and 0.0001 percent. He believed that tryptophane was needed only as a starter and afterwards the organism could get along without it. One of his strains grew with either glutamic acid or arginine but not with tryptophane, while another grew with tryptophane or lysine.

It might be emphasized further that wide variances in the nutritive requirements of the Salmonella have been recorded. S. typhosa, S. enteritidus, S. typhi-murium, and S. schottmuelleri can grow in relatively simple media. With S. paratyphi, S. pullorum and S. gallinarum, the requirements are far more complex and may be considered actually fastidious.

The most recent work which has been reported concerning the amino acid requirements of Salmonella was done by Johnson and Rettger (1943). They found that tryptophane was the only indispensable amino acid required by five strains of S. typhosa and these strains did not require vitamins or glucose. Apparently there was some utilization of the amino acids as a source of carbon. With S. pullorum and S. gallinarum, they found that the following mixture of amino acids would support growth; glycine, alanine, serine, valine, leucine, aspartic acid, glutamic acid, proline, phenylalanine, tyrosine,

lysine, methionine, arginine, histidine, cystine and tryptophane. Some strains of S. pullorum required nicotinic acid and most strains required glucose. The addition of 1 percent thioglycollic acid in 1 N HCl helped cut down the lag phase of other strains of S. pullorum. With S. gallinarum, vitamin B_1 and an atmosphere of 10 percent CO_2 was found to help in achieving quicker growth. Thioglycollates were found to be nonessential. Leucine was found to be the most important single amino acid for both species. Tryptophane was nonessential for S. pullorum and S. gallinarum. There was considerable variations in the requirements for the different strains of S. pullorum, but those of S. gallinarum appeared to be almost uniform.

More extensive work has been done to determine the carbon sources utilized by Salmonella. The first workers used different sugars. Kisch (1918), Koser and Rettger (1919), Pesch (1929), Pesch and Kramer (1930), Vasarhelyi (1934), Brawn and Guggenheim (1922), Kendell and Chinn (1938), and others have validated the use of glycerine, dextrose, d-galactose, d-fructose, raffinose, rhamnose, arabinose, and ascorbic acid as well as the alcohols sorbitol, dulcitol, and mannitol. Quite a bit of work has been done with the use of organic acids and the salts of organic acids. These include work by Brawn and Cahn-Bronner (1922), Pesch and Koch (1930), Brown et al. (1924), Bruce (1934), Jordan and Harmon (1928), Robbins and Lewis (1940), and Bruce (1935). Acetic, oxalic, lactic, succinic, malic, d-gluconic, saccharic, d-mannonic, galactonic, and arabonic acids as well as sodium citrate and rochelle salt, among others, were recorded as being used by some strains. Bruce's work was especially interesting as he found the odd numbered carbon acids pro-

duced no growth. The odd carbon hydroxy acids lactic, glyceric, and alpha-hydroxyacrylic, supported growth. Much better growth was obtained in even numbered carbon acids such as acetic, butyric, and isobutyric. However, only feeble growth could be found in the even numbered hydroxy acids such as glycollic and beta-hydroxy n-butyric. Alpha-hydroxy isobutyric acid failed to support growth.

Extensive work has been done to determine the amino acid requirements of other bacteria, including both aerobes and anaerobes. It appears that all organisms have specific amino acid growth requirements, some amino acids being more important than others. Three more or less arbitrary categories may be recognized.

In the first group are organisms which do not need many accessory growth factors. Many of this group have the ability to derive their nitrogen from simple inorganic salts of nitrogen. Ammonium salts are of special importance to this group, which includes some of the members of the genera Sarcina, Bacillus, Proteus, Vibrio, Salmonella, and also some of the luminous bacteria, such as Photobacterium fischeri.

A second group includes those organisms which need certain accessory factors such as vitamins, purines, pyrimidines and other chemical compounds. This group includes members of the genera Mycobacterium, Corynebacterium, Streptococcus, Diplococcus, Shigella, Brucella, Pasteurella, Acetobacter, Lactobacillus, Clostridium, Neisseria, and Staphlococcus. Some work done on this group might be discussed since it will show some similarity to the requirements of Salmonella.

Dunn et al. (1940), in their study of the amino acid require-

ments of Leuconostoc mesenteroides, strain P-60, found that the organism needed practically the same amino acids for growth as the strains of S. pullorum and S. gallinarum reported by Johnson and Rettger. The amino acids threonine, asparagine, and arginine were required by L. mesenteroides in addition to those required by strains of S. pullorum and S. gallinarum. Koser et al. (1941), in their studies of the genus Brucella found that a medium practically identical to that used by Dunn et al., would support the growth of members of this group. Further work on Brucella by McCullough et al. (1947), revealed that cystine, histidine, and tryptophane were essential while phenylalanine and tyrosine increased growth. Glycine, arginine, methionine, glutamic acid, isoleucine, aspartic acid, serine, and threonine appeared to be stimulatory. Drea (1940) found that his strains of Mycobacterium tuberculoses (human variety) could grow on a simple medium containing asparagine and ammonium citrate as sources of nitrogen.

The specificity of certain amino acids for growth may be inferred from the work of Burrows (1932, 1933), with strains of Clostridium botulinum. In the presence of an acid hydrolysate of casein, cystine, leucine, and proline were essential. Leucine and proline could be substituted by isoleucine and isoproline respectively. However, when other strains of Clostridium, including Cl. tetani, Cl. welchii, Cl. sporanges, and Cl. histolyticum, were placed in this medium no growth resulted. With Acetobacter suboxydans Stokes and Larsen (1945) found that isoleucine, valine, alanine, and histidine were essential. Growth could be increased by addition of either cystine or methionine. The further addition of proline increased the growth

to a level equal to that which occurred in the presence of twenty amino acids or of hydrolysed casein. Rane and Subburow (1940) working with strains of Diplococcus pneumoniae, types II, V, and VIII, found that they could be grown in a synthetic medium containing 11 amino acids. Glutamic acid, leucine, histidine and arginine seemed to be essential for all three types. However methionine was found to be nonessential for type II, necessary for type V, and actually inhibitory for type VIII. Likewise, lysine was found to be nonessential for type II, inhibitory for type V, and essential for type VIII. Types I and VII refused to grow upon any combinations of amino acids.

