CULTURAL AND METABOLIC STUDIES OF THE COLON-AEROGENES GROUP OF BACTERIA

By

Elbert Lee Treece

B.S. University of Kansas, 1916.
A.M. University of Kansas, 1919.

Submitted to the Department of Bacteriology and the Faculty of the Graduate School of the University of Kansas in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Approved by:

_____
Chairman of Department

(Date) May 24, 1926

A C K N O W L E D G M E N T

I desire to express my sincere appreciation to Doctor Noble P. Sherwood, Chairman of the Department of Bacteriology, for his unfailing aid and encouragement throughout this work.

TABLE OF CONTENTS

Table of Contents - continued

Table of Contents - continued

TABLE OF CONTENTS.

TABLES AND PLATES.

Table of Contents - continued

Table of Contents - concluded.

INTRODUCTION

The cultivation and identification of the members of the colon-aerogenes group of bacteria are of great importance from the standpoint of public health. These organisms are normal inhabitants of the intestinal tract of man, and their presence or absence in water, and to a less extent in foods, serves as an index to its sanitary quality. They are also of some importance as an etiological factor in disease, the colon bacillus being not infrequently found to be the cause of infections of the urinary tract, and of other suppurative conditions in the body. The successful cultivation and identification of these organisms depend upon a knowledge of their nutritional requirements and of their metabolism. The nutritional requirements of bacteria are relatively simple, but like other living things they require nitrogen, carbon, oxygen and hydrogen with certain inorganic salts.

A study of bacterial nutrition would involve a study of the conditions under which these elements may be supplied to allow or favor growth of the organism.

A study of the metabolism of bacteria constitutes a study of the processes and products of extra-cellular digestion, whereby complex food molecules are broken down to simpler substances preparatory to absorption by the bacterial

cell, and a study of the process and products of intracellular digestion whereby the food molecule is further disintergrated, a part being stored, or built up into bacterial protoplasm and a part being given off as secretions or as waste products. The nutrition and metabolism of bacteria are so closely allied that a study of one could not be made without considering the other.

REVIEW OF LITERATURE

A review of the literature on bacterial nutrition and bacterial metabolism might be considered under the following headings.

I. Bacterial Nutrition

- A. Nitrogen requirements
  - a. Utilization of purified proteins
  - b. Utilization of hydrolytic products
  - c. Utilization of amino acids and ammonium salts
  - d. Growth accessory substances
- B. Carbon requirements
  - a. Carbon dioxide
  - b. Carbohydrates and alcohols
  - c. Organic acid radicles
  - d. Cellulose
  - e. Proteins.
- C. Inorganic salt requirements

II. Bacterial Metabolism

A. Nitrogen metabolism

a. Qualitative study of intermediate and end products.

b. Quantitative study of intermediate and end products.

c. Effect of utilizable carbohydrate upon nitrogen metabolism

B. Carbohydrate metabolism; products of fermentation; acids and gases

C. Gaseous metabolism

I. BACTERIAL NUTRITION

Previous work on bacterial nutrition has been along two general lines with different ends in view. One with the practical purpose of supplying the nutritional requirements for the growth of some particular organism or group of organisms, without regard to the actual chemical composition or complexity of the medium; the other with the more purely scientific purpose of simplifying the medium to one of known chemical composition, for the purpose of more accurately studying the reactions of the organism.

A. Nitrogen Requirements

a. Utilization of purified proteins.

The nitrogen requirement for bacterial nutrition has been considered to be the most difficult to supply and

has received considerable attention by investigators. The early observation of Pere (1892) that certain bacteria would not grow upon media containing pure protein as the only source of nitrogen is usually overlooked. He was unsuccessful in growing B. typhosus and B. coli upon a dilute solution of albumine as the only source of nitrogen. He believed they were unable to produce suitable enzymes to transform that substance into assimilable substances. Bainbridge (1911), using purified animal proteins as the only source of nitrogen, found that certain aerobic and facultative an-- aerobic bacteria were unable to grow. Sperry and Rettger (1915) confirmed the previous work and noted in addition that putrefactive anaerobes were unable to attack pure proteins and that bacteria were unable to attack the purified vegetable protein edestin, but that decomposition was rapid if peptone or other nitrogenous food was present. Rettger, Berman and Sturges (1916) observed that B. vulgaris, B. subtilis, Staphylococcus aureus, B. coli and B. typhosus were unable to grow upon coagulated egg albumin as the sole source of nitrogen. They determined the amount of protein and peptone used by the above organisms and found that the non-gelatine liquefying group utilized them very little or not at all, while the gelatine liquefying group digested them readily. Opportunity for autolysis of the bacterial cells was given in the case of both of the above groups.

Sturges and Rettger (1922) following up this work upon autolysis, found that bacteria of the proteolytic type, such as B. prodigiosus, Ps. pyocyaneus and B. subtilis; the pathogenic cocci, such as the gonococcus, the meningococcus and the pneumococcus undergo an actual autolysis, while the autolytic changes in B. coli was very slight. Wollman (1919) observed that when B. coli was grown on a horse serum or an egg-white medium no indol was produced but if proteolytic organisms had previously grown in the medium, the growth of B. coli was accompanied by indol production.

b. Utilization of hydrolytic products.

A number of investigators have called attention to the use of acid and alkali hydrolytic products and the products of peptic and tryptic digestion of proteins, for the cultivation of bacteria. Pere (1892) reports the growth of B. coli and B. typhosus upon the products of acid and alkali hydrolysis, also upon the products of digestion by pepsin and trysin.

Dalimer and Lancereaux (1913, a &b) reported excellent results upon a protein-free product of peptic and tryptic digestion, known commercially as "opsine". It contained all the amino acids of hydrolysis, phosphorus from nucleius, cystine, and a carbohydrate, glucosamine. They obtained good growth not only with the saprophytic bacteria and fungi but also with the pathogens more difficult to cultivate. Robinson and Rettger (1918) confirmed the

results of Dalimer and Lancereaux concerning opsine and in addition prepared protein free acid hydrolytic products from casein, lacto albumin and edestin. The products from casein gave fair bacterial growth, but the products of edestin were found unsuitable. They noticed less growth in the products decolorized with charcoal. Stickel and Myers (1918) used peptic and tryptic digestion products of blood, pig or beef liver and placenta, successfully for bacterial culture. In some cases they found it necessary to add phosphates. Kligler (1919) found an autolysate of yeast a suitable medium for bacterial growth, toxin production, and observation of indol production, he noted that the amino acid content was high.

c. Amino Acids and Ammonium Salts.

While many of these products furnish nitrogen in a form which may be utilized by a great number of strains of bacteria, and have proven very useful for cultivation, they do not serve the purpose of a medium for research in bacterial metabolism so well. It is highly desirable in studying the products formed by bacterial action to have a medium of definite chemical composition. This is found in a number of synthetic media. Uschinsky (1893), Frankel (1894) and Jordan (1899(used media having as a source of nitrogen, asparagin alone or with an ammonium salt. These have been found to support the growth of many saprophytic and a few pathogenic bacteria. Studies upon the availability

of nitrogen of chemically definite compounds for certain species of bacteria have been made by several investigators. Wells, Dewitt and Long (1923) have reviewed the work on the nitrogen requirements of B. tuberculosis and in addition Long (1919) noted that while alanine in the presence of glycerol supported growth, when phenylalanine was substituted for alanine the growth was diminished. He attributed this to the inhibitive effect of the ring compound.

Dolt (1908) observed that asparagin could be used as a source of nitrogen and carbon for B. coli if phosphates were present. He recommends two media for the isolation of B. coli from water.

I.	3% agar solution	500 cc
	Glycerine	5 gms.
	Ammonium phosphate	1 gm.
	Distilled water	500 cc
II.	3% agar solution	500 cc
	Ammonium lactate	5 gms.
	Na_2HPO_4	1 gm.
	Distilled water	500 cc

Zuns and György (1916) studied the effect of certain amino acids, proteoses, peptids, purines and extractive bodies upon growth and indol production of B. coli and B. typhosus. They found that while B. coli would grow well upon mono-amino acids and peptids, B. typhosus could not utilize them but grew well upon certain proteins and nitrogen compounds such as sarcosine, xanthine and taurine. Gliadine seemed to specially favor growth of B. typhosus. Indol

production with B. coli depended upon presence of tryptophane.

Clark and Lubs (1917) recommended a medium containing aspartic acid as the only source of nitrogen for use in differentiation within the colon-aerogenes group. This has since been adopted as a standard medium in the Bacteriological Analysis of Water (1925).

Gordon (1917) used a basic medium composed of washed and dried agar 3%, K_2HPO_4 0.1%, $MgSO_4$ 0.2%, $COCl_2$ 0.01%, NaCl 0.5% to which he added various nitrogenous substances. He attempted to cultivate a number of pathogenic bacteria and found that when glucose was present as a source of carbon, B. coli, B. paratyphosus, B. pyocyaneus, and B. proteus could satisfy their nitrogen requirements with ammonium salts, amides and amino acids. The cholera vibrio and B. dysenteria could use asparagic but could not utilize ammonium salts. B. typhosus, Friedlander's bacillus, B. diphtheria, the staphylococci and the streptococci could not utilize any of these simpler nitrogeneous compounds.

Ayers and Rupp (1918) by limiting the nitrogen containing compounds to ammonium phosphate, secured a selective medium for the enumeration of colon organisms in water and milk. The colon-aerogenes group of bacteria produced typical colonies while most other bacteria failed

to develop.

Otsuka (1916) observed that staphylococcus aureus and B. prodigiosus could decompose glycyl-l-tyrosine and glycyl-tryptophane while B. coli could not.

Frances (1923) made the interesting observation that B. tularense failed to grow on beef infusion peptone agar and in media containing various proteins, peptides, amino acids and sulphur compounds other than cystine, but grew well on media containing a small amount of added cystine. Davis and Terry (1919) found that B. diphtheria would not grow on a synethetic medium containing amino acids or extractives as the only source of nitrogen, but when a small amount of beef infusion was added, growth and toxin production proceeded as usual. Cystine seemed to have a stimulating effect upon toxin production.

Of the investigations which have been of a more general nature, Lepierre (1903) prepared what he believed to be pure crystaline glucoproteins, with from 6 to 11 carbon atoms. These were added in amounts of from 1.5% to 3% to a new constant mineral solution consisting of

Sodium chloride	0.5%
Magnesium sulphate	0.05%
Calcium glycerophosphate	0.2 to 0.3%
Potassium bicarbonate	0.1 to 0.2%

He sometimes added 2.0 to 3.0% of glucose, saccharose or

glycerine. He found that of 45 organisms tried (22 pathogens and 23 saprophytic) 14 of the pathogens and 23 of the saprophytes grew on all the gluco-proteins. Seven pathogens, i.e., streptococcus, B. diphtheria, B. anthrax, B. petis, B. tetanus and Vib septique were able to grow in glucoproteins of C^8 and C^9 while a strain of human tuberculosis bacillus preferred C^{10} and C^{11}. The meningococcus required preliminary adaptation.

Galimard and Lacomme (1908), using Lepierre's basic medium studied the growth of a number of species of pathogenic bacteria using various nitrogen compounds consisting of seven different amino acids, urea, and eleven mixtures of amino acids. He found that B. pyocyaneus could utilize glycine, leucine, tyrosine, aspartic acid, lysine, arginine, urea. B. coli utilized glycine, leucine, tryorine and arginine. B. dysenteria utilized arginine only; B. paratyphosus, tyresine and arginine; B. typhosus, none; B. diphtheria, none. None of the species were able to utilize phenylalanine. Of the mixtures no one medium supported growth of all organisms used. B. coli grew on all but one, B. typhosus grew on none and B. diphtheria on none. Doryland (1916) reported growth by certain strains of bacteria upon a synthetic medium containing various nitrogen and carbon compounds and using silicate jelly as a coagulant. Hulton-Frankel, et al., (1919 a,b,c)

studied growth (a), sugar fermentation (b), virulence and antibody production (c), of bacteria upon a synthetic medium containing ammonium salts as the only source of nitrogen.

Koser and Rettger (1919), using a modified Uschinsky's basic medium studied the utilization of representative monoamino, diamino, aromatic and heterocyclic amino acids; ammonium phosphate, urea, taurine, creatin, hypoxanthin, uric acid, allantoin and various mixtures of the above, by a number of bacteria representing 9 different groups. Their results essentially confirm those of Galimard and Lacomme. They also note that with the exception of the vib. cholerae, the organisms which utilize an amino acid can use diammonium acid phosphate as readily. B. anthracis, B. dysenteriae, members of the diphtheria group and all but one of the cocci failed togrow in any of the media, while B. typhosus developed in tryptophane and several amino acid mixtures.

Diehl (1919) observed that ferments obtained from bacterial growth on mediums containing amino acids as the only source of nitrogen show considerable specificity. Those obtained from growth on asparagin, digest both gelatin and casein. Those from tyrosine do not digest gelatine.

Wyon and McLeod (1923) report an inhibition of growth of various bacteria by amino acids when added in relatively small amounts to peptone media. They found some variation in resistance of the organisms. Staphylococcus

aureus, subtilis, B. dysenteriae, Flexner, B. diphtheriae, the pneumococcus and hemolytic streptococci being the most easily inhibited, while B. typhosus, B. paratyphosus A and B were not effected by the concentrations used. Of the amino acids tried, histidine and tyrosine were inhibitive in the lowest concentration.

Gordon (1924) found that cystine inhibited the growth of delicately growing bacteria. While bacteria which split cystine with liberation of hydrogen sulphide could withstand a considerable concentration.

D. Growth Accessory Substances.

The isolation and cultivation of the more delicately growing pathogenic bacteria has always been a problem of considerable importance.

It is usually accomplished by adding blood, serum or some other body fluid to ordinary culture media. The substances necessary for usch stimulation is considered to be nitrogenous in nature, and is known by such terms as "growth accessory factors", "vitamines" and "hormones". Lloyd (1916) working with the meningococcus noted that these substances may be supplied by body fluids and possibly by eggs, starch and vegetable digests, that they are removed by filtering, but not destroyed by heat of sterilization. Huntoon (1918) and Bailey (1925) confirmedLloyd's results and described preparation of "Harmone" media. Ayers and Rupp (1920) used extracts of dry yeast.

Putman and Gay (1920) and Williams and Povitsky (1921) were able to cultivate Pfeiffer's bacillus without the presence of hemoglobin by growing in mixed culture with various organsism. Ayers and Mudge (1922) found growth promoting substances in autolysed yeast extract, cabbage extract and in animal, vegatable and mineral oils. They could not successfully identify them with the source of any of the known vitamines. Mueller (1922 a) found that boiling beef heart infusion with 2% wood charcoal removed some substance necessary for the growth of streptococcus and that this could be replaced by small quantities of peptone or acid hydrobyrate of casein or edestin.

Whitehead (1924) gave evidence to show that amino acids were not responsible for stimulation of growth.

Robertson (1924) showed that B. coli growing in a medium devoid of accessory factors produced substances necessary to the growth of yeast.

B. CARBON REQUIREMENTS.

The carbon requirements of bacteria are more easily supplied although considerable variation is found among the different species. The ability to utilize the carbon of various organic substances is believed to be one of the more stable characteristics of bacteria and has been used as a basis for classification.

Jensen (1909) in his classification recognized a

group of bacteria which were able to utilize atmospheric CO_2. The studies of Kligler (1914), Browne (1914), Winslow, Kligler and Rothberg (1919) and the classification of Holman (1916), Castelloni and Chambers (1920) and Bergey's (1923) are based more or less upon the fermentation of carbohydrates and the higher alcohols. Laybourn (1920) studied the fermentation of various poly-saccharides by B. aerogenes. The ability of certain bacteria to utilize salts of the organic acids has been studied by Sullivan (1905-06), Brown, Duncan and Henry (1924) and Koser (1923 & '24).

McBeth and Scales (1913) described a number of bacteria and fungi that decompose cellulose.

That bacteria can obtain the carbon for their energy and structural needs from protein sources has been shown by Smith (1897), Dolt (1908), Kendall (1913) and others.

C. Inorganic Salt Requirements.

There is lack of agreement as to the inorganic salts necessary for bacterial development. Phosphorus in some form is conceeded to be necessary for the development of bacteria due to its inclusion in the structure of the nucleus of the cells. Löwenstein (1913) and Kendall, Day and Walker (1914) were able to grow B. tuberculosis with phosphorus as the only inorganic constituent. Lockemann (1919) on the other hand found potassium and magnesium to be needed in addition to phosphorus. A number

of the synthetic mediums referred to above contain only a few inorganic radicles. Jordan (1899) studied the effect of inorganic salts on pigment production by bacteria. Sullivan (1905-1906) used a number of synthetic media in his study of growth and pigmentation. He used asparagin as a source of nitrogen and was able to control pigmentation by varying the qualitative inorganic salt content of the media. He considered phosphates and sulphates necessary for pigment production. Pitz (1916) reports that elemental sulphur had a harmful effect upon the growth of soil bacteria and that $CaSO_4$ increased growth of nitrogen fixing bacteria but had no effect upon the general soil flora. Holm and Sherman (1921) explain the inhibiting and stimulating effect of different salts on growth of bacteria by comparing it to the action on colloidal reactions such as coagulation, permeability, diffusion. They found that the cations Na, K, NH_4, stimulated growth while Ca, Mg and Fe inhibited growth. Of the anions, the chloride, iodide, NO_3, SO_4 and PO_4 stimulated; while the oxylate, acetate, citrate and fluoride inhibited.

Another method of approach to the problem is by a chemical analysis of the bacterial protein. The outstanding fact learned from such analyses is the marked variation of the percentage composition of the ash when the organism is grown upon a medium of varying inorganic

salt content. Wells, et al., (1923) has summarized the analyses of B. tuberculosis made by a number of investigators. The elements usually found present were phosphorus, Mg, Ca, K, Na and sometimes SO_4. Dawson (1919) made analyses of the ash of B. coli grown upon 8 different media which varied in their chemical composition. He found marked variation in the precentage composition not only of the inorganic salts but of the nitrogenous composition, fats and carbohydrate radicles as well. Vaughn (1913) summarized the work done by Leach (1906), Wheeler and others in his laboratory upon the chemical analyses of the bacterial substance. Na, K, Ca, Cu, Al, PO_4 were identified from the ash of the colon bacillus. A number of mono-amino and diamino acids were determined from the bacterial protein. They believed that the Cu and Al were from extraneous sources.

Downs (1924) has compiled a comprehensive bibliography on the effect of hydrogen ion concentration on biological reactions.

2. Bacterial Metabolism

Scientists have been aware of the reactions and products of cultures of bacteria and allied forms since the work of Pasteur. Some work was done by the early bacteriologists on the identity of the end products of bacteria grown upon proteins and upon carbohydrate media. Most of the work which we now classify under the term

bacterial metabolism awaited the perfection of chemical methods of analysis and their modification to bacteriological use, and is the result of more recent years. This work has taken two general directions: (1) a study of the various steps in the breakdown of protein and carbohydrate molecules by qualitative identification of the intermediate and end products; (2) a careful quantitative study of the end products and such of the intermediate products as are necessary for determining the manner of the decomposition.

The accumulation of such knowledge has been of great value to: (1) the public health worker in regard to food spoilage, purification of streams and surface waters of sewage pollution and industrial wastes, and the proper disposal of human excreta and other waste products; (2) the physician and medical scientific workers, in regard to the production of bacterial toxins, the relation of bacteria to disease, intestinal putrefaction and the identification of pathogenic bacteria; (3) agriculture, in this regard the study of the nitrogen and carbohydrate metabolism has been the means of establishing upon a firm scientific foundation the theory of rotation of crops, nitrifaction of the soil, nitrogen fixation by leguminous plants, and has established the great importance of bacteria in the nitrogen and carbon cycles of nature; (4) the manufacturer and industrial chemist, in the production of fermented foods

and vinegar, the scientific control of texture flavors and aromas in butter, cheese, and in the production of other dairy products, the manufacture of white lead, and the production of various alcohols, acetone and other chemical products.

A. NITROGEN METABOLISM.

A. Qualitative study of the intermediate and end products.

Indol was one of the first end product of bacterial decomposition of proteins to be determined and has long been used as an index of proteolysis and as an aid in identification of bacteria. Remy (1900) studied indol production of B. coli and B. typhosus in protein media. Taylor (1902) observed that B. coli produced proteoses and peptones and a small amount of amino acids from casein. Rettger (1903) cultured B. coli and B. aerogenes in an egg-meat medium and identified the following products of decomposition: indol, oxyacids, skatol, carbonic acid, leucin, tyrosin, tryptophane, hydrogen sulphide, mercaptan, albuminoses and peptones. He noticed some qualitative differences in the production of these substances by the two organisms. He later (1906) continued this study using a number of aerobic and anaerobic organisms and found a considerable variation in the end products produced by the various bacteria. Berthelot (1917) isolated an organism from the intestines which produced large quantities of pehnol from tyrosine. He called the organism.B. phenol-

ogenes. Herter and TenBroeck (1911) grew B. Protens vulgaris upon a meat extract peptone medium and were able to identify indol, acetic acid, aromatic oxy acids, hydrogen sulphide and volatile alkali products but were unable to demonstrate phenol, skatol, mercapton, putrescine and cadaverine. Sasaki (1912 a,b,&c) showed that the dipeptides glycyl-glycine and glycyl-l-tyrosine were split by B. coli(a), by non-liquefying(b), and certain liquefying bacteria(c). In a later paper (1914) he demonstrated P-oxyphenylethylamin as a decomposition product of B. coli grown in a synthetic medium containing tyrosine.

Hall (1921) reported an organism which he called B. tyrosinogenes, which produced tyrosine.

b. Quantative Study of Intermediate and End Products.

The quantitative study of products of bacterial metabolism is of rather recent development, due to the lack of suitable quantative methods of analysis. Kendall (1913) in his early papers, and later Kendall and Walker (1915) used ammonia nitrogen as an index of proteolysis and nitrogen utilization by bacteria. In more recent studies, Kendall (1922) has also used other nitrogen fractions of the media. Waksman (1918) studied a number of soil fungi and bacteria. He questions the use of ammonia production as an index of proteolysis. Benton (1919) cultivated several species of bacteria in plain broth, acitic fluid

and plain broth to which was added various amino acids, and compared the utilization of amino nitrogen to the coaguable protein and growth rate of organisms. Sears (1916) studied the amino acid, ammonia, creatin and creatinine in a large number of cultures in peptone and gelatine media. Wolfe (1919) measured the proteolytic action of the anaerobic bacteria, B. welchii and B. sporogenes in a cooked meat medium by means of ammonia and amino acid nitrogen determinations. Lampitt (1919) studied the nitrogen utilized and excreted by a yeast S. cerevisiae. DeBord (1923) determined amino nitrogen, hydrogen ion concentration, total count and sugar utilization in a peptone phosphate medium. He noted in the pyocyaneus culture a coincident decrease in sugar, increasing ammonia with the hydrogen ion concentration remaining about the same. He concluded from this that sugar utilization cannot be determined by measuring the acidity produced. This observation has been confirmed by Sherwood (1926). DeBord believes that amino acid nitrogen content is a more logical index of proteolysis than ammonia nitrogen. Raistrick (1919) and Raistrick and Clark (1921) have studied the utilization of the nitrogen in histidine (1919), tryptophane and tyrosine (1921). They found that of the organisms studied, B. paratyphosus A, B. paratyphosus B, B. fecalis alhaligcues, B. pyocyaneus

and B. vulgaris, all could utilize the nitrogen of the iminazole ring except B. vulgaris. This organism was also unable to break down the indole ring, while B. prodigiosus, B. fluoresceus, and B. pyocyaneus could. Morris and Ecker (1924) reported an organism isolated from chicken excreta that was able to break down large amounts of uric acid. This was alos accomplished by a few molds. Most bacteria failed to grow in the medium used.

Patty (1921) studied quantitatively the production of hydrocyanic acid by several strains of B. pyocyaneus.

c. Effect of Utilizable Carbohydrates upon Nitrogen Metabolism

The presence of utilizable carbohydrates in a culture medium has a very profound effect upon the nitrogen metabolism of bacteria. Observations to this effect have been made by many investigators. Peckham (1897) while studying the effect of envoirment upon members of the colon group of bacteria made the following statement. "When the members of the colon group are cultivated under circumstances favorable to the development of both the function of fermentation and that of proteolysis, fermentation invariably takes precedence, and no evidence of proteolysis is manifested until after fermentation has ceased". Kendall and Farmer (1921) showed that ammonia production by bacteria was much greater in sugar free broth than in one

containing a utilizable carbohydrate. This was interpreted to indicate a "protein sparing action" of the carbohydrate. This has been the theme of the many studies on bacterial metabolism by Kendall and his associates. Waksman (1917) confirmed Kendall's observation while studying the influence of available carbohydrate upon ammonia accumulation in the soil.

Jones (1916) noted that gelatine containing 0.5% sugar was not liquefied by B. proteus vulgaris. In a later communication (1920) he concludes that B. proteus shows no evidence of amino acid utilization, when carbohydrate is present, though kept at neutrality. Kligler (1916) while studying the effect of phosphates on carbohydrate utilization noted reduced ammonia production in the presence of utilizable carbohydrate.

Theobald Smith (1899) reported that when a fermentable sugar was present in appreciable amounts, toxin production by B. diphtheria was diminished. Dozier, Wagner and Meyer (1924) pointed out that this was not true of toxin production by B. botulinus. Toxin was produced earlier and in as great quantity in media containing dextrose as in sugar free media. They believe that this difference may be due to the fact that while the toxin of B. diphtheria is exocellular, the toxin of B. botulinus is released only after death of the cell. Simonds (1915)

found that B. typhosus grown in dextrose media was more easily agglutinated, more easily engulfed by leucocytes and there was some evidence to show that the potency of the endotoxin was decreased. Ruediger (1906) and later Stevens and Koser (1919) determined that fermentable carbohydrates inhibited hemolysin production by streptococci.

The influence of utilizable carbohydrate upon indol production was observed quite early and has been the subject of considerable research.

Pere (1892) noted that B. coli did not produce indol when sugar was present. He says, "La presence du sucre garantit la peptone contre la putrefaction: cette preservation se maintient autant que dure la fermentation du sucre".

Homer (1916) believes that the lack of indol production in presence of carbohydrate is caused by the production of condensation products of tryptophane and aldehyde or keto groups which are too stable for bacteria to break down. Logie (1919) gave results to support his contention that in the presence of sugar, indol was used by the bacteria to synthesize tryptophane. Wyeth (1919) studied the effects of free acids, alkalies and of sugars upon indol production. He concluded that the sugar inactivated the proteolytic enzymes. Bondo (1922) determined that the acids due to the fermentation of sugars, is not the cause of the failure of indol production. He also has shown that sugars do not inactivate the enzymes necessary for indol production.

B. Carbohydrate Metabolism
Products of Fermentation.

Some of the products of carbohydrate decomposition was recognized very early in the history of fermentation. Exact analysis of these products from pure cultures of organisms were of later date, however.

Harden and Walpole (1906) determined the percentage production of carbon compounds from glucose by B. aerogenes and B. coli. The compounds were alcohol, acetic acid, lactic acid, succinic acid, formic acid, carbon dioxide. In the culture of B. aerogenes they also found 2:3 butyleneglycol $CH_3.CH(OH).CH(OH).CH_3$ and its derivitive acetylmethylcarbinol $CH_3CO.CH(OH).CH_3$. Harden (1906) reported this substance responsible for the production of the Voges and Proskauer reaction.

The titratable acidity of cultures of bacteria in carbohydrate media has been determined by a number of investigators, for the purpose of classification. Broadhurst (1912) and Winslow (1912) studied the streptococci, Browne (1914) and Fitzgerald (1914) determined the acid production of various members of the colon aerogenes group.

The more accurate determinations of the actual acidity by hydrogen ion determination has been made of the colon aerogenes group by Clark and Lubs (1915) and Clark (1915) who established the fact that B. coli, when cultured in dextrose broth, reaches a final hydrogen ion concentra-

tion of 4.37 to 4.55. This relatively high concentration is maintained for some time. B. aerogenes, on the other hand, does not produce this degree of acidity and within a few days reverts toward an alkaline reaction. Thus B. coli when grown in a dextrose medium will react acid to methyl red indicator while aerogenes will react alkaline. Ayers and Rupp (1918) showed that this reversion may be partially due to the breaking down of organic acid salts, with the formation of carbonates and bicarbonates.

It is of interest to note that unlike the products of protein decomposition, which vary qualitatively with the protein acted upon, the products of carbohydrate fermentation are similar irrespective of the carbohydrate decompsed. However, Browne (1916) has shown that the quantitative yield of acid in cultures of B. coli varies inversely with the complexity of the carbohydrate.

The possibility of insulin effecting carbohydrate utilization by bacteria was suggested by Noyes and Estill (1924) who found an increased acid production by B. bulgaricus and B. acidophilus when grown in a glucose-skim milk solution to which insulin was added. McGuire and Falk (1924), Kendall (1925 a&b), and Kendall and Mitzutem (1925) were unable to confirm this observation

C. Gaseous Metabolism

The gaseous products of carbohydrate and protein

metabolism merit consideration.

These gases must not be confused with the gases produced by respiration. This consists in an exchange of gases between the protoplasm of the cell and the medium surrounding it. This characteristic is common to all living cells. These respiratory gases produced by bacteria are so small in amount that they escape notice unless special means are taken for their detection. A series of studies has been recently begun by Novy, et. al.(1925) who have been able, by means of a mercury manometer to follow bacterial respiration, and by suitable methods of gas analysis, to determine the respiratory quotient of the culture in the different phases of its development.

A knowledge of the gases produced by bacteria aids in determining the methods by which bacteria break down different nitrogen and carbon compounds. The gases produced from carbohydrates have long been used as a means of classification. That gas from protein substances may also be used for classification is suggested by certain results shown in this paper.

A review of the literature bearing on gaseous metabolism is reserved for later sections where the production of gas from peptone is considered.

The present investigation grew out of an early observation that certain members of the colon aerogenes group produced a visible amount of gas from a peptone gelatine medium to which no carbohydrate had been added. With the view of explaining this reaction and of determining its possible significance in classification, the work herein reported was undertaken. This has been considered in the following way.

Part I. Cultural Studies.

In this section there has been given the methods and results of the isolation and cultural reactions of the organisms used. Correlations between gas production in Difco peptone gelatine with other cultural reactions and with the source of the organisms have been observed.

Part II. Metabolic Studies.

There has been included in this section:

(1) A study of the gas production from peptones, showing the conditions under which the gas is produced, together with quantitative analyses of the gas produced under these conditions.

(2) A study of H_2S production from peptones and and from cystine, showing the influence of the presence of other amino acids upon H_2S production from cystine.

PART I

CULTURAL STUDIES

Isolation of Cultures

The cultural studies were made upon 216 strains of the colon-aerogenes group isolated from the sources as indicated by Table I.

The different types of special media which are used for the isolation of members of this group are prepared with lactose agar, making use of the fact that these organisms ferment this sugar. They vary in respect to whether they contain the following:

(1) An indicator for acid or aldehyde production, such as Endo's agar, Eosin-methylene blue agar, litmus lactose agar, etc.

(2) An inhibiting agent, such as, brilliant green agar, gentian violet agar, etc.

(3) A combination of 1 and 2 such as Conradi Drigalski's agar.

References to these media have been made in the description of the isolation of cultures.

TABLE I.

SHOWING SOURCE OF STRAINS STUDIED

Source	Number of Strains	Preliminary Enrichment	Media Used for isolation
Surface Water A & B	37	lactose broth	Endo's fuchsin agar
Ground Meat	24	lactose broth	Eosin-methylene blue
Oysters	21	lactose broth	" " "
Milk & Cream	14	lactose broth	" " "
Flour Sax	6	lactose broth	" " "
Soil(stored)	6	lactose broth	" " "
Feces A & B	46	plated direct	" " "
Feces C	63	dextrose broth with brilliant green	" " "
Total	217		

Methods of Isolation.

The 36 surface water strains were obtained by picking colonies from Endo's agar plates prepared according to the Standard Method of Water Analysis (1923) and planted as routine confirmation tests in the Kansas State Water and Sewage Laboratory. Plates were selected which showed well isolated characteristic colonies. These were planted on agar slants and subsequently purified by repeated planting upon Eosin-methylene blue plates. The Eosin-methylene blue plates in this and all subsequent isolations were prepared after the method of Holt-Harris and Teague (1916) which was slightly modified, and was prepared as follows: To 100 cc of plain meat extract, peptone agra, adjusted to P_h 7.2, was added 5 cc of sterile 20% lactose, 1 1/3 cc 3% Eosin solution and 2 cc 0.5% methylene blue, plates poured and let dry in incubator over night. Typical B. coli colonies appear on such plates as medium sized colonies, purple to black in color, quite flat on top and having usually a greenish bronze sheen when viewed by reflected light. B. aerogenes colonies are larger,(tend to become confluent after 24 hours). They are raised and convex. They have a brownish center with a lighter periphery and are very moist in appearence.

The strains from ground meat, oysters, milk and

cream, flour sacks and soil were all isolated by planting appropriate dilutions of the material in lactose fermentation tubes and plating out on Eosin-methylene blue plates from the highest dilution showing gas in 48 hours.

The 46 feces strains marked "Feces A and B" were plated out directly upon Eosin-methylene blue plates without preliminary enrichment. A small amount of the sample being first emulsified in salt solution. Where more than one type of colony was present on the plate they were picked.

The 63 "Feces C" strains were obtained after preliminary enrichment in dextrose-brilliant green broth. This was prepared by adding a measured amount of a sterile 0.1% brilliant green solution to 10 cc of sterile 0.5% dextrose broth.

Since the work of Churchman (1912), a number of different media have been developed for use in isolation of the colon-typhoid group all based upon the selective bactericidal action of the triphenylmethane dyes. Robinson and Rettger (1916), Krumwiede, et. al. (1916), Hall and Ellefsen (1919), Skinner and Murray (1924) have recommended media of this type. Winslow and Dolloff (1922), among others, noted that B. aerogenes was more resistant to brilliant green than B. coli. He used a lactose broth medium. Levine and Linton (1924) used brilliant green

in lactose broth for isolating B. aerogenes from feces.

It was found necessary to titrate each new lot of brilliant green before use. This was done by adding to a series of tubes containing 10 cc of 0.5% dextrose broth a varying amount of the brilliant green solution, (1 drop in the first tube, 2 drops in the second, etc.) then add a uniform amount of feces to each tube, and finally inoculate each tube with 0.1 cc of a broth culture of B. coli and 0.1 cc of broth culture of B. aerogenes. Incubate at 37°C for 24 hours and plate out upon Eosin-methylene blue agar plates. The amount of brilliant green necessary to cause inhibition depends upon the amount of feces added, as this neutralizes the action of the dye.

When used for isolation, the same amount of feces sample must be used as was used in the titration. Almost complete decolorization of the dye was sometimes noticed, even in cases where B. coli was apparently inhibited.

All strains were plated out from time to time during the progress of the work to insure the purity of the culture.

Cultural Characteristics and Classification.

There has been much interest in the classification of the colon-aerogenes group of bacteria, especially by American bacteriologists, because of their use in this country as an index of fecal pollution. The accurate

identification of these organisms isolated from water and foods is a necessity. As defined by the Committee on Standard Methods of Water Analysis (1925) this group includes "all-non-spore forming bacilli which ferment lactose with gas formation and grow aerobically on standard solid media."

The earlier classifications of this group such as those of McConkey (1905), Bergey, Deehan and Jackson (1911), Kligler (1914), Winslow et. al. (1919), Castellani and Chambers (1920) were all based upon the fermentation reactions in a large number of carbohydrates and alcohols. By this method the group has been divided into a large number of strains. Attempts at classification by immunological methods by Dunham (1900), Mackie (1913), Coulter (1917) and others have resulted in failures, most strains showing a great specificity for their homologous anti-serum. Dudgeon, et al (1921) (1922) have reported that B. coli isolated from infections of the urinary tract cause hemolysis of red blood cells, and that B. coli from this source form a rather homogeneous group in this respect.

Recent studies upon the correlation of certain characteristics of these organisms with sourde have given us a logical, workable, division of the group

Clark and Lubs (1915) showed that B. coli, when growing in dextrose broth, properly buffered, would reach a P_h of about 4.5 and would then remain quite constant,

while B. aerogenes did not reach such a high hydrogen ion concentration and quickly reverted toward an alkaline reaction. These organisms can then be differentiated in dextrose broth by the use of an indicator such as methyl red whose range is P_h 4.4-6.0.

Voges and Proskauer (1898) noted an eosin pink like color develop when KOH was added to a peptone dextrose culture of certain organisms. The substance responsible for the color was determined to be acetyl-methylcarbinol by Harden (1906). The test has since been used by many investigators and it is found to be characteristic of B. aerogenes, while B. coli fails to produce this substance.

Koser (1918) noted that B. aerogenes was able to utilize uric acid as a source of nitrogen while B. coli could not, and in 1923 reported that B. aerogenes was able to use citrates as a source of carbon while B. coli could not.

Attempts to correlate two or more of these tests with the source of the organism has been made by Levine (1916), Johnson (1916), Hulton (1916), Greenfield (1916), Burton and Rettger (1917), Winslow and Cohen (1918), Chen and Rettger (1920) and Koser (1924). The result of these studies has been to establish the fact that; 1, B. coli and B. aerogenes are well defined types; 2, that the B. coli type predominates in the

feces of man and animals, while the B. aerogenes type is found in greater numbers in the soil; and 3. that these two types may be successfully differentiated by the above tests. This is summarized by the following table.

TABLE 1a

Essential differences between B. coli and B. aerogenes.

Organism	Voges & Proskauer Reaction	Methyl Red Test	Growth in Uric acid Medium	Growth in Citrate Medium	Predominates in
B. coli	-	+	-	-	Feces
B. aerogenes	+	-	+	+	Soil

The interpretation of the presence of these organisms in water, foods, etc. is complicated, however, by the finding of certain intermediate strains, that partake of some of the characteristics of each of the above type species. Also a small percentage of aerogenes-like organisms have been found in the feces. Any evidence which would help to establish the origin of these intermediate strains would be of value to Sanitary Bacteriology.

METHODS.

The cultural characteristics which have been found pertinent to the classification of this group, were used. They are as follows: fermentation of the carbohydrates; dextrose, lactose and saccharose and of the alcohols, dulcitol and adonitol; the production of

indol; liquefaction of gelatine; methyl red test; the Voges Proskauer reaction; hydrogen sulphide production; and the utilization of uric acid and sodium citrate. To this list has been added the production of gas from Difco peptone gelatine.

The description of the culture media used and of tests applied in the study of the characteristics of these organisms is as follows.

Fermentation Reactions.

All fermentation reactions were made in inverted vial fermentation tubes, filled with meat extract-peptone broth to which 1% of the desired carbohydrate was added. This was adjusted to P_7 7.4 and heated in the autoclave for 20 minutes at 10 lbs. pressure. Innoculations were made from 24 hours agar slant cultures, incubated at 37°C for 48 hours. Acid production was determined by the use of phenol red as indicator. Gas production was noted and in cases where the reaction as acid with no gas or less than 10% of gas present the test was repeated. In the case of the repeated tests the incubation period was increased to 14 days in order to eliminate the possibility of a delayed fermention.

In this way the fermentation of dextrose, lactose, saccharose, dulcitol and adonitol was studied.

Indol Reaction.

Indol tests were applied after four days

incubation at 37°C in a peptone water medium. The Salkowsky and the Ehrlich tests were used. The Salkowsky test was applied as follows: To 5 c.c. of the culture was added .5 c.c. concentrated sulphuric acid, this was mixed and 2 c.c. of a 0.1% solution of sodium nitrite was carefully run down the side of the tube to stratify the two solutions. Indol is indicated by a red color forming at the juncture of the two solutions. The Ehrlich test for indol was made by stratifying 1 c.c. of the reagent (96% alcohol 380 c.c., HCl conc. C. P. 80 c.c., and paradimethylamidobenzaldehyde 4 grams) above 4 to 5 c.c. of the culture. A positive reaction is indicated by a red color appearing at the junction of the two liquids.

Liquefaction of gelatine and gas production in 2% Difco Peptone Gelatine.

The 2% peptone gelatine medium was prepared by dissolving .5% meat extract, 2% Difco peptone, .5% NaCl, and 10-12% gelatine, in distilled water. Adjust to P_h 7.2, tube and sterilize in autoclave for 20 minutes at 10 lbs. pressure, cool rapidly and store in ice-box. Innoculation was made with a straight needle, and from a 24 hour agar slant culture. Gas production was recorded after 48 hours incubation at 20°C.

The gas appeared as bubbles along the line of innoculation, and at times out near the edge of the tube. The bubbles vary somewhat in size and vary in

number from 1 to 8 or more.

Gelatine liquefaction was determined after 14 days incubation at 20°C.