Doudoroff's work (1944) with Pasteurella pestis shows an interesting adjustment of the organism. He found when this organism was inoculated from blood agar slants that cystine, phenylalanine, and proline were essential for growth. However, after the initial phase of growth had passed the cystine could be substituted by either thiosulfate, thioglycollate or sulfites, but methionine would not suffice. Phenylalanine appeared to be almost specific as far as the phenolic amino acids were concerned and only very scanty growth was noted with substitution of either tryptophane or tyrosine. Certain antagonistic effects apparently were developed by the addition of leucine when cystine was present, causing a failure of growth.

Some confusion exists in literature with regard to Neisseria intracellularis. Frantz (1942) found that growth occurred on a medium of casein hydrolysate, various salts, accessory factors, and glucose. Glutamic acid and cystine were found to be essential. This work was confirmed by Sherp and Tuttle (1945) who found that

the addition of guanine, uracil, cytosine also diminished the lag period. Boor (1942) reported, however, that his strains of Neisseria were unaffected by the presence of cystine. Grossowicz (1945) reported only scanty growth with the absence of calcium, and inhibition of growth by cystine and asparagine. Tyrosine and glycine also inhibited his strains, though to a somewhat lesser degree. Gould et al. (1944), working with strains of N. gonorrhoeae, found that glutathione, glutamic acid, and histidine were essential. Strains which failed to grow on the above compounds could be grown by the substitution of glutathione for cystine. Boor also found that cystine was stimulatory to the strains he studied.

Many nutritional studies have been made with the genus Streptococcus in the past few years. Because of the wide differences in the physiology of different species of this genus it is difficult to discuss nutritional requirements in general terms. Hence each of the Lancefield Groupes will be discussed separately. Woolley (1941), Bass et al. (1941), Rane and Subbarow (1938), Pappenheimer and Hottle (1940), and Hottle et al. (1941) worked on the nutrition of Group A. Hottle et al. found that a mixture of fifteen amino acids, gelatin hydrolysate, and biotin were essential for growth. McIlwin et al. (1939) determined that best growth was obtained with glutamine present. Others have found that pantothenic acid, riboflavin, pyridoxine, inositol, choline, pimelic acid, nicotinic acid, hemin, adenine, and thiamin among the accessory factors favoring the growth of the beta-hemolytic streptococci.

Niven (1943), Hutner (1938), and Woolley and Hutchings (1939) found that these alpha-hemolytic, Group B strains, could grow on a

medium in which twenty amino acids were substituted for the casein hydrolysate. Similar accessory factors as those found essential for the beta-hemolytic, Group A, strains were also found to be necessary for the Group B strains. Woolley and Hutchings found that best growth occurred with the addition of such reducing substances as ascorbic acid, glutathione, or thioglycollate.

Rogers (1944) found that the growth characteristics as reported necessary for the other types were identical with those needed by Group C with the exception that uracil or orotic acid (uracil-4-carboxylic acid) was required for growth.

Schuman and Farrell (1941) and Perlman (1946), among others, studied the nutrition of the enterococci. Schuman and Farrell found that arginine, glutamic acid, methionine, tryptophane, tyrosine and valine were essential for growth. Lysine slightly enhanced growth. An example of the differences in the requirements of the organisms of this last type have been noted with St. zymogenes and St. fecaelis. St. zymogenes requires isoleucine and lysine while St. fecaelis requires valine and not lysine.

The third category includes the organisms the growth factors of which are unknown or partially unknown at present. Included within this group are members of the genera Hemophilus, Leptospira, Listerella, Erysipelothrix, Bartonella, and others. Bass et al. (1941) working with the genus Hemophilus attempted to substitute Coenzyme I and cystine for hemin, a known requirement, but were unsuccessful. Further experiments have shown that Coenzyme I and hemin may be replaced by the enzyme catalase. Green (1945) found with the genus Leptospira that the peptones of Schüffner's medium

which supported growth, could be supplanted by asparagine, aspartic acid, and leucine. Arginine, glutamic acid, alanine, glycine, methionine, lysine, tryptophane, tyrosine, or valine added to the other amino acids proved to be inhibitory. However, when the serum of Schüffner's medium was removed, no growth resulted. No combinations of amino acids or accessory factors studied proved to be of value in promoting growth. Spizizen (1943) found that when glycine, glycine anhydride, and hippuric acid were added to a mixture of phage particles and E. coli there was an increase in the numbers of particles but not of the E. coli cells.

It appears from this survey that many organisms have nutritional requirements which parallel each other fairly closely. Others, though in the same genera, differ quite distinctly and no doubt in the future many of these differences will be worked out.

EXPERIMENTAL

Preliminary Methods

The organisms with which this work was carried out included several strains of different species of the genus Salmonella. These included 13 strains of S. pullorum, numbers 1 through 13; three strains of S. typhosa, numbers 14 through 16; S. paratyphi, number 17; two strains of S. schottmuelleri, numbers 18 and 19; S. hirschfeldii, number 20; S. abortivoequina, number 21; S. derby, number 22; S. gallinarum, number 23; and S. typhi-murium, number 24. The strains of S. pullorum were obtained partially from the collection at the Department of Bacteriology at Kansas State College,

and partially from the collection of the University of Illinois, through the courtesy of D. Lester Erwin. Also obtained from the collection of the Department of Bacteriology at Kansas State College were two strains of S. typhosa, one strain each of S. schottmuelleri, S. abortivoequina, and S. gallinarum. The remainder of the strains, which included a strain each of S. typhosa, S. schottmuelleri, S. paratyphi, S. typhi-murium, S. hirschfeldii, and S. derby, were obtained through the kindness of Mr. Guido Iannarelli, senior bacteriologist, at the West Virginia State Hygenic Laboratory, Charleston, West Virginia.