Methyl red and Voges and Proskauer Tests.

The media used for the methyl red and Voges and Proskauer tests is that recommended by the Standard Methods of Water Analysis (1923). Proteose peptone Difco was used and the method of preparation was carefully followed. The suggestion of Chen and Rettger that the media be autoclaved, with the tubes contained in beakers or tins instead of the usual wire baskets, was tried with excellent success. The media always came out crystal clear, however, it was necessary to sterilize at 20 lbs. added pressure and for 15 to 20 minutes to insure sterility. Incubation was carried out at 37°C and the tests made after 48 hours. The culture was divided by pouring half into another tube. Five drops of methyl red indicator was added to the media in the original tube and the reaction recorded. To the culture in the other tube was added an equal amount of 10% KOH. This was mixed thoroughly by shaking, and allowed to stand at room temperature. Readings were made after 4-5 hours and after standing over night. A positive Voges and Proskauer test is indicated by the formation of a fluorescent-eosin-like color which forms at the surface. The negative tubes remain colorless or slightly yellow.

Hydrogen Sulphide Production.

The qualitative test for H_2S production was made upon the greater part of the strains by the use of a 3% Difco peptone agar with ferrous sulphate added as an indicator. A solid medium for use in the detection of H_2S is usually prepared, with lead acetate as an indicator, Jordan and Victorson (1917).

There are a number of objections to using lead acetate, (1) when the solution is added to a medium containing a small amount of CO_2, the medium becomes cloudy, due to the precipitation of lead carbonate. This compound also forms upon exposure of the solution to the air. (2) Upon standing or upon prolonged incubation, the medium containing lead acetate becomes brownish in color due probably to the formation of lead oxides. This color may be confused with the brown to black color produced by the H_2S. In order that these objections might be overcome, the following media was devised.

Ferrous Sulphate Agar .

It was found that ferrous sulphate could be substituted for the lead acetate, and that it did not have the objectionable features of that salt. Results showing a comparison of the two salts as indicators are shown in table 9.

The agar was prepared as for plain agar except 3% Difco peptone was used instead of the usual 1%. The agar was adjusted to P_h 7.2, bottled in 100 cc amounts and sterilized in the autoclave. After sterilization there was added to each 100 cc of agar, 1 cc of a 2% solution of ferrous sulphate in distilled water. The ferrous sulphate solution may be sterilized in the autoclave and should then be kept tightly stoppered. The media was poured into sterile tubes about 6-7 cc per tube, and allowed to solidify. Stab innoculations were made with a straight needle and from 24 hour agar slant cultures. Incubation was carried on at 37°C for 48 hours. A positive reaction is indicated by an intense black color beginning at the line of innoculation and diffusing toward the sides of the tube.

Uric Acid Utilization.

The medium for testing uric acid utilization was made according to Koser (1918) with the following formula.

Distilled ammonia-free water	1000 cc
NaCl	5.0 gms.
$MgSO_4$	0.2 gms.
$CaCl_2$	0.1 gm.
K_2HPO_4	1.0 gm.
Glycerol	30.1 gms.
Uric acid	0.5 gm.

Some difficulty was had in getting the uric acid to dissolve. This difficulty was overcome by dissolving the K_2HPO_4, glycerol and uric acid in about 800 cc

of the water. The NaCl, $MgSO_4$ and $CaCl_2$ were dissolved in the remainder and the two solutions mixed. The media was filled into tubes which were chemically clean and had been thoroughly rinsed with ammonia free water. It was then sterilized in the autoclave at 15 pounds added pressure. Innoculation was made as soon after preparation of the media as possible in order to avoid the possibility of absorption of ammonia. Very light innoculations were made with a straight needle from a 24 hour agar culture. Incubation was at 37°C and the presence of growth noted at the end of 4 days. The presence of growth is interpreted as indicating utilization of the uric acid.

Citrate Utilization.

The citrate media was made according to Koser (1923) (1924) and the Standard Methods of Water Analysis (1925). It has the following formula.

Sodium ammonium phosphate	1.5 gm.
Potassium dihydrogen phosphate	1.0 gm.
Magnesium sulphate - - - -	0.2 gm.
Sodium citrate (crystals)	2.5 to 3.0 gms.
Distilled water- - - - - -	1000. cc.

The solution was filled into chemically clean tubes and sterilized in the autoclave. Very light innoculations were made with a straight needle from a 24 hour agar slant culture. The innoculated tubes were incubated at 37°C for 4 days, at the end of this time readings were made for the presence of growth. As in the

case of the uric acid medium the presence of growth is interpreted as indicating utilization of the citrate.

RESULTS

The results of the cultural studies are found in TABLE 2.

LEGEND: The + sign in the fermentations means acid and gas production. "A" indicates acid but no gas production. The + sign in liquefaction of and gas production from 2% peptone gelatine; methyl red test; Voges and Proskauer test; Indol test; hydrogen sulphide production, means a positive reaction in each case; + in uric acid and citrate media indicates visible growth.

The - sign indicates a negative reaction in each case.

TABLE 2.

RESULTS OF CULTURAL STUDIES

Culture number	Source	Gram	Dextrose	Lactose	Saccharose	Dulcitol	Adonitol	2% Peptone Gelatine		Methyl Red	Voges Proskauer	Uric Acid	Citrate	Indol	Hydrogen Sulphide
								Gas	Liq						
X 1	Feces Series A	-	+	+	+	+	-	-	-	+	-	.	.	+	.
X 2	"	-	+	+	-	A	-	-	-	+	-	.	.	+	.
X 3	"	-	+	+	-	-	-	-	-	+	-	.	.	+	.
X 4	"	-	+	+	+	+	-	-	-	+	-	.	.	+	.
X 5	"	-	+	+	+	+	-	-	-	+	-	.	.	+	.
X 6	"	-	+	+	-	+	-	-	-	+	-	.	.	+	.
X 7	"	-	+	+	-	-	-	-	-	+	-	.	.	+	.
X 8	"	-	+	+	-	-	-	-	-	+	-	.	.	+	.
X 9	"	-	+	+	+	+	-	-	-	+	-	.	.	+	.
X10	"	-	+	+	-	-	-	+	-	+	-	.	.	+	.
X11	"	-	+	+	A	+	-	-	-	+	-	.	.	+	.
X12	"	-	+	+	+	-	-	-	-	+	-	.	.	+	.
X13	"	-	+	+	-	-	-	-	-	+	-	.	.	+	.
X14	"	-	+	+	+	-	-	-	-	+	-	.	.	+	.
X15	"	-	+	+	A	+	-	-	-	+	-	.	.	+	.
X16	"	-	+	+	-	+	-	-	-	+	-	.	.	+	.
X17	"	-	+	+	-	-	-	-	-	+	-	.	.	+	.
X18	"	-	+	+	+	-	-	-	-	+	-	.	.	+	.
X19	"	-	+	+	-	+	-	-	-	+	-	.	.	+	.
X20	"	-	+	+	-	-	-	-	-	+	-	.	.	+	.
X21	"	-	+	+	-	+	-	-	-	+	-	.	.	+	.
X22	"	-	+	+	-	-	-	-	-	+	-	.	.	+	.
X23	"	-	+	+	-	+	-	-	-	+	-	.	.	+	.
X24	"	-	+	+	+	+	-	-	-	+	-	.	.	+	.
X25	"	-	+	+	-	+	-	-	-	+	-	.	.	+	.
X26	"	-	+	+	-	+	-	-	-	+	-	.	.	+	.
X28	"	-	+	+	-	+	-	-	-	+	-	.	.	+	.
X29	"	-	+	+	-	-	-	-	-	+	-	.	.	+	.
X30	"	-	+	+	-	+	+	-	-	+	-	.	.	+	.
X31	"	-	+	+	+	+	-	-	-	+	-	.	.	+	.
X32	"	-	+	+	+	+	-	-	-	+	-	.	.	+	.
X33	"	-	+	+	-	+	-	-	-	+	-	.	.	+	.
X34	"	-	+	+	+	+	-	-	-	+	-	.	.	+	.
X35	"	-	+	+	+	+	-	-	-	+	-	.	.	+	.
X36	"	-	+	+	+	-	-	-	-	+	-	.	.	+	.
X37	"	-	+	+	-	+	-	-	-	+	-	.	.	+	.

TABLE 2 - continued

Culture Number	Source	Gram	Dextrose	Lactose	Saccharose	Dulcitol	Adonitol	2% Peptone Gelatine Gas.	2% Peptone Gelatine Liq.	Methyl Red	Voges-Proskauer	Uric Acid	Citrate	Indol	Hydrogen Sulphide
	Feces														
B 1a	Series B	-	+	+	-	+	-	-	-	+	-	-	-	+	-
B 3	"	-	+	+	+	-	-	-	-	+	-	-	-	+	-
B 3a	"	-	+	+	-	-	-	-	-	+	-	-	-	+	-
B 4a	"	-	+	+	-	-	-	-	-	+	-	-	-	+	-
B 4	"	-	+	+	-	-	-	-	-	+	-	-	+	+	-
B 5a	*	-	+	+	-	-	-	-	-	+	-	-	-	+	-
B 6a	"	-	+	+	-	-	-	-	-	+	-	-	-	+	-
B 7a	"	-	+	+	-	-	-	-	-	+	-	-	-	+	-
B 7b	"	-	+	+	-	-	-	-	-	+	-	-	-	+	-
B 8	"	-	+	+	A	....	..	-	-	+	-	-	-	+	-
	Series C														
F 1	Hayden	-	+	+	-	-	-	-	-	+	-	-	-	+	-
F 3	Long	-	+	+	+	-	-	+	-	-	+	+	+	-	-
F 4	"	-	+	+	-	-	-	-	-	+	-	-	-	+	-
F 5	Wells	-	+	+	-	-	-	-	-	+	-	-	-	+	-
F 6	Fairchilds	-	+	+	+	-	-	+	-	-	+	+	+	-	-
F 8	Prewitt	-	+	+	+	-	-	-	+	-	+	+	+	-	-
F10	Davis	-	+	+	+	-	-	-	-	+	-	+	-	-	+
F11	Vermillion	-	+	+	+	+	+	+	-	-	+	+	+	-	-
F 12	"	-	+	+	-	+	-	+	-	+	-	-	-	+	-
F13	"	-	+	+	-	-	-	-	-	+	-	-	-	+	-
F14	"	-	+	+	-	+	-	-	-	+	-	-	-	++	-
F15	Treece	-	+	+	+	-	-	+	-	-	+	+	+	-	-
F17	Hosp.#167	-	+	+	-	+	-	-	-	+	-	-	-	+	-
F18	Wyatt	-	+	+	-	-	-	-	-	+	-	-	+	+	-
F19	Giffin	-	+	+	+	+	-	+	-	-	+	+	+	-	-
F21	"	-	+	+	-	-	+	-	-	+	-	-	-	+	-
F22	Holcomb	-	+	+	-	+	-	-	-	+	-	-	-	+	-
F23	Giffin	-	+	+	+	-	-	+	-	-	+	+	+	-	-
F24	Dyche	-	+	+	+	+	+	+	-	-	+	+	+	+	-
F25	Luke	-	+	+	+	+	+	+	-	-	+	+	+	+	-
F26	"	-	+	+	+	+	+	+	-	+	+	+	+	+	-
F28	Kettlecamp	-	+	+	-	-	-	-	-	+	-	-	-	+	-

TABLE 2 - continued

Culture Number	Source	Gram	Dextrose	Lactose	Saccharose	Dulcitol	Adonitol	2% Peptone Gelatine: Gas	2% Peptone Gelatine: Liq	Methyl Red	Voges-Proskauer	Uric Acid	Citrate	Indol	Hydrogen Sulphide
F29	Fairchild	-	+	+	-	-	-	-	-	+	-	-	-	+	-
F30	"	-	+	+	+	+	+	+	-	+	+	+	+	+	-
F31	"	-	+	+	+	+	+	-	-	+	-	+	+	+	-
F32	Brennen	-	+	+	+	-	+	+	-	+	-	+	+	-	-
F33	"	-	+	+	-	-	+	-	-	+	-	+	-	+	-
F34	"	-	+	+	+	-	+	+	-	+	-	+	+	+	+
F35	Hollingswth	-	+	+	-	+	-	-	-	+	-	+	-	+	-
F36	Prewitt	-	+	+	+	+	-	-	-	+	-	+	-	+	-
F37	"	-	+	+	A	+	-	-	-	+	-	-	-	+	-
F38	Loudon	-	+	+	+	-	+	+	-	-	+	+	+	+	-
F39	"	-	+	+	+	-	+	+	-	-	+	+	+	-	-
F40	"	-	+	+	+	-	+	-	-	+	-	-	-	+	-
F41	Stegman	-	+	+	-	-	-	-	-	+	-	-	-	+	-
F42	"	-	+	+	A	+	-	-	-	+	-	+	-	+	-
F43	Giffin	-	+	+	-	-	-	-	-	+	-	-	-	+	-
F44	"	-	+	+	-	-	-	-	-	+	-	-	-	+	-
F45	Longwood	-	+	+	-	+	-	-	-	+	-	-	-	+	-
F47	Treece	-	+	+	+	-	+	+	-	+	-	+	+	+	-
F 48	Monkey	-	+	+	-	-	+	-	-	+	-	+	-	+	-
F49	"	-	+	+	+	-	-	-	-	+	-	-	-	+	-
F50	Millett	-	+	+	A	+	-	-	-	+	-	-	-	+	-
F52	Hayden	-	+	+	-	+	-	+	-	+	-	+	-	+	-
F53	Westland	-	+	+	-	+	-	+	-	+	-	-	-	+	-
F54	"	-	+	+	+	-	+	-	-	+	-	+	+	+	-
F55	Hamlin	-	+	+	-	+	-	-	-	+	-	-	-	+	-
F56	"	-	+	+	-	-	-	-	-	+	-	+	+	+	-
F57	Haines	-	+	+	-	-	-	-	-	+	-	-	-	-	-
F58	Wells	-	+	+	-	-	-	-	-	+	-	-	-	+	-
F59	"	-	+	+	A	+	-	+	-	+	-	-	-	+	-
F60	Gaston	-	+	+	A	+	-	-	-	+	-	-	-	+	-
F61	"	-	+	+	-	+	-	-	-	+	-	-	-	+	-
F62	"	-	+	+	+	+	-	+	-	+	-	-	-	+	-
F63	Bailey	-	+	+	+	-	+	-	-	+	-	-	-	+	-
F64	Vermillion	-	+	+	-	+	-	-	-	+	-	-	-	+	-
F65	Kettlecamp	-	+	+	-	+	-	+	-	+	-	-	-	+	-
F66	Long	-	+	+	-	-	-	-	-	+	-	+	-	+	-
F68	Anderson	-	+	+	-	-	-	-	-	+	-	-	-	+	-

TABLE 2 - continued

Culture Number	Source	Gram	Dextrose	Lactose	Saccharose	Dulcitol	Adonitol	2% Peptone Gelatine: Gas	2% Peptone Gelatine: Liq	Methyl Red	Voges-Proskauer	Uric Acid	Citrate	Indol	Hydrogen Sulphide
F69	Steichen	-	+	+	-	+	-	-	-	+	-	-	-	+	-
F70	"	-	+	+	-	+	-	+	-	+	-	+	+	+	-
F72	Hosp.227	-	+	+	+	-	-	+	+	-	+	+	+	+	-
F73	Hosp.238	-	+	+	A	+	-	-	-	+	-	-	-	+	-
	Surface Water Series A														
V 1	"	-	+	+	+	-	-	+	-	-	+	...	...	...	...
V 2	"	-	+	+	A	-	-	-	-	+	-	...	...	...	...
V 3	"	-	+	+	+	-	+	+	-	-	+	...	...	...	...
V 4	"	-	+	+	-	+	-	-	-	+	-	...	...	...	...
V 5	"	-	+	+	-	-	-	+	-	+	-	...	...	...	...
V 6	"	-	+	+	-	-	+	-	-	+	-	...	...	...	...
V 7	"	-	+	+	-	-	+	-	-	+	-	...	...	...	...
V 8	"	-	+	+	-	-	+	-	-	+	-	...	...	...	...
V 9	"	-	+	+	-	+	-	-	-	+	-	...	...	...	...
V10	"	-	+	+	+	-	+	+	-	+	+	...	...	...	...
V11	"	-	+	+	A	...	-	-	-	+	-	...	...	...	...
V12	"	-	+	+	-	+	-	-	-	+	-	...	...	...	...
V13	"	-	+	+	-	+	-	-	-	+	-	...	...	...	...
W 1	Series B	-	+	+	-	+	-	-	-	+	-	-	-	+	-
W 2	"	-	+	+	-	+	-	-	-	+	-	-	-	+	-
W 3	"	-	+	+	-	-	+	-	-	+	-	+	-	+	-
W 4	"	-	+	+	+	-	-	-	-	+	-	-	-	+	-
W 5	"	-	+	+	-	...	-	-	-	+	-	-	-	+	-
W 6	"	-	+	+	+	...	-	-	-	+	-	-	+	+	-
W 7	"	-	+	+	+	+	-	-	-	+	-	+	-	-	-
W 8	"	-	+	+	A	+	-	-	-	+	-	+	-	+	-
W 9	"	-	+	+	-	+	-	-	-	+	-	-	-	+	-
W10	"	-	+	+	-	-	-	-	-	+	-	-	-	+	-
W11	"	-	+	+	+	+	-	+	-	+	-	+	+	-	-
W12	"	-	+	+	-	+	-	-	-	+	-	+	-	+	-
W13	"	-	+	+	-	+	-	-	-	+	-	+	-	+	-
W14	"	-	+	+	+	A	-	-	-	+	-	+	-	+	-
W15	"	-	+	+	A	...	-	-	-	+	-	-	-	+	-
W16	"	-	+	+	+	-	-	+	-	-	+	+	+	-	-
W17	"	-	+	+	+	+	-	-	-	+	-	+	-	+	-
W18	"	-	+	+	A	+	-	-	-	+	-	+	-	+	-
W19	"	-	+	+	+	+	-	-	-	+	-	-	-	+	-

TABLE 2 - continued

Culture Number	Source	Gram	Dextrose	Lactose	Saccharose	Dulcitol	Adonitol	2% Peptone Gelatine		Methyl Red	Voges-Proskauer	Uric Acid	Citrate	Indol	Hydrogen Sulphide
								Gas	Liq						
W20	Series B	-	+	+	-	: :	-	-	-	+	-	+	-	+	-
W21	"	-	+	+	+	+	+	-	-	-	+	+	+	+	-
W22	"	-	+	+	A	: :	-	-	-	+	-	+	-	+	-
W23	"	-	+	+	-	+	-	-	-	+	-	+	-	+	-
W24	"	-	+	+	A	: :	-	-	-	+	-	+	-	+	-
D 1	Foods	-	+	+	-	+	-	-	-	+	-			-	-
D 2	"	-	+	+	+	-	-	+	-	-	-				
D 3	"	-	+	+	+	+	-	+	-	-	+				
D 4	"	-	+	+	+	-	+	+	-	-	+				
D 5	"	-	+	+	+	+	-	+	-	-	+				
D 6	"	-	+	+	+	+	+	+	-	-	+				
D 7	"	-	+	+	-	-	-	-	-	+	-				
D 8	"	-	+	+	+	-	-	-	-	+	-				
D 9	"	-	+	+	+	-	+	+	-	-	+				
D10	"	-	+	+	-	+	-	-	-	+	-				
D11	"	-	+	+	+	-	+	+	-	-	+				
D12	"	-	+	+	+	-	-	-	-	+	+				
D13	"	-	+	+	-	-	-	-	-	+	+				
D14	"	-	+	+	-	+	-	-	-	+	-				
D15	"	-	+	+	+	-	+	+	-	-	+				
D16	"	-	+	+	+	-	+	+	-	-	+				
D17	"	-	+	+	+	-	+	+	-	-	+				
D18	"	-	+	+	+	-	+	-	+	-	+				
D19	"	-	+	+	+	-	-	-	-	+	-				
D20	"	-	+	+	+	-	-	-	-	+	-				
D21	"	-	+	+	+	-	+	+	-	-	+				
D22	"	-	+	+	+	-	A	+	-	-	+				
D23	"	-	+	+	+	-	+	+	-	-	+				
D24	"	-	+	+	-	+	+	-	-	-	+				
D25	"	-	+	+	-	-	-	-	-	+	-				
D26	"	-	+	+	-	-	-	-	-	+	-				
D27	"	-	+	+	+	-	+	+	-	-	+				
D28	"	-	+	+	+	-	-	+	-	-	+				
D29	"	-	+	+	+	-	+	+	-	-	+				
D30	"	-	+	+	+	-	+	+	-	-	+				
D31	"	-	+	+	-	-	-	+	-	+	-				
D32	"	-	+	+	-	-	-	-	+	-	+				

TABLE 2 - continued

Culture Number	Source	Gram	Dextrose	Lactose	Saccharose	Dulcitol	Adonitol	2% Peptone Gelatine Gas	2% Peptone Gelatine Liq	Methyl Red	Voges-Proskauer	Uric Acid	Citrate	Indol	Hydrogen Sulphide
D33	Foods	-	+	+	-	-	-	-	-	+	-			-	
D34	"	-	+	+	-	-	-	-	+	+	+			-	
D35	"	-	+	+	-	-	-	+	-	+	-			-	
D36	"	-	+	+	+	-	+	+	-	-	+			+	
D37	"	-	+	+	+	-	-	+	-	+	-			+	
D38	"	-	+	+	+	-	+	+	-	+	+			+	
D39	"	-	+	+	+	-	-	-	-	+	-			+	
D40	"	-	+	+	+	-	-	-	-	+	-			+	
D41	"	-	+	+	+	-	-	-	-	+	-			+	
D42	"	-	+	+	+	-	+	+	-	+	-			+	
D43	"	-	+	+	+	-	+	+	-	+	-			+	
D44	"	-	+	+	-	+	-	-	-	+	-			+	
D45	"	-	+	+	+	+	-	-	-	+	-			+	
D46	"	-	+	+	-	+	-	-	-	+	-			+	
D47	"	-	+	+	+	-	-	-	-	+	-			+	
D48	"	-	+	+	+	+	+	+	-	+	-			+	
D49	"	-	+	+	+	-	+	+	-	+	-			+	
D50	"	-	+	+	+	+	+	+	-	+	-			+	
D51	"	-	+	+	+	+	-	+	-	+	-			+	
D52	"	-	+	+	+	+	+	+	-	-	+			+	
D53	"	-	+	+	+	-	-	-	+	+	-			+	
D54	"	-	+	+	+	-	-	-	-	+	-			+	
D55	"	-	+	+	+	-	-	-	-	+	-			+	
D56	"	-	+	+	-	-	-	-	-	+	-			+	
D57	"	-	+	+	-	-	-	-	-	+	-			+	
D58	"	-	+	+	+	-	+	+	-	-	+			-	
D59	"	-	+	+	+	-	+	+	-	-	+			-	
H 1	Flour Sacks	-	+	+	+	+	+	+	-	+	+			-	
H 2	"	-	+	+	-	-	-	+	-	+	-			+	
H 3	"	-	+	+	+	-	+	+	-	+	+			+	
H 4	"	-	+	+	+	+	+	+	-	-	+			-	
H 5	"	-	+	+	+	-	+	+	-	-	+			-	
H 6	"	-	+	+	+	+	+	+	-	-	+			+	
S 1	Soil	-	+	+	+			-	-	+	-			+	
S 2	"	-	+	+	-			-	-	+	-			-	
S 3	"	-	+	+	-			-	-	+	-			+	
S 4	"	-	+	+	-			-	-	+	-			-	
S 5	"	-	+	+	-			-	-	+	-			+	
S 6	"	-	+	+	+			-	-	+	-			-	

Discussion.

The separation of the above strains into the type species; B. coli; B. aerogenes; and B. cloacae on the basis of the methyl red test, the Voges and Proskauer reaction and gelatine liquefaction, gives the following percentage incidence of these species from the various sources studied. (See Table 3).

Any comparative study of the percentage incidence of species of bacteria isolated from various sources is of limited value, for the reason that the conditions of collection of material and the methods of isolation are seldom duplicated. Also the personal equation as to the choice of the colonies to be picked are all factors which enter very largely into the results.

In regard to the strains tabulated above, the fecal strains were accumulated by picking representative colonies on the plate that had the appearence of being a lactose fermenting organism. Sometimes two or three different colony types would be found. The food and water strains, however, were collected under more routine conditions. One typical colony being selected from a plate in the confirmation of a positive presumptive test.

The percentage of B. coli (M.R. +, V.P. -) found in surface water is 83.8%. This is in the main

TABLE 3

SHOWING PERCENTAGE INCIDENCE OF B. COLI, B. AEROGENES, and B. CLOACAE, FROM DIFFERENT SOURCES, UPON THE BASIS OF METHYL RED AND VOGES PROSKAUER TESTS AND GELATINE LIQUEFACTION.

Source	Number of Strains	B. coli M.R.+ V.P.- Gel - percent	B.aerogenes M.R.- V.P.+ Gel - percent	B.cloacae M.R.-V.P.+ Gel + percent
Feces Series A & B	46	100	0	0
Feces Series C	63	74.6	22.2	3.2
Surface Water Series A & B	37	83.8	16.2	0
Ground Meat	24	70.8	25.0	4.2
Oysters	21	33.3	47.6	19.0
Milk & Cream	14	64.3	35.7	0
Flour Sacks	6	50	50	0
Soil	6	100	0	0

higher than that reported by other investigators using direct plating methods.

The results reported have varied from 70% to 80.4% as reported by Greenfield (1916), Koser (1924) and Winslow and Cohen (1918).

The summary shown in Table 4 was copied from the published report of the last named authors.

A study of this table shows that B. aerogenes-like organisms have been very seldom found in feces. This is especially true when the isolations are made by direct plating. No aerogenes types were found in the 46 feces strains isolated by direct plating. However, when special means of isolation were employed a relatively large percent of the organisms isolated are of the B. aerogenes type.

When brilliant green broth was used for preliminary enrichment, 22.2% of the organisms isolated were of the B. aerogenes type, as shown by the methyl red and Voges-Proskauer tests. These results are shown in Table 3. These strains were isolated from 11 individuals, from 3 of which M.R. -, V.P. + organisms were isolated each time the feces were examined. Three of the 16 strains were isolated from stools following cathartics. The remainder were from normal stools. Two other isolations from stools following a cathartic produced B. coli only.

TABLE 4

SHOWING COLON-AEROGENES AS RELATED TO SOURCE
(Copied from table of Winslow & Cohen)

Authors	Source	Number of Strains	Percentage V.P.+ Or M.R.-
Ferriera, Horton & Paredes 1908	Feces Human	117	6.8
MacConkey 1905	Human	36	0.
" 1909	"	178	6.2
" 1909	Horse	67	11.9
" 1909	Other animals	87	0.
Clemesha 1912	Human	1207	6.1
" 1912	Cow	1029	10.7
Rogers, Clark & Evans 1914	Cow	150	0.7
Rogers, Clark & Lubs 1916	Human	113	5.6
*Rogers, Clark & Lubs (1918)	Human	177	26.0
Levine 1916	Sewage	----	23.0
Levine 1916	Feces	187	0.0
*Chen & Rettger (1920)	Feces	173	0.0
* " " 1920	Soil	467	93.6
MacConkey 1909	Cereals, water etc.	121	56.2
Hanston, A.C 1911	Raw water	243	12.9
" 1911	Stored water	133	5.3
" 1911	Stored & filtered H_2O	156	3.2
Greenfield 1916	Water	405	30.2
Rogers 1916	Water	137	33.3
Winslow & Cohen 1918	Polluted H_2O	94	23.0
" "	Unpolluted H_2O	80	24.0
*Koser (1924)	Polluted H_2O	170	19.6
* " 1924	Unpolluted H_2O	90	26.7
Rogers, Clark & Evans (1915)	Grains	166	91.0
Johnson (1916)	Soil	363	72.0

* Added to list by author.

The soil strains shown in Table 3 merit some discussion. The strains were isolated from soil obtained from the Agronomy Department of the Kansas State Agriculture College and had been sealed in glass fruit jars for about 8 years. As no water had been added in the meantime, the soil was very dry. These results are of interest since it is generally assumed that the B. coli type is able to survive in the soil only a relatively short time. Skinner and Murray (1926) report 197 days and Young and Greenfield (1923) almost 3 years as the period of storage which they will survive.

The present series is too small to allow any general conclusions to be drawn, but they would at least indicate that B. coli was able to exist under the conditions of storage found in this experiment.

If it is true that B. coli can adapt itself to survive in the soil for long periods of time, then the finding of this organism in surface water which contains soil washings should not have the sanitary significance which is now attached to it.

B. Aerogenes Isolated From Feces (M.R. -, V.P. + Group.)

These strains have been collected together and their principle characteristics tabulated in order to facilitate their study.

TABLE 5

CULTURAL CHARACTERISTICS OF THE METHYL RED - VOGES PROSKAUER+GROUP ISOLATED FROM FECES

Strain	M.R.	V.P.	2% Difco Peptone Gelatine		Adonitol	Uric Acid	Citrate	Indol
			Gas	Liq				
F 3	-	+	+	-	-	+	+	-
F 6	-	+	+	-	-	+	+	-
F 8	-	+	-	+	-	+	+	-
F11	-	+	+	-	+	+	+	-
F15	-	+	+	-	-	+	+	-
F19	-	+	+	-	-	+	+	-
F23	-	+	+	-	-	+	+	-
F24	-	+	+	-	+	+	+	+
F25	-	+	+	-	+	+	+	+
F26	+	+	+	-	+	+	+	+
F30	-	+	+	-	+	+	+	+
F32	-	+	+	-	+	+	+	-
F38	-	+	+	-	+	+	+	+
F39	-	+	+	-	+	+	+	-
F47	-	+	+	-	-	+	+	-
F72	-	+	+	+	-	+	+	-

These organisms shown in Table 5 are typical strains of B. aerogenes, with a few exceptions. F 8 and F 72 are slow liquefyers of gelatine but inasmuch as the sanitary significance of these cloacae-like organisms is usually considered to be the same as B. aerogenes, they have been included here. F 26 is like B. aerogenes in every respect except that it is methyl red positive. This characteristic has persisted through many transfers and after many attempts at purification.

Cultural Study of Gas Production from Difco Peptone Gelatine.

A study of the fecal aerogenes strains in Table 5, and of B. aerogenes from other sources shown in Table 2, shows that gas production is uniformly positive for members of this group. This gas may be demonstrated in a number of ways which will be found described in Part II. When innoculated into 2% peptone gelatine*, and incubated at 20°C the gas appears usually within 24 hours but the bubbles seem to reach their maximum size after about 48 hours. The bubble may appear along the line of innoculation or out along the sides of the tube with no apparent connection with the line of growth. They usually appear

- - - - - -

*The 2% peptone gelatine medium was retained throughout the cultural study because it could also be used for observation of gelatine liquefaction, thus serving two purposes.

first near the bottom of the tube but may later extend to within ½ inch of the top. The bubbles vary in number from one or two small bubbles in the case of certain atypical B. coli strains to seven or eight in typical B. aerogenes.

B. aerogenes strains which had been isolated for more than a year still retained their ability to produce gas from peptone. This would indicate that the characteristic was a permanent one.

B. cloacae-like organisms have been found variable in gas production. Some strains showed gas production comparable in amount to that produced by B. aerogenes, while other strains produced no visible gas. This suggests the possibility that a more thorough study of this group of bacteria in regard to gas production from peptone might reveal important relationships between this group and others and within the group itself, as this study has shown for the gelatine non-liquefying members of the group.

Adonitol Fermentation.

Of the sixteen strains listed in Table 5, eight fermented adonitol while eight were negative. The fact that adonitol negative strains of B. aerogenes may be found in feces is of considerable interest, since the fermentation of this alcohol has been used as a criterion to differentiate fecal strains of B. aerogenes from non-fecal strains. (Standard Methods of Water Analysis,1923.)

The adoption of this standard was based largely upon the results of Rogers, Clark and Lubs (1918) who found all of their strains of fecal aerogenes to be adonitol fermenters, while the strains isolated from other sources were variable. Levine and Linton (1924) also report similar results.

The value of the test has been questioned, however, by Chen and Rettger (1920), Winslow and Cohen (1918), Perry and Montfort (1921) and others, who were unable to successfully apply the test to organisms obtained from water. Since the actual sources of their organisms were unknown, their results were based on the supposed distribution of B. coli and B. aerogenes in water as determined by statistical methods. Such evidence is, therefore, indirect.

The isolation of adonitol negative strains of aerogenes from feces would, on the other hand, be direct proof that such organisms existed.

The fermentation of adonitol as a criterion of the fecal source of B. aerogenes has been omitted from the last edition of the Standard Methods of Water Analysis (1925), awaiting additional evidence as to its value.

Inasmuch as Rivas (1912) had reported a loss of fermentative power in a strain of B. coli after repeated transfers into brilliant green broth; the possibility of the brilliant green, used in the isolation of these

aerogenes strains, effecting the fermentation of adonitol could not be ignored. The fact that the organisms were normal in all the other fermentation reactions argued against such a probability. Nevertheless, several strains of B. aerogenes which had been isolated by direct plating were innoculated into brilliant green dextrose broth using the same technic as had been employed in the enrichment method. These were incubated for 24 hours and streaked out on Eosin-methylene blue agar plates. A number of colonies were picked from each plate and planted into adonitol broth. Without exception these organisms fermented the adonitol with acid and gas production within 24 hours. This eliminated the probability that such an attenuation had occurred in the fecal aerogenes strains.

This observation is also of interest in connection with the discussion of atypical B. coli strains, which follows.

Definition of Typical B. coli.

The direct plating of suspensions of feces usually yield a large proportion of typical B. coli. These may be described as short gram negative rods, fermenting dextrose and lactose with acid and gas production, positive or negative fermentation of saccharose and dulcite, non-liquefaction of gelatine, positive methyl red,

negative Voges-Proskauer, indol positive, and as has been recently shown by Koser, fail to grow in uric acid and in citrate media. Such organisms are seen to predominate in the feces series A and B. (See Table 2) These organisms are the most numerous of the colon-aerogenes forms found in feces and are the forms usually picked from direct plating.

Atypical Strains Isolated From Feces.

However, when some method, such as enrichment in brilliant green broth, is used which inhibits this type, allowing the less numerous forms to develop, there is found an increased number of organisms which fail in one or more characteristics to conform to the description given above. Many of these colonies on Eosin-methylene blue plates are atypical in appearence, being larger, slightly raised, with a dark center, and showing little or no metalic luster.

While only 2 atypical strains, 1 in Series A, number X 10, and 1 in Series B, number B 4, were isolated from feces by direct plating. These irregular strains amount to 40% of the methyl red +, Voges-Proskauer - organisms picked when preliminary enrichment in brilliant green dextrose broth was used. A comparison of the characteristics of these organisms is found in the following Table.

TABLE 6

CULTURAL CHARACTERISTICS OF ATYPICAL B. COLI STRAINS FROM FECES

Strain	M.R.	V.P.	2% Difco Peptone Gelatine		Adonitol	Indol	Uric Acid	Citrate	Colonies on Eosin Methylene blue agar
			Gas	Liq					
F12	+	-	+	-	-	+	-	-	Medium raised, dark center
F18	+	-	-	-	-	+	-	+	Medium, flat light colored
F31	+	-	-	-	+	+	+	+	Coli-like
F33	+	-	-	-	+	+	+	-	small, almost clear
F34	+	-	+	-	+	+	+	+	Small, dark blue center, no sheen
F35	+	-	-	-	-	+	+	-	Coli-like
F36	+	-	-	-	-	+	+	-	Coli-like
F42	+	-	-	-	-	+	+	-	Coli-like
F48	+	-	-	-	+	+	+	-	Large, soft, reddish center
F49	+	-	-	-	-	+	+	-	Coli-like
F52	+	-	+	-	-	+	+	-	Coli-like
F53	+	-	+	-	-	+	-	-	Coli-like
F54	+	-	-	-	+	+	+	+	Aerogenes-like
F56	+	-	-	-	-	+	+	+	Medium, dark, slightly raised
F59	+	-	+	-	-	+	-	-	Large, dark, center, no sheen
F62	+	-	+	-	-	+	-	-	Large, dark, center, no sheen
F65	+	-	+	-	-	+	-	-	Large, dark center, no sheen
F66	+	-	-	-	-	+	+	-	Large, dark
F70	+	-	+	-	-	+	+	+	Medium, raised, almost clear

All of the organisms in feces "Series C" were isolated by the use of brilliant green broth which had been titrated so that the amount of brilliant green used would just inhibit B. coli and allow B. aerogenes to develop. However, this balance is easily tipped one way or the other by a slight variation in the amount of feces used for the innoculation. It is conceivable that in certain cases conditions would exist when the concentration of the dye would inhibit B. coli strains which were more sensitive and would permit more resistant ones to develop, while in other cases the concentration would be such as would completely inhibit all coli forms, then only B. aerogenes would be found. Upon inspection of Table 1 this is often found to be the case. F 32, F 33 and F 34 were all picked from one plate. F 32 is a typical B. coli while F 33 and F 34 are atypical and differ from each other in their characteristics. Another interesting case is shown in F 53 and F 54. The innoculated brilliant green broth was incubated 24 hours as usual, and plated out on Eosin-methylene blue plates. The colonies after 24 hours were all of the coli type. F 53 was picked. This culture is atypical only in its positive gas production from peptone gelatine. Several loops full of the brilliant green broth culture, which was now 48 hours old, were planted into another dextrose brilliant green tube and incubated 24 hours. It was then

plated out as usual and the colonies were now found to be all aerogenes-like. Strain F 54 was picked. It does not produce gas but grows on uric acid and citrate media. Cultures number F 55 and F 56 were obtained under the same circumstances. F 55, obtained from the first planting, gave typical coli-like colonies and is typical coli in every way, while F 56, from the replant, gave dark, slightly raised colonies with no sheen. As may be seen in Table 1 or Table 6 the first organism picked in both cases (F 53 and F 55) is coli-like in its characteristics anf fails to grow in either uric acid or citrate media. The organisms picked from the replants, F 54 and F 56, are typical coli which retain their methyl red and Voges-Proskauer characteristics but also have the ability to utilize uric acid and citrate, which are attributes of B. aerogenes. In each case the dye concentration was greater in the replant tube since no feces was used, which probably accounts for the appearence of the strains which were more like B. aerogenes.

A study of the above table shows that these strains have several characteristics common to all, namely, M.R. +, V.P. -, failure to liquefy gelatine and indol production. These characteristics would ordinarily be sufficientto identify the organism with the B. coli group. However, it is seen that they also have one or more of

the characteristics such as gas production in peptone gelatine, uric acid utilization or citrate utilization which are typical of the B. aerogenes section. These characteristics together with the fact that they are found only after preliminary enrichment in brilliant green broth, of a concentration which inhibits B. coli and does not affect B. aerogenes would cause these strains to be considered rather close physiologically to B. aerogenes.

The ability of these M.R. +, V.P. - strains to produce gas from peptone gelatine is not well developed. The amount is small as compared to that produced by the M.R. -, V.P. + types. In every case the visible gas consisted of one or two small bubbles, while B. aerogenes produces 4 to 8 medium to large bubbles.

That such organisms may be found in surface water is shown by the following group of strains selected from water Series B.

Atypical Strains Isolated From Water

The similarity of these organisms shown in Table 7 and those of the atypical fecal strains is apparent. With the exception of one, they are indol producers and all but one failed to ferment adonitol. None of the group produced gas in peptone gelatine. The

TABLE 7

CULTURAL CHARACTERISTICS OF ATYPICAL B.COLI STRAINS FROM SURFACE WATER

Strain	M.R.	V.P.	2% Peptone Gelatine		Adonitol	Indol	Uric Acid	Citrate	Colonies on Eosin-Methylene blue agar
			Gas	Liq					
W22	+	-	-	-	+	+	+	-	Small, reddish, no sheen
W25	+	-	-	-	-	+	-	+	Small, dark center, no sheen
W27	+	-	-	-	-	-	+	-	Small dark center, light periphery
W28	+	-	-	-	-	+	+	-	Small, reddish, no sheen
W33	+	-	-	-	-	+	+	-	Small, reddish, some sheen
W34	+	-	-	-	-	+	+	-	Small, dark center, light periphery
W35	+	-	-	-	-	+	+	-	Coli-like
W39	+	-	-	-	-	+	+	-	Small, reddish, some sheen
W41	+	-	-	-	-	+	+	-	Small, dark center, light periphery
W44	+	-	-	-	-	+	+	-	Small, reddish, no sheen
W50	+	-	-	-	-	+	+	-	Small, reddish, some sheen

results of growth in uric acid and citrate media show that but one strain developed in citrate media and that this strain was the only one which failed to grow in uric acid media. These atypical strains have become especially interesting in view of Koser's (1924) interpretation of citrate utilization by members of this group. He found that 90.7% of 118 fecal organisms failed to utilize citrate, while 97.2% of 72 soil strains utilized it. The soil was collected from apparently unpolluted sources. He, therefore, correlates lack of utilization with the fecal source of the organism.