This group contains a number of serologically related bacteria. Some members of the group are motile, while others are nonmotile. Those which are motile have peritrichous flagella. The cells are gram negative bacilli which do not form either capsules or spores. Bergy's Manual Sixth Edition states that:

> They produce acid and gas from glucose, mannitol and sorbitol (Except S. typhosa and S. gallinarum no gas is produced). Lactose, sucrose and salacin are not attacked. They do not clot milk, form indol or liquefy gelatin. They reduce trimethylamine oxide to trimethyamine. All species are pathogenic for warm blooded animals, including man causing food infections and enteric fevers.

It was of primary importance before preceding with the major experiments to determine whether all cultures belonged to the genus Salmonella and whether they were pure.

Each culture was streaked upon a nutrient agar plate and one colony was picked and restreaked upon a second nutrient agar plate. Upon examination, each culture plate appeared to be pure. Then differential media were inoculated including eosin methylene blue plates, bismuth sulfite plates, and sugar fermentation tubes. Gram stains were done upon each culture as well as motility tests.

Each culture was inoculated into Kligler's iron agar slants and urea broth. Microslide agglutination tests were done with the strains of S. pullorum, S. typhosa, S. paratyphi, and S. schott-muelleri.

Preliminary Results

The colonies of the group appeared small and greyish-opaque to transparent. Minute differences in the different strains appeared under the microscope, but all fell within the descriptions found in Bergy's Manual. The Gram stains showed typical cells and only S. pullorum and S. gallinarum were not motile. The results of the sugar fermentation tubes parallel the results which have been described in literature. There was no growth in the urea broth tubes and Kligler's slants showed typical results with certain strains of S. pullorum producing hydrogen sulfide following prolonged incubation. Strains of S. pullorum, S. typhosa, S. paratyphi, and S. schottmuelleri were agglutinated by their respective antisera. Serologic differentiation was not attempted because the necessary sera were not available.

Methods and Procedures

The materials and chemicals used in these experiments were the best obtainable. The amino acids were commercial preparations of highest purity purchased from a biological supply house. The other materials were supplied by the Department of Bacteriology.

The first work with S. pullorum, included a combination of the available amino acids at a concentration of 200 gamma/ml. These were

added separately to each tube constituting a test. The source of carbon was glucose. The basal medium was a modification of Burrows (1942) which omitted ammonium sulfate. The basal medium consisted of sodium chloride 2.87 grams, potassium dihydrogen phosphate 5 grams, and glucose 6 grams dissolved in 100 ml of double distilled water. The pH of 6.8 to 7 was adjusted with M/10 dipotassium hydrogen phosphate using brom thymol blue as an indicator.

After the first series of experiments, (Table 1), it was decided to use the method of Johnson and Rettger and the amino acids were used in the following concentration: beta-alanine, threonine, aspartic acid, glutamic acid, glycine, leucine, proline and serine were employed in a concentration of M/1500. Arginine, cystine, histidine, lysine, methionine, phenylalanine, tyrosine, and glutathione, were used in a concentration of M/4000. Tyrosine and cystine were first dissolved in a small amount of concentrated HCl. Tryptophane was prepared in a concentration of M/10,000, thiamin in a concentration of 1 mg/ml, and nicotinic acid in a concentration of 10 mg/ml. For convenience stock solutions of each amino acid were made 100 times stronger than employed. The modification of the basal medium of Burrows which had proved satisfactory was used in all subsequent studies.

The second set of experiments, (Table 2), were of the same nature as the first set and consisted of a series of 23 tests. The first test concerned the availability of inorganic nitrogen (ammonium sulfate) as a source of nitrogen. Tests 2 to 22 inclusive consisted of mixtures of the amino acids with the omission of one amino acid in each successive test. Test 23 consisted of a complete mix-

ture of the above mentioned amino acids.

The third set of experiments, (Table 3), included the use of one amino acid as the source of nitrogen, each test containing a different amino acid or vitamin. The basal medium and the pH was the same as previously described.

The fourth set of experiments (Tables 4 through 15) dealt with only five strains of S. pullorum. Twelve amino acids and vitamins were employed and a series of combinations (121) were made. Included were leucine, threonine, methionine, phenylalanine, histidine, tyrosine, tryptophane, proline, arginine, glutamic acid, thiamin and nicotinic acid. The combinations began with leucine and threonine in test one, leucine, threonine, and methionine in test two, and so on.

Inoculations were carried out in the following manner: Five ml of sterile distilled water was pipetted into a Kligler's iron agar slant upon which the cultures were carried. With a sterile one ml pipette, the solution was mixed until a heavy suspension resulted. One-tenth ml was pipetted into a tube containing five ml of sterile distilled water. This was well mixed and then one-tenth of this mixture was used as an inoculum for the different tests. A control on growth was also run by adding one-tenth ml of the above mixture to a sterile petri dish and adding five ml of melted and cooled nutrient agar. All the tests were incubated at 37° C. The tests were read by comparison of the tubes with standard nephlometer tubes. Nephlometer tube number two had a concentration of 600 million bacteria/ml and a value of five was assigned it. Tube number one had a concentration of 300 million bacteria/ml

and was given a value of three. Those with a concentration of approximately 150 million bacteria/ml were given the value of two. Those with a trace of growth were given the value of one.

Experimental Results

The data obtained can be shown best by a series of tables. In the first table, the results of the first set of experiments are recorded. In this experiment 13 strains of S. pullorum were introduced into combinations of amino acids from which one acid was omitted from each test. It may be observed that ammonium sulfate proved incapable of supplying the necessary nitrogen. However, in experiment 2, growth occurred in the case of strain 2, 3, and 13. When rechecked no growth occurred and, therefore, it was concluded that the growth recorded was due to contamination. Tryptophane was apparently essential for strain one. Leucine was apparently the most important amino acid for several strains of S. pullorum since strains 9, 10, 12, and 13 failed to grow in its absence. Strain 10 failed to grow with any combinations of either amino acids or vitamins. It may be observed further that of the other strains of Salmonella tested only one of S. typhosa, number fourteen, and S. paratyphi and S. gallinarum failed to grow in the presence of ammonium sulfate. With strain 14 of S. typhosa the omission of either tryptophane or leucine resulted in failure of growth. S. paratyphi was the most fastidious of the strains tested. The omission of any of the following amino acids from the medium: glutathione, cystine, phenylalanine, leucine, threonine, methionine, nicotinic acid, thiamin, valine, and beta-alanine did

not inhibit growth, though in most cases growth was significantly delayed. Absence of leucine and alanine proved deleterious to growth of S. gallinarum.