These atypical strains have also been reported by others. Among their soil strains, Chen and Rettger (1920) found 20 that were M.R. +, V.P. - of which 10 utilized uric acid. They considered them as possible intermediate strains between B. coli and B. aerogenes. Perry and Montfort (1921) found these same types to be prevalent in water and call attention to the difficulty of the interpretation of their sanitary significance.

That organisms of the colon type which utilize citrate are found in the feces is shown by the above Table 7. Their number in the feces must be small as compared to the typical B. coli which fails to utilize citrate. Nevertheless, feces cannot be disregarded as a possible source of these organisms in soil and water.

The relative importance of feces as a source of such atypical colon-aerogenes forms found in soil and water can be determined only by more extensive work on this group, using some method for their isolation, such as preliminary enrichment in brilliant green broth.

Summary of Strains.

The following Table, number 8, has been arranged to compare the principal characteristics of all strains in relation to their source.

This work was begun in 1918 and the strains indicated as Feces A, Water A, Foods, Flour sacks and soil were isolated at that time. These cultures were lost after completing the work then contemplated. Uric acid and citrate utilization was unfortunately not run on these strains.

A consideration of Table 8 will again emphasize the fact that gas production in peptone gelatine is a constant characteristic of B. aerogenes. Of the 52 cultures of the M.R. +, V.P. - group only 6 fail to produce gas and of these 6 strains, 5 are gelatine liquefiers and would, therefore, not be considered as aerogenes.

Treece (1920) in a preliminary report called attention to the close correlation of gas production in peptone gelatine to fermentation of adonitol in the food

TABLE 8

SHOWING CORRELATION OF CULTURAL CHARACTERISTICS WITH SOURCE

Source No. of Strains	Methyl Red and Voges-Prsk	Uric Acid No of Strains		Citrate No.of Strains		2% Pept. Gel.gas Strains		Adonitol. No.of Strains		Indol No. of Strains	
		+	-	+	-	+	-	+	-	+	-
Feces A 36	MR+ VP- 36	..	....	....	.....	1	35	0	36	36	0
Feces B 10	MR+ VP- 10	0	10	1	9	0	10	1	9	10	0
Feces C 63	MR+ VP- 47	13	34	6	41	8	39	9	38	45	2
	MR- VP+ 16	16	0	16	0	15	1	8	8	5	11
Water A 13	MR+ VP- 10	..	....	....	.....	1	9	2	8	9	1
	MR- VP+ 3	..	....	....	.....	3	0	2	1	1	2
Water B 24	MR+ VP- 21	10	11	1	20	0	21	1	20	18	3
	MR- VP+ 3	3	0	3	0	3	0	1	2	1	2
Foods 58	MR+ VP- 32	..	....	....	.....	8	24	5	27	17	15
	MR- VP+ 26	..	....	....	.....	21	5	21	5	4	22
Flour Sacks 6	MR+ VP- 2	..	....	....	.....	1	1	0	2	2	0
	MR- VP+ 4	..	....	....	.....	4	0	3	1	1	3
Soil	MR+ VP- 6	..	....	....	.....	0	6	0	6	3	3
Totals 116	MR+ VP- 164	23	55	8	70	19	145	18	146	140	24
	MR- VP+ 52	19	0	19	0	46	6	35	17	12	40
		42	55	27	70	65	151	53	163	152	64

strains, Feces A and Water A series studied. This correlation is probably a coincidence since a study of a greater number of strains has shown fermentation of adonitol to be a more variable factor than production of gas from peptone gelatine. This is particularly evident in Feces C where of the 16 M.R. -, V.P. + strains, 8 were negative in adonitol, only one (gelatine liquefier) failed to produce gas in peptone gelatine. This characteristic is very uncommon among the organisms of the B. coli group, (19 out of 164 strains) and many of these were found to be atypical in other respects. (See Table 6)

It should be emphasized that the expression "gas production" as used in the above discussion has the same significance that it has in the usual tests for gas production in fermentation of carbohydrates, i.e., that it refers to visible gas as evidenced by bubbles in the peptone gelatine, or in the case of the usual fermentation tests, by gas collecting in the closed arm of the tube. Gas will collect, in either case, only after the medium has become saturated with the gas produced. It is well known that small amounts of gaseous products are produced by the respiration of bacteria. These gases are ordinarily in such small quantities that the medium does not become saturated. Special methods must be employed for their demonstration and collection for analysis. Tests for visible gas then becomes a quantitative rather than

a qualitative test.

Hydrogen Sulphide Production.

A review of the literature given in a later section shows that statements regarding hydrogen sulphide production by members of the intestinal group, especially of the colon-aerogenes section, do not agree. It was, therefore, thought advisable to include this test with others made upon this group.

As shown by Table 2 only two strains in the entire series produced blackening in the 48 hour incubation period. This short incubation period was selected for the readings as this is the usual period used in differential tests of the intestinal organisms for hydrogen sulphide production. A number of the organisms were incubated for a longer period, however, and the results are shown in Table 9.

It is seen that the results are somewhat variable after several days incubation. In no case was there any considerable amount of hydrogen sulphide produced. It was difficult to distinguish small amounts of blackening in the lead acetate agar due to the fact that after several days the media becomes slightly brown due perhaps to the production of a brown lead oxide*.

*Attention has been previously called to this, as well as other objections to the use of lead acetate agar.

TABLE 9

SHOWING COMPARISON OF H_2S PRODUCTION IN FERROUS SULPHATE AND IN LEAD ACETATE AGAR

	3% Difco peptone agar & lead acetate					3% Difco peptone agar & ferrous sulphate				
	Incubation period					Incubation period				
	24 hr	48 hr	72 hr	5 da	7 da	24 hr	48 hr	72 hr	5 da	7da
B.typhosus	++	++++	++++	++++	++++	+++	++++	++++	++++	++++
B.paratyphosus B	++	+++	++++	++++	++++	++	+++	++++	++++	++++
B.coli F 10	+++	+++	++++	++++	++++	+++	+++	++++	++++	++++
F 28	-	-[a]	-	sl+	+	-	-	-[b]	-	-
F 31	-	-	-	sl+	sl+	-	-	-	sl+	+
F 35	-	-	-	sl+	+	-	-	-	sl+	+
F 37	-	-	-	sl+	sl+	-	-	-	-	-
F 50	-	-	-	+	+	-	-	-	+	+
F 61	-	-	-	sl+	sl+	-	-	-	+	+

Legend: Sl+ indicates slight blackening; +, blackened along stab; ++, blackening slightly diffused; +++, blackening considerably diffused; ++++, blackening diffused throughout tube.
a, surface growth slightly dark. This appeared after 48 hours on all the coli strains and persisted until end of experiment.
b/ a narrow brown ring about 1/4 inch beneath the surface appeared after 72 hours and persisted until end of experiment.

The Ferrous sulphate agar, on the other hand, is clearer and, except for the brown ring which forms near the surface, the media retains its normal color. The brown ring is probably caused by the precipitation of ferric hydroxide.

The results from this series would indicate that B. coli, which produces more than traces of hydrogen sulphide from Difco peptone in 48 hours, is comparatively rare in feces. But the fact that certain strains are found which produce abundant hydrogen sulphide in 24 hours might explain the disagreement of the results of different investigators.

An explanation of this delayed hydrogen sulphide production by the B. coli and B. aerogenes strains will be found in Part II of this thesis under the heading "Metabolic study of H_2S production".

PART II

METABOLIC STUDIES

GAS PRODUCTION FROM PEPTONE.

The production of gases from carbohydrates, by bacteria, was early recognized, and accurate analyses of the gases produced, have been made by a number of investigators as shown in the review of the literature previously given.

The fact that bacteria may produce a considerable amount of gas from protein sources is often overlooked, due, no doubt, to the fact that the gases are not in sufficient amount or of a character such as to be demonstrated by the usual methods of determining gas production.

Grimpert and Legros (1900) noted bubbles of gas which formed in nutrient gelatine with cultures of B. aerogenes. They attributed this to the native sugar present in the meat bullion. Van Slyke and Hart (1903) observed continuous CO_2 production in Cheddar cheese from the beginning of the ripening process until the end of the experiment 32 weeks later. They believed that the gas was produced from such compounds as tyrosine and arginine by micro-organisms. Eldridge and Rogers (1904) found a large percentage of cultures of bacteria isolated from Emmenthaler cheese produced gas in a whey medium rendered sugar free by B. bulgaricus.

Using a special device they determined that the gas consisted entirely of CO_2.

In a preliminary report the author (1920) called attention to the production of visible gas in a 2% Difco peptone gelatine medium by B. aerogenes.

Ayers, Rupp and Mudge (1921) demonstrated CO_2 production by certain streptococci from Difco peptone. They believed the source of the gas was other than the carbohydrate radicles in the peptone. Nichols (1921) showed that B. typhosus produced measurable amounts of gas from dextrose broth.

The gaseous metabolism of certain anaerobes has been carefully worked out by several investigators. Smith et. al., (1905) observed gas production by anaerobic bacteria in sugar free media. More recently Wolf and Harris (1917), Bushnell (1922) and Anderson (1924) have reported analyses of the gases produced by anaerobes under various conditions. Anderson considers the main gases produced from peptone to be CO_2, H_2, N_2 and a small amount of H_2S.

Early in the present investigation it was noted that bubbles of gas were formed in the depths of certain nutrient gelatine cultures. Upon investigation it was found that the amount of the gas could be increased by increasing the amount of peptone and that increasing the percentage of gelatine had very little effect.

Table 10 shows the relative amounts of gas produced by B. aerogenes in a medium containing .5% meat extract and varying amounts of peptone and gelatine incubated at 20°C for 72 hours.

When such a medium is placed in Smith fermentation tubes and incubated at 37°C there is usually a failure to produce visible gas, although at times as much as 10% of gas in the closed arm has been observed. This has also been observed in the inverted vial type of fermentation tubes filled with a carbohydrate broth containing 1% Difco peptone, and innoculated with a strain of B. aerogenes which does not ferment the carbohydrate. This might become a source of error where gas production alone is taken as an indication of fermentation of the carbohydrate. When tested with phenol red or other suitable indicator, however, such broth is found to be alkaline.

In order to determine whether the peptone alone was the source of the gas, a number of tests were made eliminating one by one the other ingredients in the media. These have been summarized in Table 11. It is to be noted that in each case where the peptone was present there was gas production. Where it was absent there was no gas production, even though other proteins substances such as meat extract and gelatine were present. This proved beyond doubt that the source of the gas was the peptone. Where the peptone solution was used alone the

TABLE 10

SHOWING RELATIVE AMOUNTS OF GAS PRODUCED BY B. AEROGENES IN A MEDIUM CONTAINING .5% MEAT EXTRACT AND VARYING AMOUNTS OF PEPTONE AND GELATINE, INCUBATED AT 20° C. 72 hours

Difco peptone percent	Percent Gelatine		
	5	10	12
2	7-10 medium size bubbles	6-10 medium size bubbles	3-5 medium size bubbles
3	7-14 medium size bubbles	6-28 medium to small bubbles	4-7 medium size bubbles
5	14-16 medium size bubbles	6-8 large bubbles	11-14 large bubbles

TABLE 11

SHOWING PRODUCTION OF GAS BY B. AEROGENES IN MEDIA CONTAINING VARIOUS INGREDIENTS OF NUTRIENT AGAR AND NUTRIENT GELATINE.

Difco peptone + .5% extract + 10% gelatine	Difco peptone + 10% gelatine	Difco peptone + 1-1/2% agar	.5% extract + 1-1/2% agar	.5% extract + gelatine	2% Difco peptone solution
Gas	Gas	Gas	No gas	No gas	Gas

medium was placed in ordinary culture tubes to which was added a layer of sterile vaseline. Gas forms beneath this plug forcing it up, the gas being collected above the medium. It was noticed that gas production was slightly increased when a small amount of K_2HPO_4 was added, due perhaps to its buffer effect.

A Comparative Study of Gas Production from Different Peptones

A number of experiments were made upon different commercial peptones to determine whether this property was peculiar to Difco peptone or was common to all.

The media contained 4% of the peptone to be tested, with the exception of Difco, which was used in the usual 2% solution. Five-tenths percent K_2HPO_4 was added and this solution heated in the Arnold steam sterilizer for several minutes. This was then filtered and adjusted to P_h 7.2 to 7.4. One and five-tenths percent agar was added and the media was sterilized in the autoclave. Andrades' indicator was added to the sterilized media, adjustment to neutrality to this indicator being made when necessary. The media was then poured into sterile tubes and allowed to solidify. Innoculation was also made into plain nutrient agar, containing 0.5% meat extract, 1% Difco peptone and

1.5% agar prepared in the usual way and adjusted to Andrades' indicator. Innoculations were made by deep stabs. The results of the experiment are shown in Table 12.

The readings were made at short intervals to follow the acid production. Where gas was produced the surrounding medium was invariably acid. The surface of the medium never became acid. It was neutral for the first 24 hours then became alkaline. This alkalinity gradually extended toward the butt of the tube. The appearance of B. aerogenes growing in the Difco medium described above, is strikingly like that of B. paratyphosus in New Russell's medium and where acid but no visible gas is produced as in 6a the appearance is like that of B. typhosus.

The appearance of the tube may be explained by assuming that the gas produced, is a mixture of CO_2 and H_2. The CO_2 produced would first neutralize the slight amount of alkali in the media and would then be absorbed as such. This would cause the media to react acid. The CO_2 being quite soluble would be found dissolved in the medium while H_2 being almost insoluble would tend to force the media apart and would collect in bubbles.

The reversion to an alkaline reaction of the surface of the agar, may be explained by the diffusion of the CO_2 from the agar into the air and the increase of alkalinity due to production of NH_3. This interpre-

TABLE 12

RESULTS OF COMPARATIVE STUDY OF GAS PRODUCTION FROM DIFFERENT PEPTONES BY B. AEROGENES AND B. COLI

Culture	Plain Agar				Difco Peptone				Park Davis Peptone				Witte's Peptone				Armour's Peptone			
	6hr	12hr	24hr	48hr	6hr	12hr	24hr	48hr	6hr	12hr	24hr	48hr	6hr	12hr	24hr	48hr	6hr	12hr	24hr	48hr
C F 47	-	A	AG	AG	-	AG	AG	AG					-	-	-	-				
F 19	-	A	AG	AG	-	AG	AG	AG					-	-	-	-				
F 6	-	A	AG	AG	-	AG	AG	AG	-	A	AG	AG	-	-	-	-	-	-	-	-
F 15	-	A	AG	AG	-	AG	AG	AG	-	A	AG	AG	-	-	-	-	-	-	-	-
F 3	-	A	AG	AG	-	AG	AG	AG	-	-	A	AG	-	-	-	-	-	-	-	-
F 23	-	A	AG	AG	-	AG	AG	AG					-	-	-	-				
F 11	-	A	AG	AG	-	AG	AG	AG	-	A	AG	AG	-	-	-	-	-	-	-	-
F 50	-	A	A	A	-	AG	AG	AG					-	-	-	-				
6a	-	A	A	A	-	A	A	A	-	-	-	-	-	-	-	-	-	-	-	-

tation is similar to that given by Nichols (1921) to explain the reversion found in Russell's medium by B. typhosus.

The acid reaction might also be caused by the formation of fatty acids or from the breaking down of carbohydrates radicles, found in the peptone. The small amount of acidity produced, however, is evidence against the presence of any great amount of fatty acids.

As may be seen from Table 12 B. aerogenes produced acid and gas from Difco peptone and from Park-Davis peptone. The production was always much less in the Park-Davis peptone although the concentration used was double that used for Difco. No acid or gas could be demonstrated in either Wittes' peptone or Armour's peptone. This would indicate that the substance responsible for the acid and gas was either not present in these peptones, or was not available for use by the organisms used. It is also noted that strains of B. coli F 50 and 6a are able to produce small amounts of acid and in some cases gas from Difco peptone. When gas is produced the amount is very small as compared to that produced by B. aerogenes. As has been stated before, it is believed that the difference in the reaction of these two organisms in peptone is a quantitative rather than a qualitative one.

That the source of acid and gas is the same for both B. coli and B. aerogenes is indicated by the fact that this reaction is produced in the same peptones by both organisms.

Additional evidence to this fact is shown by the following experiment.

A flask of 4% Difco peptone and 0.5% K_2HPO_4 was innoculated with B. aerogenes F 11 and incubated 24 hours. The culture was then boiled and filtered. The reactionwas found to be P_h 7.2. One and one-half per cent was added and the media was sterilized, adjusted to Andrade's indicator and tubed. This media was innoculated with several strains of B. aerogenes and B. coli.

In no case was there a perceptible production of acid or gas. Growth of all the strains was less in this media than in normal peptone solution agar.

This would indicate (1) that the substance in the peptone that is the source of the acid and gas produced is present in small quantities and (2) that incubation with one strain of B. aerogenes will remove the source of acid gas production for other strains of B. aerogenes and for strains of B. coli as well.

Analyses of the Gas produced from Difco Peptone, by B. aerogenes and B. coli.

Methods Used in Gas Analysis.

In selecting a method for an analysis of the

gases produced from peptone, a number of factors had to be considered. (1) Whether sufficiently accurate results would be obtained. (2) Whether the method would lend itself to this particular problem. (3) Whether or not the necessary apparatus was available.

A survey of the literature showed that a number of methods had been used in the analyses of gas produced by bacteria.

Keys (1909), Fisher (1913), Rogers et al (1914), Bushnell (1922), Anderson (1924) used the method of collecting the gas in a vacuum and then transfering it to a train of appropriate absorbents, where each gas could be determined. This method gives accurate results, when a comparatively large sample of gas is taken. Anderson believed that in order to get consistent results, samples of at least 100 c.c. should be used and that there should be sufficient gas for duplicate analyses. This method was rejected because of the very small amounts of gas produced under the conditions of the experiment and because the apparatus was at the time not available. Eldridge and Rogers (1904) used a special culture tube for the determination of CO_2 by bacteria. This consists of two compartments, connected with a "U" shaped tube, eadh compartment having an exterior opening. The culture is placed in one compartment and in the other is placed a quantity of N/10 $Ba(OH)_2$. The CO_2 produced by

the bacteria passes over and is absorbed by the alkaline solution. The amount neutralized is determined by titration. This method has been used by Ayers et al (1921), Nichols (1921) and others. This method was not suitable, because: (1), there is no means of measuring the volume of gas produced; (2), there is no means of determining other gases than CO_2. The method used by Smith (1895) for the rough approximations of gas percentages was not seriously considered because of the inaccuracies of the method mentioned earlier in this paper.

METHOD USED IN THIS EXPERIMENT.

The method introduced by Brown (1922) was selected and used with a few modifications. It is given here in some detail.

The method consists in collecting the gas under vaseline, from a known amount of media, the gas pushing the plug of vaseline up as the gas collects. This may then be measured in c.c. in terms of the amount of media used. The CO_2 in the collected gas is analysed by aspirating out a measured quantity of the gas by means of a short piece of capillary rubber tubing with a long needle. The syringe and needle are first rinsed with dilute acid then the needle is passed through the vaseline plug at the side of the tube, and as the gas is withdrawn the plug moves down. Care must be taken to seal the opening left by the needle as it is withdrawn. This is

done by applying a hot spatula or forceps to the side of the tube until the vaseline softens and closes the hole.

The syringe is then used as an absorption pipette. When the needle is withdrawn it is quickly transferred to a dish of dilute acid (2 -5%). Holding the syringe upright, the acid is drawn up until it reaches the graduations on the syringe. The amount of gas (and air) in the syringe is read. The plunger is then pushed down until only a small amount of the acid remains in the needle. The needle is then plunged into a solution of alkali (4% $\overline{Na}OH$) and this is drawn into the syringe. Keeping the end of the needle in the alkali the syringe is rocked back and forth to aid the absorption. It is then held upright as before and the amount of remaining gas and air read. The difference in this amount and that of the previous reading represents the amount of CO_2 absorbed by the alkali. Knowing the amount of gas sample originally taken the percentage of CO_2 is readily obtained. The fact that the system is filled with air at the beginning and therefore a mixture of the gas and air is really analysed is not believed to introduce an appreciable error since the percentage of CO_2 in the air (.06%) is well within the limit of experimental error.

The CO_2 in the medium as carbonates and dissolved CO_2 is determined with the same apparatus. The syringe

and needle are thoroughly rinsed with water and with the plunger placed at one of the lower graduations the needle i is lowered through the vaseline plug into the medium. One-tenth c.c. of the medium is aspirated into the needle. It is then removed, using the same precautions as before to seal the hole in the vaseline. A small bubble of air is allowed to enter the needle, then a small amount of Caprylic alcohol is drawn up, followed by another bubble of air. The end of the needle is then placed in a 5% solution of H_2SO_4 containing methyl red indicator. The plunger is raised until the 0.1 - 0.2 c.c. of the acid is seen to enter the barrel of the syringe. With the end of the needle remaining in the acid, the syringe is inverted. The needle end of the syringe is closed by pressing a finger over the rubber tubing. The plunger is then drawn down creating a partial vacuum. As this is increased the air leaks by the plunger and bubbles up through the media, aerating it. The plunger is held down until the pressure is equalized. The syringe is then held upright and the amount of the contained gas and air determined. The CO_2 is then absorbed and determined as in the preceding operation. Since the amount of the media in the culture tube is known, the amount of CO_2 in the culture may be calculated.

It is necessary to conduct the entire experiment under uniform terperature conditions, and where handling of the syringe is necessary during the manipulation, the

contents allowed to return to room temperature before making the reading.

Oxygen may be determined in a similar way to CO_2 except that the system is filled with dilute acid instead of air, and that sodium pyrogallate is used as an absorbent instead of NaOH.

It is evident that no provision is made for the analysis of other gases than CO_2 and O_2 which might be found above the medium. Of these H_2 has been found in the greatest quantity by other investigators making complete gas analysis under other conditions such as carbohydrate fermentation. Roger, et al, using a dextrose medium, found that all, except a mere trace, of the gas not absorbed by NaOH was H_2. Other gases which have been reported as being produced by bacteria are N_2, CO, CH_4, NH_3 and H_2S.

The NH_3 would be found combined as ammonium salts in the media when the reaction was neutral or acid. There was no evidence found in the literature to show that either CO or methane occur in more than traces. N_2 may under certain conditions be produced in considerable quantities by certain anaerobes as shown by Anderson. It was therefore believed that while working with the colon-serogenes group no considerable error would be introduced by assuming the collected gas which was unabsorbed by NaOH to consist largely of H_2.

The accuracy of the method and apparatus was determined in the following way.

(1) Tubes containing 10 c.c. of 1.0% dextrose broth were prepared and innoculated with cultures of B. aerogenes and B. coli and 2 c.c. of sterile vaseline added. After incubation at 37°C for 48 hours the tubes were removed to the laboratory, allowed to cool to room temperature, and the volume of gas measured. The CO_2 in the collected gas and in the media was determined. These results are found in table #13.

(2) A number of analyses of the collected gas from carbohydrate fermentation was made upon the same culture to determine how close duplicate analyses would check. These results are shown in table #14.

The CO_2/H_2 ratio calculated from the above results was found to be characteristic of the organisms used. Rogers, et al, with their more elaborate methods of gas analysis found the average CO_2/H_2 ratio of many strains of B. coli to be approximately 1.0 varying from 0.95 to 1.4 while the average CO_2/H_2 ratio of aerogenes was approximately 2. varying from 1.7 to 2.8.

Thus the method applied to gases produced by carbohydrate fermention is seen to give consistent results in the hands of the author and to give results which are comparable to those obtained by more elaborate methods used by others. Having thus determined that the method

TABLE 13

RESULTS OF ANALYSIS OF GAS PRODUCED FROM CARBOHYDRATE BROTH BY B.COLI & B.AEROGENES

Culture	Media	Gas Collected	CO_2 in Collected gas	CO_2 in Media	H_2 in Collected Gas	Total gas produced	Gas ratio $\frac{CO_2}{H_2}$	PH
B.coli 6a	10cc	5.7cc	37.5% 2.14cc	2.0 cc	3.56cc	7.7cc	1.13	less than 5.0
B.aerogenes F11	10cc	14.6cc	56% 8.18cc	4.0 cc	6.42cc	18.6cc	1.89	5.4

TABLE 14

RESULTS OF DUPLICATE ANALYSES OF CO_2 IN COLLECTED GAS FROM DEXTROSE BROTH

Organism	Amount sample used	Gas plus air	After absorption	CO_2 Absorbed	CO_2 %
F 15	.4cc	.95cc	.73cc	.22cc	55
	.4cc	.85cc	.63cc	.22cc	55
	.4cc	.87cc	.65cc	.22cc	55
F 10	.4cc	.87cc	.71cc	.16cc	40
	.4cc	.97cc	.85cc	.12cc	30*
F 28	.2cc	.71cc	.64cc	.07cc	35
	.5cc	.96cc	.77cc	.19cc	38

* The hole made in the vaseline seal by the previous test had not been completely sealed.

was reasonably accurate for CO_2 and O_2, the application of this method to the analysis of gases produced from a peptone medium to which no carbohydrate was added, seemed warranted. The assumption that the remaining gas was largely H_2 was proven correct by more elaborate methods, as seen by the footnote to table #15.

It was therefore adopted with the following modifications. Brown used a long metal needle which he had made especially for the purpose. There was substituted for this a glass needle, made from a piece of glass tubing of the proper diameter and drawn out to the proper capillary size. This is inexpensive and in addition allows the progress of bubble of gas, which is being drawn up, to be followed. This is especially helpful in the case of determination of the carbonates and CO_2 in the medium where bubbles of air precede and follow the small amount of caprylic alcohol. It also allows obstructions in the needle to be more easily detected.

The media used was a 4% Difco peptone solution to which was added 0.5% K_2HPO_4. This gave a P_h of about 7.4 without adjustment. This was sterilized in flasks and 50 c.c. measured into sterile "9 gm." Babcock bottles. These were selected so that 50 c.c. of the media filled up the bottle into the graduations. Since the neck between the graduations (from 0 to 5%) has 5 c.c. volume, the amount of gas may be directly determined after it

has collected. The media was innoculated and 1 c.c. of sterile vaseline added. The bottles were incubated at 37°C for 48 hours. They were then removed to the laboratory for at least 30 minutes, before the volume of gas was read and analysis made.

RESULTS.

A sufficient number of analyses of the gas from cultures of typical B. aerogenes and B. coli were made to determine the proportion of the gases produced. A number of innoculations of B. coli strains were made and in no case was there more than a small bubble of gas collected. This was too small to be measured or to be analysed. Therefore, for these cultures the determination of the CO_2 as carbonates and dissolved CO_2 in the medium was all that could be made. The B. coli strain 6a was selected because it had been isolated from directly innoculated plates and concerning which there could be no question of its being a typical strain. The results of a series of determinations are found in table #15.

It is to be noted that the B. aerogenes strains were quite consistent in the manner by which the peptone was broken down with the production of gas, as indicated by the CO_2/H_2 ratio. While there is some variation, there is no more than might be expected among different strains.

While the CO_2/H_2 ratio of the B. coli type could not be determined because of the small amount of H_2 pro-

TABLE 15

RESULTS OF ANALYSES OF THE GAS PRODUCED FROM 4% PEPTONE MEDIUM BY B. AEROGENES AND B. COLI

Organism	F 11		F 15			F 3	6 a	Control
Media Cc	50	50	50	50	50	50	50	50
Gas collected cc	0.7cc	1.8cc	.8cc	1.2cc	.6cc	.6cc	small bubble	0
CO_2 in collected gas	0	.045cc	0	0	0	.015cc	?	
CO_2 in media	5cc	10cc			6cc	7.5	10.cc	0
*H_2 in collected gas	0.7cc	1.755cc	.8cc	1.2cc	.6cc	.585cc	?	
Total gas produced	5.7cc	11.8cc			5.6cc	8.1cc	10+cc	
Gas ratio $\frac{CO_2}{H_2}$	7.1	5.7			6.3	12.8	?	
P H	7.2	7.2	7.1	7.1	7.1	7.2	7.1	7.4

* An analysis kindly made by Dr. H. C. Allen of the Department of Chemistry, upon a sample of the collected gas, showed:

F 11 86.8% H_2, 3.61% CO_2

F 15 89.0% H_2, 4.85% CO_2, 1.19% O_2

The remainder in each case was considered to be N_2. In the case of F 15, the O_2 was believed to be due to air leakage.

duced, it is readily seen that the proportion of CO_2 to H_2 would be much greater than that found with B. aerogenes. The total amount of CO_2 produced by B. coli is as great as that produced by B. aerogenes. This difference in the gas ratio is then due to the difference in the amount of H_2 produced by the two organisms. Since it has been shown that the source of the acid and gas is the same for both B.coli and B. aerogenes, this difference in the gas ratio might be taken to indicate a fundamental difference in the manner of attack of this substance in peptones.

This evidence of the difference in the proteolytic nature of the two organisms, is in line with the known differences in the products formed in a protein medium in which B. coli is known to produce indol, indol acetic acid, skatol, mercaptan, etc., in considerable quantities while in cultures of B. aerogenes, they are found in very small amounts or not at all.

A comparison of the CO_2/H_2 ratios of these organisms in peptone media and in one containing dextrose shows that the relation is just reversed. The possibility of the coincident production of gas from the peptone and from the dextrose in the usual dextrose broth, would raise a question as to the accuracy of the interpretations placed upon analyses of gas produced from peptone sugar broths.

The reaction of the medium dertmines to a large extent whether the CO_2 will remain in the medium or will collect above it. If the medium becomes acid as in the

dextrose fermentation a large part of the CO_2 is found above the medium. When the medium is neutral or slightly alkaline the CO_2 is found largely in the medium as dissolved CO_2 and as carbonates.

The demonstration of a considerable CO_2 ppoduction B. coli as well as B. aerogenes would seem to explain the production of the slight amount of acidity, noted in the Difco peptone agar.

Although the two media are not entirely comparable due to the fact that the agar media was open to the air and no doubt diffusion of the gases through the media to the air would slowly occur.

This high percentage of CO_2 might be explained by considering the amino acids as a source. When no other source of carbon is present, these are broken down to supply the energy requirements of the organisms. The carbon is obtained by an attack upon the COOH radicle. This is broken down with the production of CO_2. The decarboxylization may precede the utilization of the amino nitrogen radicle, producing an amine, or the deamination may take place first producing a fatty acid, which in turn may be broken down with the production of CO_2 and H_2. It is thus seen that the relative amount of CO_2 and H_2 produced depends upon the type of reaction by which the amino acid is broken down.

That the characteristic of visible gas production in solid media, which is due to the presence of the mond

insoluble H_2, correlates with other established characteristics of the organisms has been shown in the preceding section.

m The fact that the source of the gas was found, only in two of the four peptones would indicate a selective utilization of the amino acids by the bacteria. Further evidence that such selective utilization may occur is given in the following section under the study of H_2S production.

All of the peptones used by the author gave a strong Molisch test. This is a general test for carbohydrates; a positive reaction being due to the production of furfural from the carbohydrate radicle, by concentrated sulphuric acid and the reaction of this with a-naphthol to produce a purple color. This test was still strongly positive in a Difco peptone culture of B. aerogenes after several days incubation.

If a carbohydrate is present in all of the peptones, the fact that B. aerogenes could produce acid gas from only two of the four would indicate if it was the source of the gas that either the same carbohydrate was not present in the four peptones or that it was bound up in such a way that it was not available for the organisms. Whether the source of the gas is a carbohydrate or an amino acid it must be present in quite small amounts as shown by the fact that it can be quantitatively removed by a 24 hour culture of an appropriate organism.

Evidence presented in this study, that would indicate a protein source of the gas, may be summarized as follows.

(1) Visible gas production from 2% Difco peptone gelatine, with no added carbohydrate.

(2) Amount of gas produced is increased by increasing percentage of peptone.

(3) While all peptones gave a positive Molisch test, gas was produced from two only.

(4) The substance responsible for the gas, is present in small amounts, as evidenced by the fact that it ben be readily removed by a 24 hour culture of B. aerogenes.

(5) The Molisch test for carbohydrates shows a strong reaction, after the substance responsible for the gas has been removed. And lastly but probably the most significant, (6))A gas ratio is produced which is unlike that produced by carbohydrate fermentation.

The gas ratio of these organisms is practically the same for all carbohydrates. In a carbohydrate medium this CO_2/H_2 value is consistently found to be from 1. to 2.

The CO_2/H_2 ratio in a peptone medium is found to be from 6.0 $\nearrow$ 12.0 for aerogenes, and very much higher for B. coli.

One of the outstanding factors in the classification of bacteria, has been their action on carbohydrates, alcohols, and glucosides. Aside from the liquefaction of gelatine and the production of indol and H_2S,

very little attention has been given to the use of protein decomposition products in classification.

The specificity shown by B. aerogenes in gas production from peptone gelatine and the work which is here reported on amino acids and sulphur metabolism, would suggest that amino acids and simple cleavage products might be found of great value in the field of Bacteriological taxonomy.

HYDROGEN SULPHIDE PRODUCTION FROM PEPTONE.

The production of H_2S from sulphur compounds by bacteria has been observed and reported by many investigators. This characteristic is not peculiar to any one species of bacteria, but may be demonstrated by a variety of species when proper methods are used for its production.

Many of the inconsistences shown in the literature may be explained by the difference in sulphur compounds present in the media used and in the time of incubation.

Herter (1907) stated that, B. aerogenes, B. coli, B. dysenteriae and B. typhosus produced abundant hydrogen sulphide when grown in bullion containing cystine. Sasaki and Otsuka (1912) reported hydrogen sulphide production by many bacteria including B. coli, B. typhosus, B. paratyphosus A and B, B. dysenteria,

from cystine, elemental sulphur and sodium thisulphate. A few organisms including B. coli produced hydrogen sulphide from sodium sulphite while more produced it from sodium sulphate or taurine. Lederer (1913) noted hydrogen sulphide production by the different members of the intestinal group when 10% peptone was used and when incubation was prolonged but noted considerable differences in the quantity produced by the different organisms. Kligler (1917) found 6% of his coli strains produced hydrogen sulphide in meat infusion, dextrose agar with lead acetate added as indicator. Jordan and Victorson (1917) used a meat infusion 3% Witte's peptone agar with lead acetate added, and found it of differential value in the paratyphoid enteritidis group. Myers (1920 compared the hydrogen sulphide production of a number of bacteria on several peptones and found that a greater number of species of bacteria produced hydrogen sulphide from Witte's peptone than from Difco or Fairchild's. He found that B. aerogenes and B. coli both produced H_2S from Witte's peptone while B. aerogenes was negative in Difco, and B. coli only slightly positive after 48 hours. Thompson (1921) used anerobic plate cultures made from Difco peptone agar with lead sub-acetate as indicator and found that B. typhosus B, paratyphosus B, B. enteritides, B. proteus vulgaris and

B. prodigiosus gave black colonies while B. coli, B. paratyphosus A, B. alcaligenes and B. dysenteriae had little or no color. Paola (1922) reported fermentation and blackening of dextrose and lactose broth by B. coli. Tilley (1923 a) found hydrogen sulphide production, from different commercial peptones, extremely variable. He also found variation between strains of the same species in regard to their ability to produce hydrogen sulphide. In a later paper (1923 b) he studied the "unoxidized", party "oxidized" and "oxidized" sulphur content of a number of peptones and determined the availability of these for H_2S production by bacteria. He found that the "partly oxidized" and "unoxidized" portions were available while the oxidized was not. He recommended plain agar with the addition of sodium thiosulphate as a medium for H_2S production in differential tests. Wilson (1923) using plain agar with sodium sulphite, glucose and ferric chloride added, reported blackening by B. typhosus, B. enteritidis, B. paratyphosus B, but no reaction with B. paratyphosus A, B. dysenteria, B. aerogenes, B. coli, B. vulgaris and others. He suggests that the H_2S production in peptones is due tosulphite impurities. Fellers et al (1924) found that B. coli produced abundant H_2S from Difco peptone, B. paratyphosus B, B. enteritidis and B. typhosus produced

moderate amounts, while B. aerogenes, B. paratyphosus A, and B. dysenteriae produced none in one hundred forty-four hours incubation.

Attention has been called to the fact that of the colon-aerogenes strains reported in this paper, only two produced H_2S in 48 hours. This is apparently a delayed reaction, however, for when incubation is carried out over a more extended period, H_2S is produced.

Experimental work for the purpose of explaining this delayed reaction was undertaken. The experiments were designed to answer the following questions.

(1) How does the incubation period necessary for H_2S production from peptone of B. aerogenes and B. coli differ from that of other organisms of the intestinal group?

(2) How would these incubation periods compare, when sulphur compounds of known chemical composition were used?

(3) What effect would other amino acids have upon H_2S production from cystine, when used in a synthetic medium?

METHODS.

In making such a study it was necessary to have a method which would give qualitative and at least roughly quantitative results, and which would

allows readings to be made upon a large number of cultures at short intervals of time. An investigation was made of the methods which might be used for quantitative H_2S determinations such as those used by Mecklenberg and Rosenkränzer (1914), Meyers (1920), Morrison and Tanner (1922), Anderson (1924), Fellers et al. (1924) and qualitative tests such as those of Kligler (1917), Jordan and Victorson (1917). It was found that these could not be applied to this particular problem. The following method was accordingly devised.

A 1.5% agar medium, containing the peptone or sulphur compound required, was prepared, with ferrous sulphate added as an indicator of H_2S production.

A system of recording comparative degrees of blackening of the medium was used as follows: - indicates no reaction, Sl / indicates a slight perceptible blackening along the line of innoculation, 1 / indicates a plainly visible blackening along the line of innoculation, 2 / indicates some diffusion of color from the growth along the stab; 3 / indicates blackening considerably diffused; 4 / indicates a complete blackening of the tube.

Duplicate tests were made on a number of cultures and it was found possible to check the readings on the duplicate tubes consistently.

RESULTS.

In order to determine the incubation period necessary for H_2S production from peptone, the following experiment was made.

A medium containing 1.5% agar, 0.5% meat extract and 3% Witte's peptone and sterilized as for ordinary agar. One c.c. of a 2.0% $FeSO_4$ solution was added to each 100 c.c. of media. This was tubed in sterile tubes, innoculated from 24 hour agar cultures and incubated at 37°C.

Innoculations were also made into a plain agar containing 3 c.c. of N/50 sodium thiosulphate per 100 c.c. of media and containing $FeSO_4$ as stated above. The results are shown in Table #16.

A consideration of the above table shows that the time of incubation necessary for H_2S production for the organisms studied varies greatly.

In the case of B. paratyphosus B. and B. vulgaris, there is evident H_2S production in 12 to 24 hours, while in the case of B. coli, B. aerogenes, B. cloacae, B. dysenteriae, Shiga, staphylococcus aureus, and microspira comma, visible blackening was not apparent until after 3 to 10 days incubation. B. typhosus and B. avisepticus appeared to be intermediate between the two extremes. The delayed reaction was not due to

TABLE 16

RESULTS SHOWING RELATIVE H_2S PRODUCTION BY VARIOUS BACTERIA FROM WITTE'S PEPTONE AND $Na_2S_2O_3$

Organism	3% Witte's Peptone Agar — Days incubation									Sodium thiosulphate Agar — Days incubation								
	12hr	1	2	3	4	5	6	7	10	12hr	1	2	3	4	5	6	7	10
B. alcaligenes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. Typhosus	-	--	1+	2+	2+	2+	2+	2+	2+	-	1+	3+	3+	3+	3+	3+	3+	3+
B. dysenteriae Shiga	-	-	-	-	-	-	-	1+	3+	-	-	-	-	-	-	-	-	-
B. dysenteriae Flexner	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. Paratyphosus B.	-	1+	2+	3+	3+	3+	3+	3+	3+	1+	2+	4+	4+	4+	4+	4+	4+	4+
B. Paratyphosus A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. Vulgaris	Sl+	1+	2+	3+	3+	3+	3+	3+	3+	1+	2+	4+	4+	4+	4+	4+	4+	4+
B. cloacae	-	-	--	sl+	sl+	sl+	1+	1+	2+	-	-	-	-	-	-	-	-	-
B. aerogenes F 23	-	-	-	-	-	-	-	-	1+	-	-	-	-	-	-	-	-	-
B. coli F 50	-	-	-	1+	1+	1+	1+	1+	2+	-	-	-	-	-	-	-	-	-
Msp. Comma	-	-	-	-	-	-	-	-	1+	-	-	-	-	-	-	-	-	-
Staph Aureus	-	-	-	sl+	sl+	sl+	1+	1+	1+	-	-	-	-	-	-	-	-	-
Staph albus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. aviseptícus	-	-	2+	2+	2+	2+	2+	2+	2+	-	-	3+	3+	3+	4+	4+	4+	4+
Control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Legend: Sl+ indicates slight blackening; +, blackened along stab; ++, blackening slightly diffused; +++, blackening considerably diffused; ++++, blackening diffused throughout tube/

lack of growth, as the growth was luxuriant within 12 to 24 hours in every case. That it was not due to any selective germicidal action of the $FeSO_4$ is shown later by the fact that B.coli, B. aerogenes and B. cloacae all produce H_2S within 12 hours in the cystine medium, containing the same amount of $FeSO_4$.