The method used by Burrows in his work with S. typhosa was subsequently employed and 18 different amino acids were tested. These were tested singly employing glucose, glycerol, lactic, pyruvic and succinic acids as sources of carbon. While some variations in results were found, either glucose or glycerol proved to be better than the other sources of carbon for growth. It was thus decided to use glucose as the source of carbon and test the available amino acids singly. The results may be seen in Table 3. It is readily discernable that there is wide variability in the strains of S. pullorum. Cystine and histidine provided the best single sources of nitrogen for these strains. However, some strains failed to grow with these amino acids and grew instead with tyrosine, thiamin, and beta-alanine. Another grew with cystine, tyrosine, valine, glutathione, threonine, and beta-alanine. An interesting difference in the strains of S. typhosa might be noted. Strain number 14 grew only in phenylalanine and tryptophane. Strain 15 grew much better, failing to utilize only aspartic acid, methionine, proline, serine, and nicotinic acid. A third strain, number 16, utilized all the sources of nitrogen tried except nicotinic acid. S. paratyphi grew only in arginine, cystine, glycine, histidine, leucine, and phenylalanine; growth was delayed in all except leucine. With S. abortivoequina growth occurred with only glutamic acid, histidine, leucine, phenylalanine, tryptophane, tyrosine, valine, and thiamin. Of the two strains of S. schott-

Table 1. Ammonium salts and/or amino acids as source of nitrogen for strains of S. pullorum.

Strains	Media																																			
	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17		18		19	
*:	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72
1	-	-	4	5**	4	5	3	4	-	-	4	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5	3	4	3	3
2	-	-	3	4	4	5	4	4	4	5	4	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	-	4	-	4	-	4	-	4	-	4	-	4
3	-	-	3	4	4	5	3	3	4	5	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4
4	-	-	4	5	4	5	2	3	3	4	4	4	3	4	4	4	4	4	4	4	4	4	5	4	4	4	4	4	5	4	4	4	4	4	4	4	4	4
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6	-	-	4	5	4	5	4	4	3	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
7	-	-	4	5	4	4	4	4	4	4	4	5	4	4	2	2	3	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	3	4	4	4	4	4
8	-	-	4	5	4	4	4	4	4	4	3	4	4	4	3	4	3	4	4	4	4	4	4	4	4	4	3	4	3	4	3	4	3	4	3	4	3	4
9	-	-	4	5	3	3	3	3	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	5	-	4	-	4	-	4	-	4	-	4	-	4
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	3	4	3	3	2	3	3	4	2	3	3	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
12	-	-	3	4	3	4	4	4	4	4	4	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	4	-	4	-	4	-	4	-	4	-	4
13	-	-	3	5	4	5	4	5	4	4	4	4	4	4	3	4	3	4	4	5	3	5	3	4	3	5	2	4	3	4	4	4	4	4	3	4	4	4

*Refers to hours incubated

**Refers to the amount of growth. 5 = 600 million bacteria/ml.

1. Ammonium sulfate as source of nitrogen
2. A mixture of amino acids
   A mixture of amino acids omitting
3. Glutathione
4. Cystine
5. Tryptophane
6. Tyrosine
7. Phenylalanine
8. Methionine
9. Nicotinic acid
10. Histidine
11. Glycine
12. Alanine
13. Glutamic acid
14. Aspartic acid
15. Serine
16. Lysine
17. Arginine
18. Thiamin
19. Asparagine

Table 2. Ammonium salts and/or amino acids as source of nitrogen.