The sulphur found in proteins is usually considered to be in the form of cystine according to Plimmer (1912). That this does not account for all the sulphur present, and the possibility of the presence of other sulphur compounds, has been shown by Johnson (1911). This organic sulphur may exist in the proteins in different states of oxidation. This is often referred to as "unoxidized" or loosely combined sulphur and as oxidized sulphur. Cystine with the linking -S-S- is considered as an example of the unoxidized sulphur, and Taurine with the grouping $CH_2.SO_3H$, analagous to the inorganic sulphates, is considered as an example of the oxidized sulphur. Mathews (1920) believes that there is a possibility of a cysteine linkage also. The sulphur occurs here as an SH group and is in the reduced state.

Tilly (1923) has recently determined the "oxidized", "partly oxidized" and "unoxidized" sulphur in a number of commercial peptones and correlated these with H_2S production by bacteria, he found that H_2S production

was dependent upon the presence of the unoxidized and partly oxidized sulphur.

Cystine was, therefore, selected for study as the probable state of the sulphur in peptone.

Hydrogen sulphide production was studied with cystine as the only source of nitrogen and with cystine together with other nitrogen compounds.

A medium was prepared, similar to that used by Clark and Lubs (1917) except that aspartic acid was omitted. This has a composition as follows:

Disodium hydrogen phosphate	7. gms.
Potassium acid phthalatev	2. gms.
Dextrose	4. gms.
Water	1000 c.c.

This medium was used as a base to which was added the various nitrogen compounds.

The amino acids used were aspartic acid, glycine, alanine, leucine, phenylalanine and tyrosine. Creatinine was included in the list, because of its presence in meat extracts and meat infusions and therefore in standard culture media. The nitrogen compounds other than cystine ware all used in 0.1% amounts.

The cystine was used in 0105% concentration* owing to its relative insolubility in water, special

*Except in the study with aspartic acid where cystine in a concentration of 0.1% used as shown by table #17.

TABLE 17

RESULTS OF HYDROGEN SULPHIDE PRODUCTION FROM CYSTINE AND FROM CYSTINE TOGETHER WITH ASPARTIC ACID

Organism	Base medium and Cystine .1%					Base medium and Cystine .1% and aspartic acid .1%					Base medium and aspartic acid .1%					Base medium				
Hours incubation	12	24	36	48	192	12	24	36	48	192	12	24	36	48	192	12	24	36	48	192
B. alcaligenes	sg	sg	sg	s	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	0	sg	sg	sg	sg
B. typhosus	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	0	0	0	0	0
B. dysenteriae Shige	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	0	0	0	0	0	0	0	0	0	0
B. dysenteriae Flex	-	-	-	-	-	-	-	-	-	-	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg
B. paratyphosus B.	1+	3+	4+	4+	4+	sl+	1+	3+	3+	4+	sg	-	-	-	-	0	-	-	-	-
B. paratyphosus A.	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	0	0	0	0	0	0	0	0	0	0
B. vulgaris	sg	2+	4+	4+	4+	sg	1+	4+	4+	4+	sg	-	-	-	-	0	sg	sg	-	-
B. cloacae	1+	3+	4+	4+	4+	-	1+	3+	4+	4+	-	-	-	-	-	-	-	-	-	-
B. aerogenes	sl+	2+	4+	4+	4+	-	-	1+	3+	3+	-	-	-	-	-	-	-	-	-	-
B. coli	1+	3+	3+	3+	3+	-	-	1+	1+	1+	-	-	-	-	-	-	-	-	-	-
Msp. Comma	0	0	0	0	sg	0	0	sg	sg	sg	0	0	0	0	sg	0	0	0	0	sg
Staph aureus	sg	sg	sg	sg	sg	sg	sg	sg	sg	sg	0	0	0	0	0	0	0	0	0	0
Staph albus	-	-	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0	0	0
B. aviseptícus	sg	sg	-	-	sl+	0	sg	-	-	-	0	0	0	0	0	0	0	0	0	0

Legend: In this and the following tables, 0 indicates no visible growth, sg indicates slight growth, - indicates growth but no blackening.

Sl+ indicates slight blackening; +, blackened along stab; ++, blackening slightly diffused; +++, blackening considerably diffused: ++++, blackening diffused throughout tube.

means had to be used to get it in solution in the medium.

This was accomplished by dissolving 1 gm. of the cystine in 12 c.c. N/1 HCl in a sterile tube. Six-tenths c.c. of this solution added to 100 c.c. media, gave a 0.05% concentration of cystine. The media was so adjusted that the addition of this amount of acid gave the desired P_h. This also avoided the necessity of heating the cystine, eliminating that possibility of altering its sulphur linkage. The other amino acids were added to the base medium before sterilizing. All the media was adjusted to P_h 6.8. This slight acidity was found sufficient to keep the cystine in solution. One and one-half percent agar was used to produce a solid medium. One c.c. of 2% sterile $FeSO_4$ was added toeach 100 c.c. of medium just before tubing. The medium as finally prepared had the following composition.

Base medium	100.0 c.c.
Amino acid) or Cystine)	0.1 gm. 0.05 gm.
Agar	1.5 gm.
$FeSO_4$ 2%	1.0 c.c.

Innoculations were from 24 hour agar cultures and were made by stabbing with a straight needle.

The results of this experiment are given in the following Tables 17 to 23 and plates 1 to 11.

TABLE 18

SHOWING COMPARISON OF H_2S PRODUCTION FROM CYSTINE ALONE AND WITH GLYCINE

Organisms	Base medium +0.05% cystine					Base medium +.05% cystine +.1% glycine					Base medium +.1% glycine				
Hours incubation	12	24	36	48	192	12	24	36	48	192	12	24	36	48	192
B.typhosus	sg	sl+	sl+	1+	1+	sg	sg	sg	sg	sg	0	sg	sg	sg	sg
B.vulgaris	1+	3+	4+	4+	4+	sg	1+	3+	4+	4+	sg	-	-	-	-
B.cloacae	2+	3+	3+	3+	4+	-	2+	3+	3+	3+	-	-	-	-	-
B.coli F 50	2+	3+	3+	3+	3+	-	2+	2+	3+	3+	-	-	-	-	-
B.aerogenes F 23	2+	2+	3+	3+	3+	-	1+	2+	2+	3+	-	-	-	-	-
B.aviseptícus	sl+	2+	2+	2+	3+	sg	sg	sl+	1+	3+	0	sg	-	-	-
B.paratyphosus B.	2+	3+	3+	3+	3+	-	2+	3+	3+	3+	-	-	-	-	-

TABLE 19

SHOWING COMPARISON OF H_2S PRODUCTION FROM CYSTINE ALONE AND WITH LEUCINE

Organisms	Base medium +0.05% cystine					Base medium +0.05% cystine +0.1% leucine					Base medium +0.1% leucine				
Hours incubation	12	24	36	48	192	12	24	36	48	192	12	24	36	48	192
B.typhosus	sg	sl+	sl+	1+	1+	0	-	-	-	sl+	0	-	-	-	-
B.vulgaris	1+	3+	4+	4+	4+	2+	3+	4+	4+	4+	-	-	-	-	-
B.cloacae	2+	3+	3+	3+	4+	1+	2+	3+	3+	4+	-	-	-	-	-
B.coli F50	2+	3+	3+	3+	3+	1+	2+	2+	2+	2+	-	-	-	-	-
B.aerogenes F23	2+	2+	3+	3+	3+	1+	2+	3+	3+	3+	sg	-	-	-	-
B.aviseptícus	sl+	2+	2+	2+	3+	sl+	1+	2+	2+	2+	-	-	-	-	-
B.paratyphosus B.	2+	3+	3+	3+	3+										

TABLE 20

SHOWING COMPARISON OF H_2S PRODUCTION FROM CYSTINE ALONE AND WITH ALAMINE

Organisms	Base medium +0.05% cystine					Base medium +0.05% cystine +0.1% alanine					Base medium +0.1% alamine				
Hours incubation	12	24	36	48	192	12	24	36	48	192	12	24	36	48	192
B.typhosus	sg	sl+	sl+	1+	1+	0	-	-	-	sl+	0	-	-	-	-
B.vulgaris	1+	3+	4+	4+	4+	1+	3+	4+	4+	4+	sg	sg	-	-	-
B.cloacae	2+	3+	3+	3+	4+	1+	2+	3+	3+	3+	-	-	-	-	-
B.coli F50	2+	3+	3+	3+	3+	1+	2+	2+	2+	2+	-	-	-	-	-
B.aerogenes F 23	2+	2+	3+	3+	3+	1+	2+	3+	3+	4+	-	-	-	-	-
B.avisepticus	sl+	2+	2+	2+	3+	sg	sl+	1+	2+	2+	sg	-	-	-	-
B.paratyphosus B.	2+	3+	3+	3+	3+	1+	3+	3+	3+	3+	-	-	-	-	-

TABLE 21

SHOWING COMPARISON OF H_2S PRODUCTION FROM CYSTINE ALONE AND WITH PHENYLALANINE

Organisms	Base medium +0.05% cystine					Base medium +0.05% cystine + 0.1% phenylalanine					Base medium +0.1% phenyl-alanine				
Hours incubation	12	24	36	48	192	12	24	36	48	192	12	24	36	48	192
B.typhosus	sg	sl+	sl+	1+	1+	sg	sg	sg	sg	sg	0	sg	sg	sg	sg
B.vulgaris	1+	3+	4+	4+	4+	sg	1+	2+	2+	3+	sg	-	-	-	-
B.cloacae	2+	3+	3+	3+	4+	-	-	1+	1+	1+	-	-	-	-	-
B.coli F 50	2+	3+	3+	3+	3+	-	sl+	1+	1+	1+	-	-	-	-	-
B.aerogenes F 23	2±	2+	3+	3+	3+	-	-	-	-	sl+	-	-	-	-	-
B.avisepticus	sl+	2+	2+	2+	3+	sg	-	-	sl+	1+	0	-	-	-	-
B.paratyphosus B.	2+	3+	3+	3+	3+	-	2+	2+	2+	2+	-	-	-	-	-

TABLE 22

SHOWING COMPARISON OF H_2S PRODUCTION FROM CYSTINE ALONE AND WITH TYRONINE

Organism	Base Medium +0.05% cystine					Base Medium +0.05% cystine +0.1% tyronine					Base Medium +0.1% tyronine				
Hours incubation	12	24	36	48	192	12	24	36	48	192	12	24	36	48	192
B.typhosus	sg	sl+	sl+	1+	1+	sg	sl+	sl+	sl+	sl+	0	-	-	-	-
B.vulgaris	1+	3+	4+	4+	4+	2+	3+	3+	3+	4+	sg	sg	sg	sg	--
B.cloacae	2+	3+	3+	3+	4+	1+	2+	2+	3+	3+	-	-	-	-	-
B.coli F 50	2+	3+	3+	3+	3+	1+	2+	2+	3+	2+	-	-	-	-	-
B.aerogenes F 23	2+	2+	3+	3+	3+	1+	2+	3+	3+	3+	-	-	-	-	-
B.aviseptieus	sl+	2+	2+	2+	3+	sg	sl+	sl+	1+	1+	sg	-	-	-	-
B.paratyphosus B.	2+	3+	3+	3+	3+	2+	3+	3+	3+	3+	-	-	-	-	-

TABLE 23

SHOWING COMPARISON OF H_2S PRODUCTION FROM CYSTINE ALONE AND WITH CREATININE

Organism	Base Medium +0.05% cystine					Base Medium +0.05% cystine +0.1% creatinine					Base Medium +0.1% creatinine				
Hours incubation	12	24	36	48	192	12	24	36	48	192	12	24	36	48	192
B.vyphosus	sg	sl+	sl+	1+	1+	sg	-	-	-	-	sg	-	-	-	-
B.vulgaris	1+	3+	4+	4+	4+	sl+	2+	2+	3+	4+	-	-	-	-	-
B.cloacae	2+	3+	3+	3+	4+	sl+	1+	2+	3+	3+	-	-	-	-	-
B.coli F 50	2+	3+	3+	3+	3+	-	1+	1+	1+	1+	-	-	-	-	-
B.aerogenes F 23	2+	2+	3+	3+	3+	sl+	1+	1+	1+	2+	-	-	-	-	-
B.aviseptieus	sl+	2+	2+	2+	3+	sg	1+	1+	2+	2+	sg	-	-	-	-
B.paratyphosus B.	2+	3+	3+	3+	3+	sl+	1+	3+	3+	3+	-	-	-	-	-

PLATE I EFFECT OF ASPARTIC ACID UPON H_2S PRODUCTION FROM CYSTINE BY B. COLI.

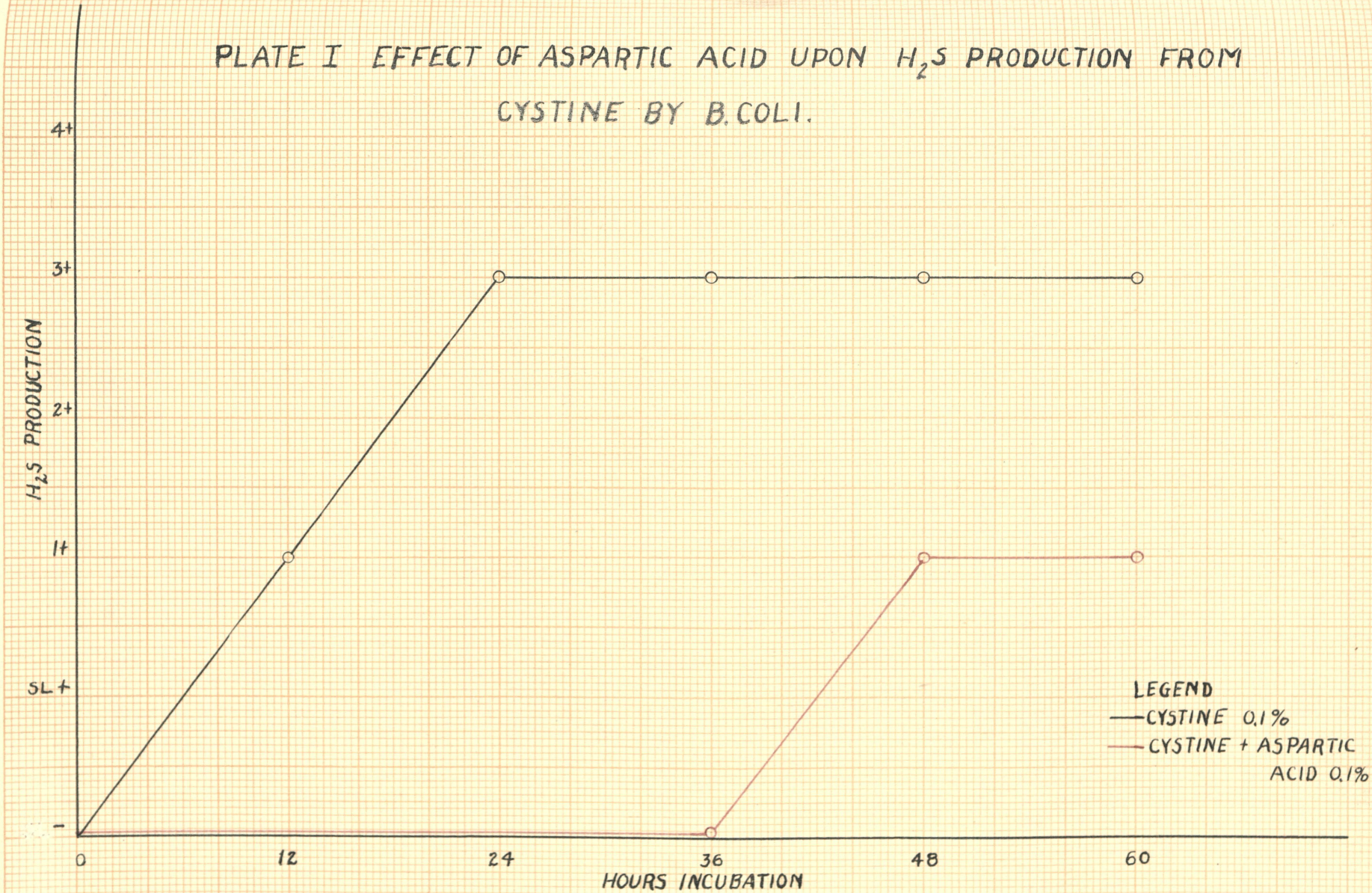
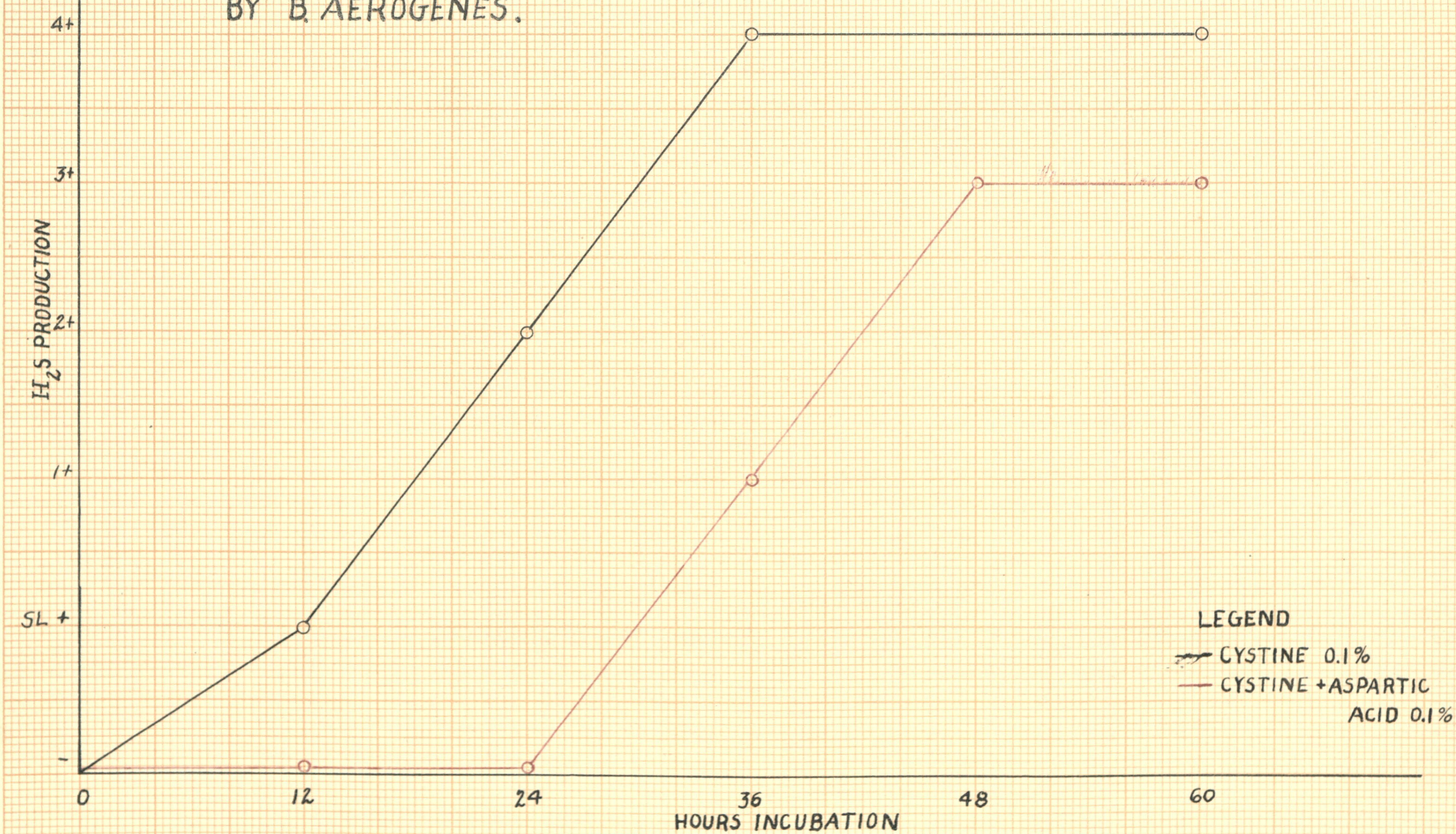


PLATE II EFFECT OF ASPARTIC ACID UPON H_2S PRODUCTION FROM CYSTINE BY B. AEROGENES.