Strains	Medium 1		2		3		4		5		6		7		8		9		10		11		12		13		14		15		16		17		18		19		20		21		22		23	
	24*	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72	24	72
1	-	-	-	4	3	4	-	-	-	4	-	4	1	4	-	4	4	4	-	4	3	3	-	3	3	3	-	3	-	3	2	3	3	3	-	3	2	3	3	3	2	3	3	3	3	4
2	-	4	4	4	4	4	-	3	2	4	2	4	-	4	4	4	4	4	4	4	3	3	-	3	3	3	2	4	-	4	2	3	3	3	3	3	2	3	3	4	4	4	3	3	4	4
3	-	2	2	4	3	4	-	3	-	4	4	4	-	4	4	4	3	3	4	4	3	3	-	3	-	3	2	3	-	3	2	3	2	2	3	4	2	3	3	3	4	4	4	4	4	4
4	-	-	3	4	2	4	2	2	2	4	2	3	-	3	2	4	4	4	3	4	3	4	3	3	3	3	2	3	3	3	3	4	3	3	3	3	4	4	3	3	4	4	4	4	4	4
5	-	-	2	4	3	4	-	4	-	4	2	4	-	2	-	4	4	4	4	4	4	4	-	3	3	3	-	3	-	3	3	3	3	3	3	3	-	4	3	3	3	4	4	4	3	4
6	-	-	4	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	3	3	-	3	3	3	3	4	4	4	3	3	3	3	3	4	4	4	3	3	3	4	4	4	4	4
7	-	-	4	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4	4	3	3	-	3	3	3	2	4	-	4	3	3	3	3	3	3	4	4	3	3	4	4	4	4	4	4
8	-	-	4	5	4	4	4	4	3	3	4	5	-	3	-	4	3	3	4	5	3	3	3	3	3	3	3	3	-	3	3	3	3	4	3	3	4	4	3	3	4	4	3	3	3	4
9	-	-	-	-	4	-	4	-	4	-	3	2	4	-	-	2	4	3	4	4	4	-	2	-	3	1	1	-	4	-	4	3	-	3	-	3	-	4	-	3	4	4	4	4	3	4
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	4	5	4	5	4	4	4	5	4	5	-	3	-	4	3	4	4	4	4	4	3	3	2	4	2	3	3	4	3	3	3	3	3	3	4	4	3	4	4	4	3	5	4	4
12	-	-	4	5	4	4	4	4	4	5	4	5	-	-	4	4	2	4	4	4	-	2	-	3	-	2	2	3	2	4	2	3	-	3	-	4	-	3	3	3	3	4	3	4	3	4
13	3	4	3	4	4	4	4	4	4	4	4	4	-	-	4	4	4	4	4	4	2	3	-	3	2	3	-	3	-	3	2	3	2	3	3	3	-	3	3	3	4	4	3	3	3	4
14	-	-	4	4	4	4	-	-	4	4	2	4	-	-	2	4	4	4	4	4	2	3	4	4	-	3	4	4	4	5	-	3	3	3	-	3	3	3	3	3	4	4	4	5	4	4
15	-	3	4	4	4	5	4	4	4	4	4	5	2	3	2	3	4	4	4	4	2	3	4	4	-	2	4	4	3	3	-	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4
16	2	4	4	5	4	5	4	5	4	5	4	5	2	3	4	5	4	4	3	3	3	3	3	4	3	3	4	4	3	4	4	4	3	3	3	3	3	4	3	3	4	4	4	4	4	4
17	-	-	-	3	1	2	-	-	1	1	-	3	1	2	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	4	-	2	-	3
18	3	3	4	5	4	5	4	5	4	5	4	5	4	4	4	5	4	4	4	4	3	3	3	4	3	3	4	4	3	3	3	3	2	3	4	4	3	3	4	4	4	4	3	3	3	4
19	3	3	4	5	4	5	4	5	4	5	4	5	4	5	2	3	4	4	4	5	3	3	4	4	3	3	3	3	3	3	3	3	4	4	4	4	3	3	3	3	3	3	4	5	4	5
20	4	4	4	5	4	5	4	5	4	5	4	5	4	5	2	3	4	5	3	3	3	3	3	3	4	4	3	3	3	3	3	3	3	3	3	3	3	3	3	3	4	4	4	5	4	5
21	-	2	4	4	4	4	-	-	4	4	2	3	4	4	2	3	4	4	4	4	3	4	2	4	-	3	3	3	3	3	-	3	-	3	4	4	4	4	3	3	3	3	3	3	3	4
22	2	3	4	5	4	5	4	5	4	5	4	5	4	5	2	4	2	3	4	5	3	4	3	3	3	3	3	4	3	3	3	3	3	4	3	4	3	3	3	4	4	5	3	5	4	5
23	-	-	-	4	3	3	4	4	4	4	4	4	-	-	-	3	4	4	4	4	3	4	3	3	-	-	3	4	3	4	-	1	-	2	3	3	3	4	3	4	4	4	4	5	4	4
24	2	3	4	5	4	5	4	5	4	5	4	5	4	4	4	5	3	4	3	4	2	4	3	4	3	3	3	4	3	4	3	3	4	4	4	4	3	3	3	3	4	5	4	5	4	5

*Refers to hours growth

Media
1. Ammonium sulfate as only source of nitrogen
   Containing a mixture of amino acids omitting
2. Glutathione
3. Cystine
4. Tryptophane
5. Tyrosine
6. Phenylalanine
7. Leucine
8. Threonine
9. Methionine
10. Nicotinic acid
11. Histidine
12. Glycine
13. Alanine
14. Glutamic acid
15. Proline
16. Serine
17. Lysine
18. Arginine
19. Thiamin
20. Aspartic acid
21. Valine
22. Beta-alanine
23. Omitting none

Organism used
1-13. S. pullorum
14-16. S. typhosa
17. S. paratyphoid "A"
18-19. S. schottmuelleri
20. S. hirshfeldii
21. S. abortusequi
22. S. derby
23. S. gallinarum
24. S. typhi-murium

Table 3. Single amino acids as the source of nitrogen.

Strains	1		2		3		4		5		6		7		8		9		10		11		12		13		14		15	
**	48	96	48	96	48	96	48	96	48	96	48	96	48	96	48	96	48	96	48	96	48	96	48	96	48	96	48	96	48	96
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3
3	-	-	-	-	-	-	-	-	-	-	-	-	-	2*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	4	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	2	4	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
6	-	-	-	-	-	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
7	-	-	-	-	-	-	3	4	-	2	-	-	-	3*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	4	4	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	4	-	-	-	-	-	3*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-	-	3*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	4	-	-	-	-	-	2*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
13	-	-	2	3	-	1	3	4	-	-	-	-	3	4	-	-	1	3	-	-	-	-	-	-	-	-	-	1	-	1
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	1	3	-	-
15	-	2*	-	2	-	-	4	4	-	2*	-	3	3	3	-	2*	-	2*	-	-	-	2*	-	-	-	-	1	2	1	2
16	3	3	2	2	3	3	4	5	-	-	3	3	3	3	3	3	3	3	3	3	-	2	2	3	3	3	-	4	2	3
17	-	-	-	2*	-	-	-	2*	-	-	-	2*	2	3	-	2	-	-	-	-	-	2*	-	-	-	-	-	-	-	-
18	4	4	2	2	2	3	4	4	2	2	3	3	3	3	3	3	3	3	-	2	3	3	2	3	-	3	3	3	3	3
19	3	4	2	3	2	4	4	5	2	2	3	3	4	4	3	3	3	3	-	2	3	3	2	2	-	-	3	3	3	3
20	3	3	2	2	2	2	5	5	3	3	3	3	3	3	-	3	3	3	3	3	3	3	2	3	-	4	3	3	3	4
21	-	-	-	-	-	-	-	-	-	2*	-	-	-	1	-	2*	-	-	-	-	-	2	-	-	-	-	2	2	2	2
22	2	3	2	2	2	2	5	5	2	2	2	2	3	4	2	3	3	3	2	2	2	2	1	2	-	4	2	3	2	3
23	-	-	-	-	-	-	-	3*	-	-	-	-	-	-	-	-	-	1*	-	-	-	-	-	-	-	-	-	-	-	-
24	3	4	2	3	2	2	5	5	2	2	3	3	3	3	3	4	3	4	1	2	2	3	2	2	2	3	2	2	2	2

Strains	16		17		18		19		20		21	
**	48	96	48	96	48	96	48	96	48	96	48	96
1	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	1	1	-	-	-	-	1	1
3	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-
6	-	1	-	-	-	-	1	3	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	1
12	-	-	-	-	-	-	-	-	-	4	-	-
13	-	1	-	1	-	-	-	-	-	1	-	2
14	-	-	-	-	-	-	-	-	-	-	-	-
15	-	3	-	2	2	2	-	-	2	3	2	3
16	2	3	3	3	3	3	-	-	2	2	2	2
17	-	-	-	-	-	-	-	-	-	-	-	-
18	3	3	3	4	1	1	-	-	2	2	2	2
19	3	3	3	4	1	1	-	-	1	1	2	2
20	3	3	4	4	1	1	-	-	3	3	2	2
21	2	2	-	-	1	1	-	-	-	-	-	-
22	2	3	2	3	1	1	-	-	2	2	1	2
23	-	-	-	-	-	-	-	-	-	-	-	-
24	1	2	2	3	1	1	-	-	1	2	1	2

*Refers to delayed growth up to 10 days.
**Refers to hours of readings.

Contains basal medium and following amino acid
1. Alanine
2. Arginine
3. Aspartic acid
4. Cystine
5. Glutamic acid
6. Glycine
7. Histidine
8. Leucine
9. Lysine
10. Methionine
11. Phenylalanine
12. Proline
13. Serine
14. Tryptophane
15. Tyrosine
16. Valine
17. Glutathione
18. Thiamin
19. Nicotinic acid
20. Threonine
21. Beta-alanine

Organisms used
1-13. S. pullorum
14-16. S. typhosa
17. S. paratyphoid "A"
18-19. S. schottmuelleri
20. S. hirschfeldii
21. S. abortusequi
22. S. derby
23. S. gallinarum
24. S. typhi-murium

muelleri, one utilized serine the other strain did not, while both utilized all the other amino acids. S. gallinarum utilized only cystine and lysine. All other strains, with one exception, of the species of Salmonella tested utilized all the sources of nitrogen with the exception of nicotinic acid. The lone exception which has been previously mentioned was one strain of S. pullorum.

Since it was found that little growth of S. pullorum occurred with the single amino acids as the source of nitrogen, with the exception of cystine, it was decided to try combinations of certain amino acids in an effort to find the minimum number of amino acids necessary to support growth. The results of this work are recorded in Tables 4 to 15. It may be noted that histidine is by far the most important amino acid for the growth of the five strains of S. pullorum employed in these tests. When histidine was used with the different combinations employed, growth resulted in all except one case, that of strain number one with a combination of histidine and leucine. Growth was delayed, however until a combination of histidine, leucine, threonine, methionine, glutamic acid and phenylalanine was tried. In the absence of histidine, growth occurrs in all five strains when a combination of arginine, leucine, threonine, methionine, glutamic acid, phenylalanine, tyrosine, tryptophane, and proline. With addition of nicotinic acid to the different combinations, some delay in growth occurred in some tests while in others, a complete failure of growth was recorded.

DISCUSSION

From the experimental data, it may be concluded that Salmonella

Table 4. Amounts of growth secured with various combinations of amino acids.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
1. Leucine and threonine	-	-	-	-	-	-	-	-	-	-
2. As 1, and methionine	-	-	-	-	-	-	-	-	-	-
3. As 2, and glutamic acid	-	-	-	-	-	-	-	-	-	-
4. As 3, and phenylalanine	-	-	-	-	-	-	-	-	-	-
5. As 4, and tyrosine	-	-	-	-	-	-	-	-	-	-
6. As 5, and tryptophane	-	-	-	-	-	-	-	-	-	-
7. As 6, and proline	-	-	-	-	-	-	-	-	-	-
8. As 7, and histidine	2	3	3	3	3	4	2	2	3	3
9. As 8, and arginine	1	4	4	4	3	4	3	3	3	3
10. As 9, and thiamin	-	-	4	4	-	4	3	3	-	4
11. As 10, and nicotinic acid	-	-	-	4	-	-	-	2	-	-

Table 5. Amounts of growth secured with different combinations of amino acids in the absence of leucine.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
12. Threonine and methionine	-	-	-	-	-	-	-	-	-	-
13. As 12, and glutamic acid	-	-	-	-	-	-	-	-	-	-
14. As 13, and phenylalanine	-	-	-	-	-	-	-	-	-	-
15. As 14, and tyrosine	-	-	-	-	-	-	-	-	-	-
16. As 15, and tryptophane	-	-	-	-	-	-	-	-	-	-
17. As 16, and proline	-	-	-	-	-	-	-	-	-	-
18. As 17, and histidine	-	1	1	1	-	1	-	1	-	1
19. As 18, and arginine	-	2	3	3	1	2	2	3	2	4
20. As 19, and thiamin	2	2	2	2	1	1	2	2	3	4
21. As 20, and nicotinic acid	-	2	-	2	-	1	-	2	-	1

Table 6. Amounts of growth secured with various combinations of amino acids in the absence of threonine.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
22. Methionine and leucine	-	-	-	-	-	-	-	-	-	-
23. As 22, and glutamic acid	-	-	-	-	-	-	-	-	-	-
24. As 23, and phenylalanine	-	-	-	-	-	-	-	-	-	-
25. As 24, and tyrosine	-	-	-	-	-	-	-	-	-	-
26. As 25, and tryptophane	-	-	-	-	-	-	-	-	-	-
27. As 26, and proline	-	-	-	-	-	-	-	-	-	-
28. As 27, and histidine	1	2	2	3	3	4	2	2	-	2
29. As 28, and arginine	1	2	2	2	2	2	2	2	2	2
30. As 29, and thiamin	1	4	2	4	4	4	2	3	2	3
31. As 30, and nicotinic acid	-	1	-	-	1	2	-	-	2	3

Table 7. Amounts of growth secured with these combinations of amino acids in the absence of methionine.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
32. Glutamic acid and leucine	-	-	-	-	-	-	-	-	-	-
33. As 32, and threonine	-	-	-	-	-	-	-	-	-	-
34. As 33, and phenylalanine	-	-	-	-	-	-	-	-	-	-
35. As 34, and tyrosine	-	-	-	-	-	-	-	-	-	-
36. As 35, and tryptophane	-	-	-	-	-	-	-	-	-	-
37. As 36, and proline	-	-	-	-	-	-	-	-	-	-
38. As 37, and histidine	-	1	1	2	-	2	-	4	1	3
39. As 38, and arginine	1	2	1	2	-	2	-	1	1	2
40. As 39, and thiamin	1	2	1	2	1	2	1	2	1	2
41. As 40, and nicotinic acid	1	1	1	1	2	2	2	3	1	3

Table 8. Amounts of growth secured with various combinations of amino acids in the absence of glutamic acid.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
42. Phenylalanine and leucine	-	-	-	-	-	-	-	-	-	-
43. As 42, and threonine	-	-	-	-	-	-	-	-	-	-
44. As 43, and methionine	-	-	-	-	-	-	-	-	-	-
45. As 44, and tyrosine	-	-	-	-	-	-	-	-	-	-
46. As 45, and tryptophane	-	-	-	-	-	-	-	-	-	-
47. As 46, and proline	-	-	-	-	-	-	-	-	-	-
48. As 47, and histidine	1	3	2	3	2	3	1	2	1	2
49. As 48, and arginine	1	3	2	4	2	4	1	3	1	3
50. As 49, and thiamin	1	3	2	4	2	4	1	3	1	4
51. As 50, and nicotinic acid	2	4	2	4	-	3	2	3	1	2

Table 9. Amounts of growth secured with different combinations of amino acids in the absence of phenylalanine.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
52. Tyrosine and leucine	-	-	-	-	-	-	-	-	-	-
53. As 52, and threonine	-	-	-	-	-	-	-	-	-	-
54. As 53, and methionine	-	-	-	-	-	-	-	-	-	-
55. As 54, and glutamic acid	-	-	-	-	-	-	-	-	-	-
56. As 55, and tryptophane	-	-	-	-	-	-	-	-	-	-
57. As 56, and proline	-	-	-	-	-	-	-	-	-	-
58. As 57, and histidine	2	4	2	3	2	3	1	3	1	3
59. As 58, and arginine	2	4	2	4	2	4	1	4	2	3
60. As 59, and thiamin	2	4	2	4	2	4	1	3	2	4
61. As 60, and nicotinic acid	2	4	2	4	2	4	2	4	2	4

Table 10. Amounts of growth secured with different combinations of amino acids in the absence of tyrosine.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
62. Tryptophane and leucine	-	-	-	-	-	-	-	-	-	-
63. As 62, and threonine	-	-	-	-	-	-	-	-	-	-
64. As 63, and methionine	-	-	-	-	-	-	-	-	-	-
65. As 64, and glutamic acid	-	-	-	-	-	-	-	-	-	-
66. As 65, and phenylalanine	-	-	-	-	-	-	-	-	-	-
67. As 66, and proline	-	-	-	-	-	-	-	-	-	-
68. As 67, and histidine	2	4	2	3	2	3	2	2	2	2
69. As 68, and arginine	3	3	2	3	2	3	2	3	3	4
70. As 69, and thiamin	2	3	2	3	2	3	2	3	2	3
71. As 70, and nicotinic acid	1	1	1	1	1	1	1	1	2	2

Table 11. Amounts of growth secured with different combinations of amino acids in the absence of tryptophane.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
72. Proline and leucine	-	-	-	-	-	-	-	-	-	-
73. As 72, and threonine	-	-	-	-	-	-	-	-	-	-
74. As 73, and methionine	-	-	-	-	-	-	-	-	-	-
75. As 74, and glutamic acid	-	-	-	-	-	-	-	-	-	-
76. As 75, and phenylalanine	-	-	-	-	-	-	-	-	-	-
77. As 76, and tyrosine	-	-	-	-	-	-	-	-	-	-
78. As 77, and histidine	-	1	-	1	-	1	-	1	-	1
79. As 78, and arginine	1	1	-	1	-	1	-	3	1	2
80. As 79, and thiamin	1	1	-	-	1	2	-	2	2	2
81. As 80, and nicotinic acid	-	-	-	-	-	-	-	-	-	-

Table 12. Amounts of growth secured with different combinations of amino acids exclusive of proline.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
82. Histidine and leucine	-	1	-	1	-	-	-	1	-	1
83. As 82, and threonine	-	1	-	1	-	1	-	1	-	1
84. As 83, and methionine	-	1	-	1	-	1	-	2	-	1
85. As 84, and glutamic acid	-	1	1	1	-	1	-	1	1	1
86. As 85, and phenylalanine	-	1	1	1	-	1	-	1	2	1
87. As 86, and tyrosine	1	1	1	1	1	1	1	1	1	1
88. As 87, and tryptophane	1	1	1	1	1	1	1	1	1	1
89. As 88, and arginine	1	2	1	2	2	2	1	2	1	4
90. As 89, and thiamin	1	3	1	2	2	2	1	2	1	4
91. As 90, and nicotinic acid	1	3	1	3	2	3	1	3	1	4

Table 13. Amounts of growth secured with different combinations of amino acids exclusive of histidine.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
92. Arginine and leucine	-	-	-	-	-	-	-	-	-	-
92. As 92, and threonine	-	-	-	-	-	-	-	-	-	-
94. As 93, and methionine	-	-	-	-	-	-	-	-	-	-
95. As 94, and glutamic acid	-	-	-	-	-	-	-	-	-	-
96. As 95, and phenylalanine	1	1	-	-	-	-	-	-	-	-
97. As 96, and tyrosine	1	1	1	1	-	1	-	-	-	-
98. As 97, and tryptophane	1	1	1	1	-	1	-	-	-	-
99. As 98, and proline	1	1	1	1	1	1	1	1	1	1
100. As 99, and thiamin	1	2	1	2	1	1	1	1	1	1
101. As 100, and nicotinic acid	1	1	1	1	1	1	1	1	1	1

Table 14. Amounts of growth secured with different combinations of amino acids exclusive of arginine.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
102. Thiamin and leucine	-	-	-	-	-	-	-	-	-	-
103. As 102, and threonine	-	-	-	-	-	-	-	-	-	-
104. As 103, and methionine	-	1	-	1	-	1	-	-	-	-
105. As 104, and glutamic acid	-	1	-	1	-	1	-	-	-	-
106. As 105, and phenylalanine	-	1	-	1	-	1	-	-	-	-
107. As 106, and tyrosine	-	1	-	1	-	1	-	-	-	-
108. As 107, and tryptophane	-	1	-	1	-	1	-	-	-	-
109. As 108, and proline	1	1	1	1	1	1	-	-	-	-
110. As 109, and histidine	1	2	1	2	1	2	1	2	1	2
111. As 110, and nicotinic acid	1	2	1	2	-	2	-	1	-	1

Table 15. Amounts of growth secured with different combinations of amino acids exclusive of thiamin.

Media	Strains of S. Pullorum									
Basal medium and the following amino acids	1		2		3		5		6	
	24	96	24	96	24	96	24	96	24	96
112. Nicotinic acid and leucine	-	-	-	-	-	-	-	-	-	-
113. As 112, and threonine	-	-	-	-	-	-	-	-	-	-
114. As 113, and methionine	-	-	-	-	-	-	-	-	-	-
115. As 114, and glutamic acid	-	-	-	-	-	-	-	-	-	-
116. As 115, and phenylalanine	-	-	-	-	-	-	-	-	-	-
117. As 116, and tyrosine	-	-	-	-	-	-	-	-	-	-
118. As 117, and tryptophane	-	-	-	-	-	-	-	-	-	-
119. As 118, and proline	-	-	-	1	-	-	-	-	-	-
120. As 119, and histidine	1	2	1	2	1	2	1	1	1	2
121. As 120, and arginine	1	3	1	3	1	2	1	3	1	3

can be grown in a purely artificial medium. Many workers also have found this to be the case. Considerable variability in the nutritional requirements of S. pullorum was observed but in general the results were similar to those reported by Johnson and Rettger (1943). Leucine was found to be the most important single amino acid in combination with others, but cystine, histidine, tyrosine, thiamin, beta-alanine, valine, threonine, lysine, serine, tryptophane, glutathione, aspartic acid, arginine, and nicotinic acid employed singly supported growth of some strains. Cystine and histidine when employed singly supported growth of the greatest number of strains. Strains of S. pullorum studied apparently differed from those studied by Johnson and Rettger in that none of these strains required nicotinic acid. Tryptophane proved to be essential for growth of strain one when used in a mixture of amino acids but it failed to support growth of this strain when used singly as the source of nitrogen. Strain number 10, a varient isolated from a turkey, failed to grow in any combinations of amino acids. Johnson and Rettger also studied several strains which refused to grow in synthetic media.

The effect of nicotinic acid in combinations with other amino acids on the growth of different strains of S. pullorum was interesting. Some strains refused to grow in its presence while some showed delayed growth. Two possible explanations may be suggested relative to the influence of nicotinic acid. First, it is possible that the combinations resulted in a mixture which exerted some antagonistic effect on the organisms and prevented growth. A second possibility might lie in the fact that nicotinic acid might have combined with an essential substance, such as histidine, forming a

complex which could not be utilized by the organism. In any case, no growth occurred with several such combinations.

Only one strain of S. typhosa failed to grow in the presence of inorganic nitrogen as the sole source of nitrogen, and it readily grew on a medium containing the proper amino acids. With this strain the absence of tryptophane and also leucine in a mixture of amino acids resulted in failure of growth. This was not the case with the strains studied by Johnson and Rettger (1943). Furthermore, this strain could grow with phenylalanine or tryptophane as the only source of nitrogen. Burrows found similar results with some of his strains though threonine was essential for most of them. Threonine was not found to be essential for any strains in this study.

The data obtained with S. paratyphi, relative to its nutritional requirements, are rather confusing. Koser and Rettger (1919) and Kisch (1918) have previously reported this organism as being more or less fastidious in its food requirements. The strain of S. paratyphi studied in these experiments was peculiar in that while it would grow in the presence of histidine, glycine, arginine, cystine, leucine, or phenylalanine as the sole source of nitrogen, and would grow in a mixture of all twenty one amino acids and vitamins studied, it would not grow in a mixture of the remaining nineteen amino acids if any one of the following was left out: tryptophane, methionine, histidine, glycine, alanine, glutamic acid, proline, serine, lysine, arginine, or aspartic acid. No explanation for this peculiar phenomenon is available. It is possible that in the presence of certain combinations of amino acids an antago-

nistic reaction develops.

S. gallinarum failed to grow when leucine and alanine were absent from a mixture and the only single amino acids that proved adequate for growth were cystine and lysine. Apparently, with this organism, a combination of several amino acids are needed for growth. Thiamin apparently exerted no effect towards decreasing the time required for growth.

CONCLUSIONS

From the data submitted several basic conclusions may be drawn relative to the nutritional studies of Salmonella. S. pullorum appears to require a mixture of amino acids for best growth, but some strains may grow with several amino acids used singly. Cystine and histidine proved to be the most important single amino acids in this respect. A combination of eight amino acids including histidine appear to be sufficient for growth. When nicotinic acid was added to the medium growth was delayed or failed completely with some of the strains. Two possibilities have been suggested as an explanation for this phenomenon, although neither was proved.

The strains of S. gallinarum, S. abortivoequina and S. paratyphi studied appeared to require a mixture of amino acids for growth. S. gallinarum utilized singly cystine and lysine. S. abortivoequina grew in either glutamic acid, leucine, lysine, proline, tryptophane, tyrosine, valine, or thiamin. S. paratyphi grew in arginine, cystine, glycine, histidine, leucine, or phenylalanine.

Different strains of S. typhosa varied in their nutritional requirements though only one strain failed to utilize inorganic

nitrogen. This strain grew in a mixture of amino acids if tryptophane or leucine was present and also grew if only phenylalanine or tryptophane was available.

The other cultures studied grew readily with inorganic sources of nitrogen, in mixtures of amino acids, and, for the most part, with a single amino acid as the sole source of nitrogen.

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