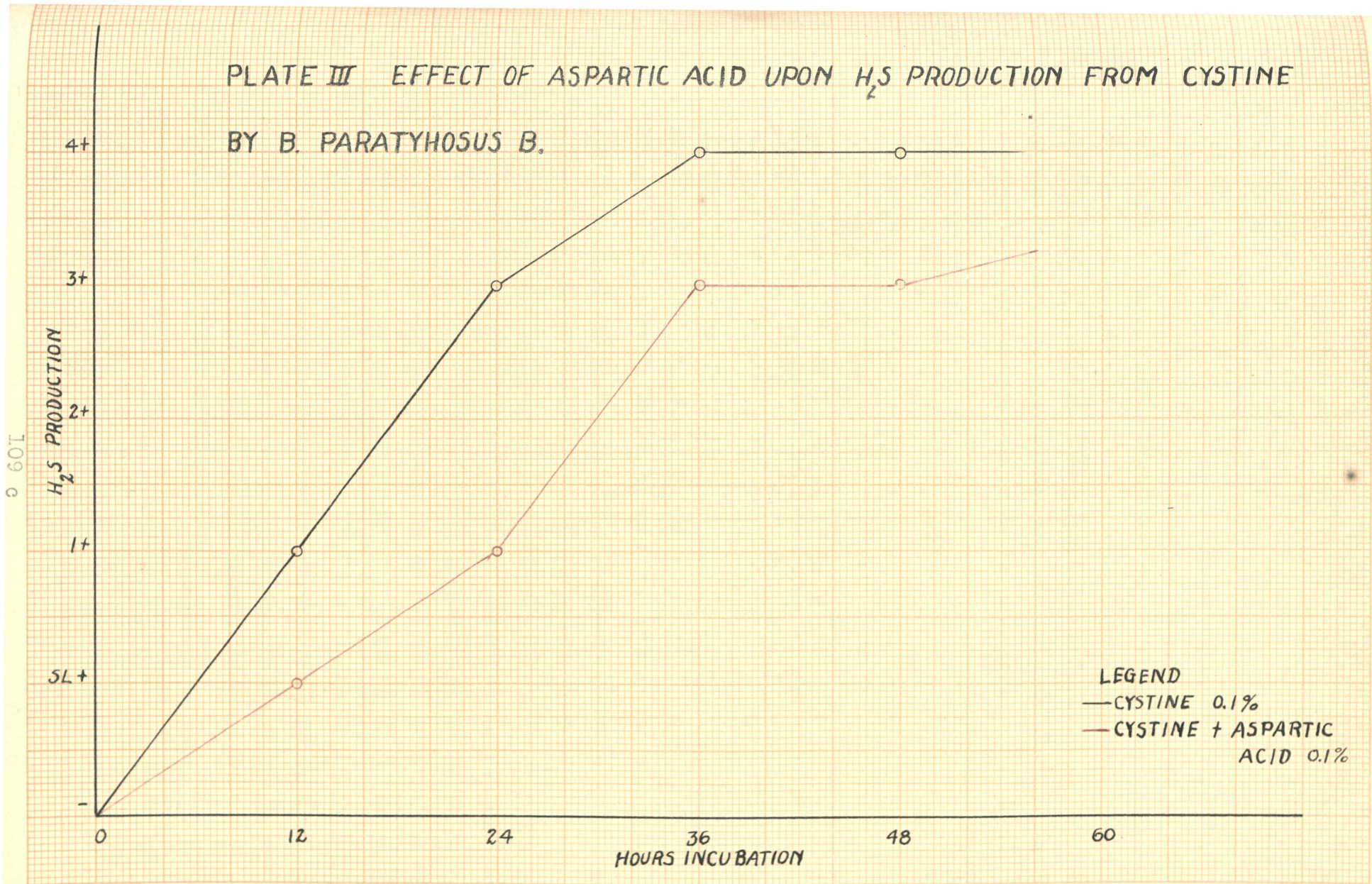


PLATE III EFFECT OF ASPARTIC ACID UPON H_2S PRODUCTION FROM CYSTINE
BY B. PARATYHOSUS B.
4+
3+
2+
1+
SL+
-
H_2S PRODUCTION
0
12
24
36
48
60
HOURS INCUBATION
LEGEND
—CYSTINE 0.1%
—CYSTINE + ASPARTIC ACID 0.1%

PLATE IV EFFECT OF VARIOUS AMINO ACIDS UPON H_2S PRODUCTION FROM CYSTINE BY B. COLI.

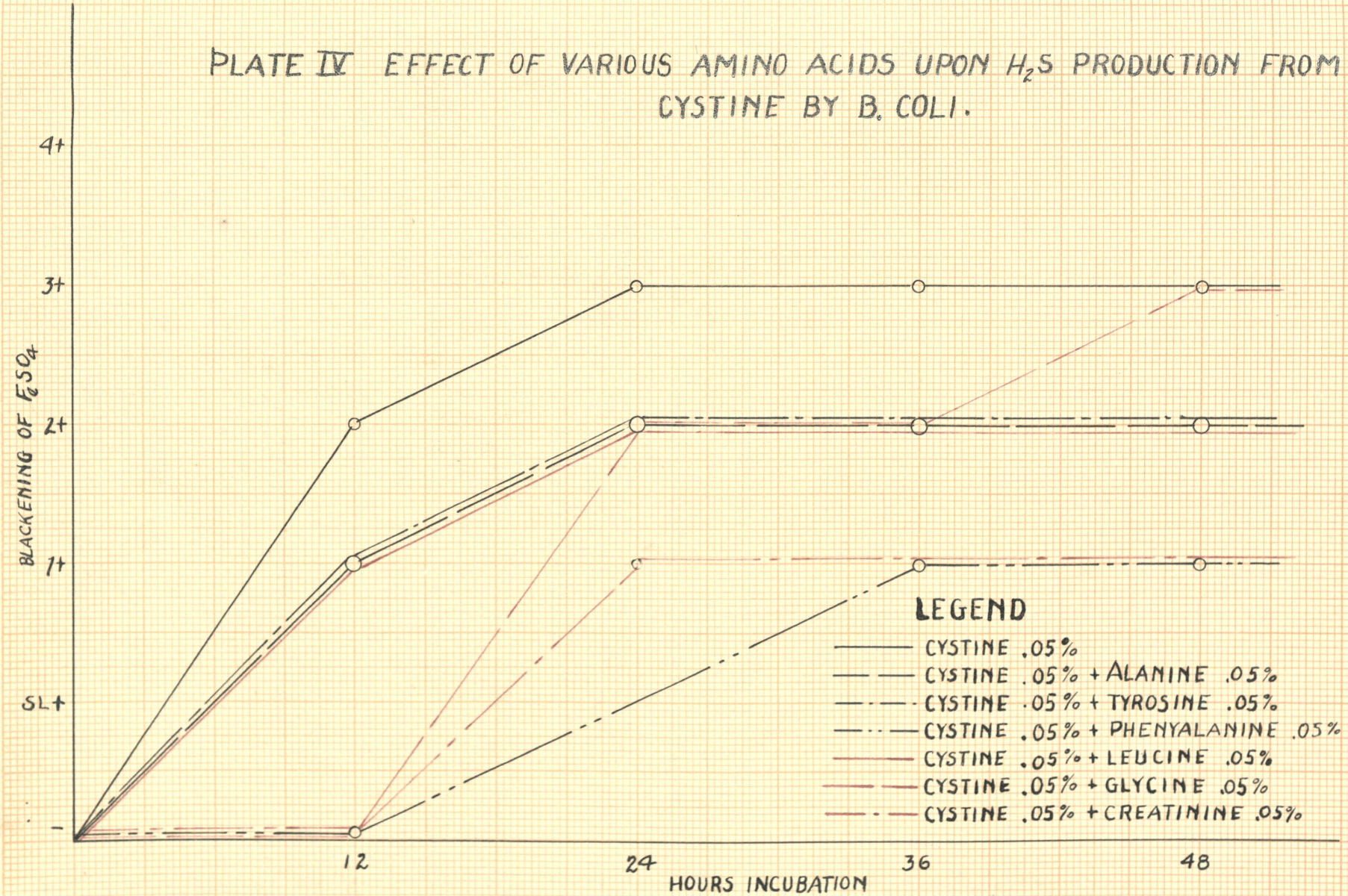


PLATE V EFFECT OF VARIOUS AMINO ACIDS UPON H_2S PRODUCTION FROM CYSTINE BY B. AEROGENES.

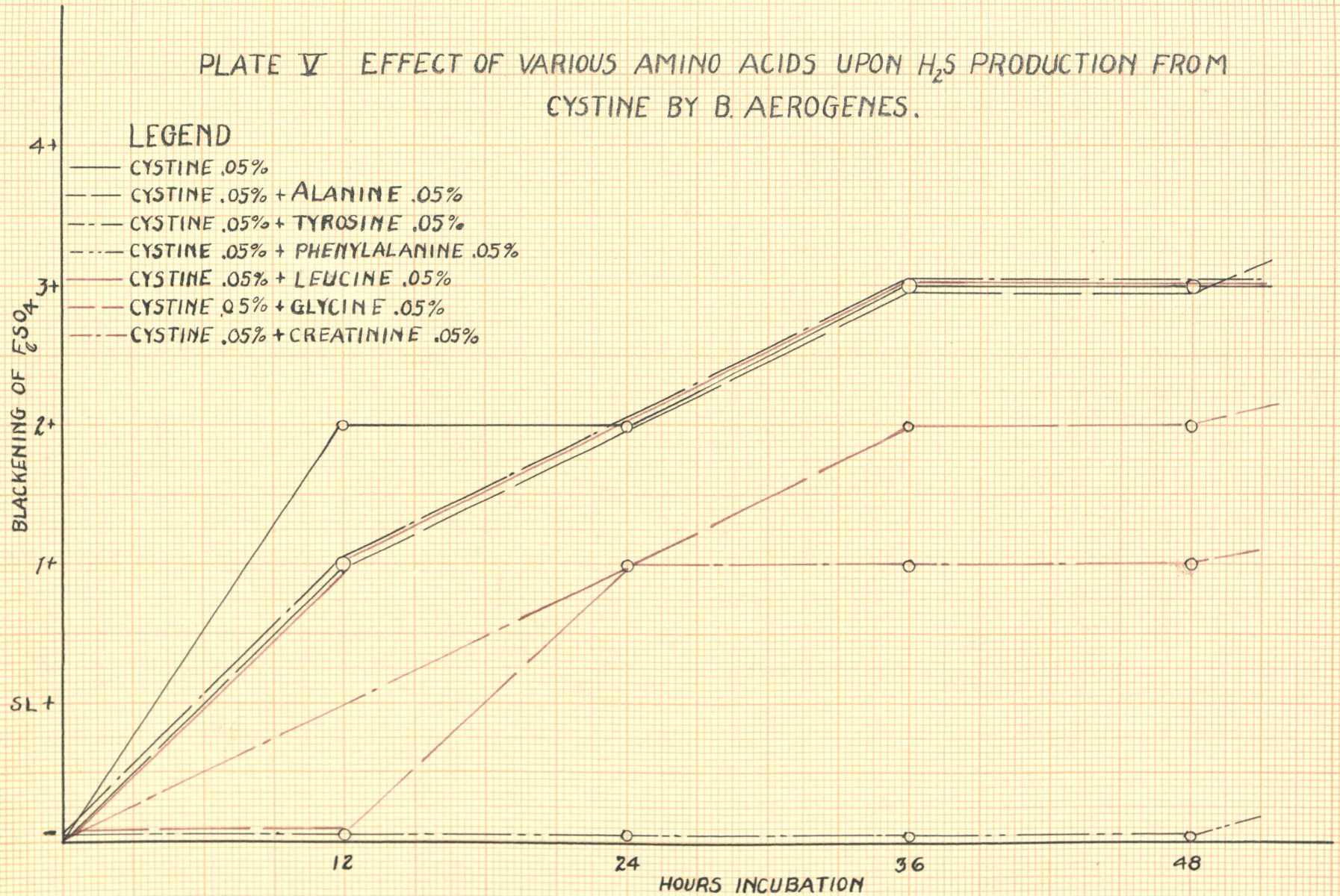
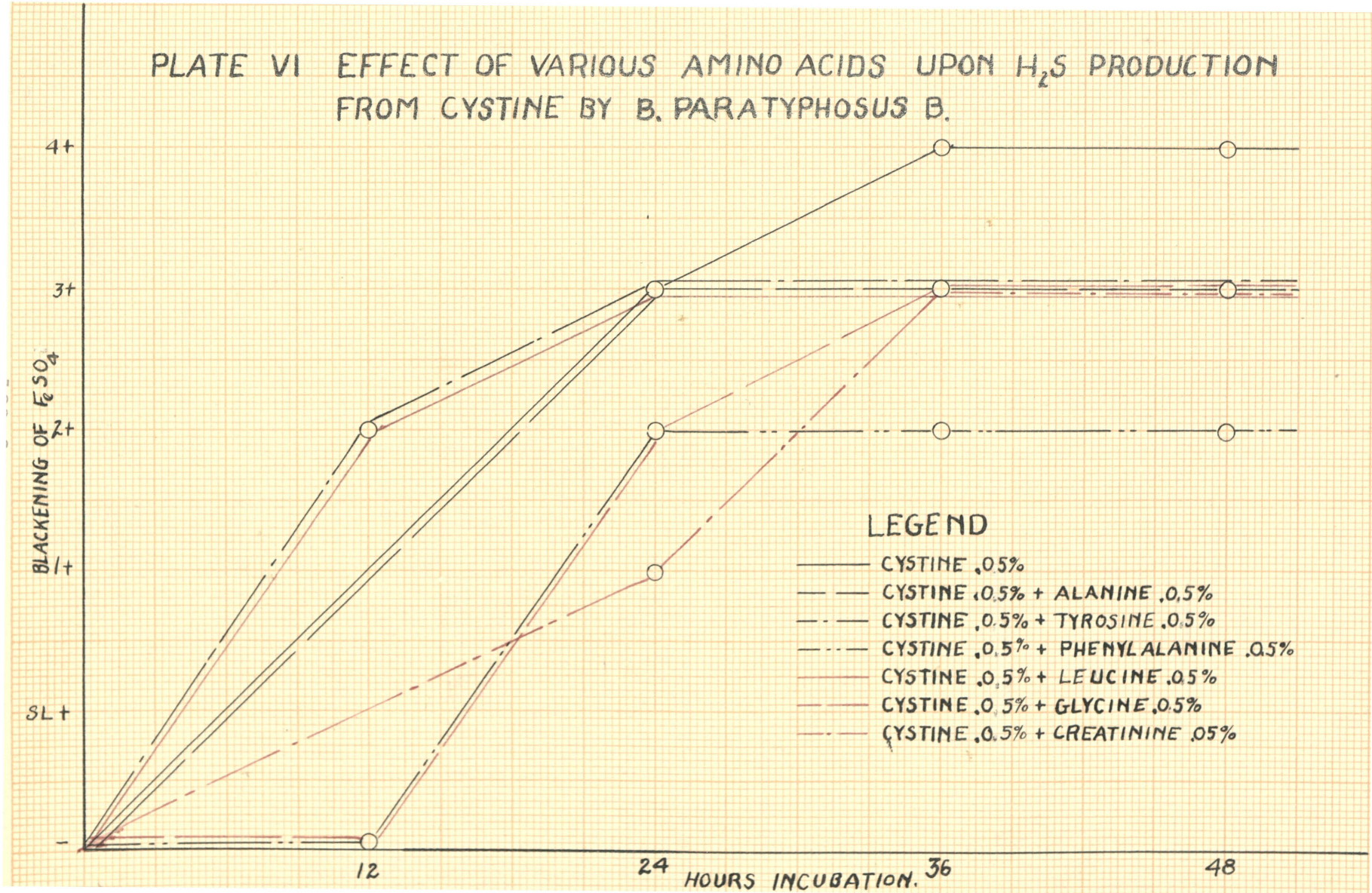


PLATE VI EFFECT OF VARIOUS AMINO ACIDS UPON H_2S PRODUCTION FROM CYSTINE BY B. PARATYPHOSUS B.

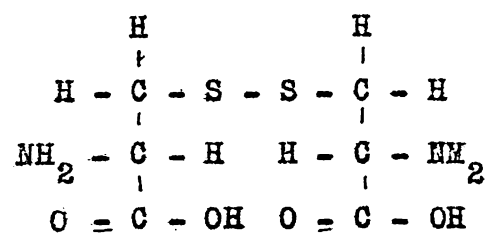


Attention has been called, previously, to the fact that B. paratyphosus B. and B. vulgaris produced a visible blackening of $FeSO_4$ in a peptone medium in 12 to 24 hours indicating an early attack upon the sulphur compounds in the medium. B. coli and B. aerogenes, however, showed no reaction until much later which would indicate a delayed action on the sulphur compounds.

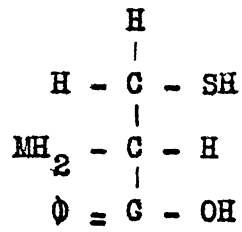
A consideration of table #17 shows that when cystine is present as the only source of nitrogen, H_2S is produced by these organisms within 12 hours, and compares with B. paratyphosus B in this respect. This is shown in plates 1, 2, and 3. This would seem to indicate that some other substances in the peptone were responsible for the delayed H_2S production. This action might take the form of an interference with the reduction of the sulphur to H_2S or it might be an interference with the utilization or breaking down of the cystine.

Any attempt at a solution of these questions must include a consideration of the manner by which bacteria may break down cystine with the production of H_2S.

The formula of cystine shows the sulphur to occur in a partially oxidized state:



For bacteria to release H_2S from such a compound, two reactions would seem to be necessary; (1) A reudction of the sulphur and (2), a splitting off of the sulphur radicle. The reduction of cystine would produce two molecules of cysteine.



In which the sulphur is reduced and H_2S would result upon its being split off. Cysteine is quite unstable, oxidizing spontaneously to cystine as shown by Mathews and Walker (1909) and probably would not occur as such in culture media.

That compounds similar to cysteine may be broken down without the release of H_2S is shown by the presence of mercaptans in bacterial culture media and in the feces.

Rey-Pailhaide (1888) demonstrated a reductase from yeast juice which would reduce elementary sulphur to H_2S. Petri and Maassen (1893) attributed H_2S production by bacteria, to the reduction of sulphur compoundsby the nascent hydrogen formed. That organisms may produce considerable hydrogen in a peptone medium without H_2S being formed would tend to rule out this possibility.

It is well known, however, that the production of redutases is quite common among bacteria.

That the presence of a reductase is not alone responsible for H_2S production by bacteria is shown by the following experiment.

Media was prepared as indicated in Table #24. B. coli and B. paratyphosus B. was cultured in plain broth and after 24 hours incubation 1 c.c. of this broth was transferred to 10 c.c. of the media. This was repeated after 48 hours and 72 hours. One set of media contained 0.5% phenol, which will stop bacterial growth, but will allow enzyme action. Another set containing no phenol was used as a control. Uninnoculated controls were incubated also.

Table #24 shows the results of the 72 hour broth innoculations. It is evident that the reductase which is sufficient to decolorize methylene blue, will not produce H_2S from peptone or $Na_2S_2O_3$. B. coli which will completely decolorize methylene blue agar in 30 minutes fails to produce H_2S after 16 hours incubation. That the preformed enzyme has some effect is suggested by the shortened incubation period in the case of B. paratyphosus B, without phenol. Other chemicals such as Toluol and sodium fluoride were used which allow enzyme action but inhibit bacterial gorwth. The results were the same. This experiment would

TABLE 24

RESULTS SHOWING EFFECT OF REDUCTASE UPON H_2S PRODUCTION FROM PEPTONE AND $NA_2S_2O_3$

	With 0.5% phenol		Without phenol	
	B.coli	Para B.	B.coli	Para B
Methylene blue agar 30' at 45° C	Completely reduced	completely reduced	completely reduced	completely reduced
3% Witte's peptone agar & $FeSO_4$ 6 hrs at 45°C	not blackened	not blackened	not blackened	2+ blackened
16 hrs at 37°C	not blackened	not blackened	not blackened	black
$Na_2S_2O_3$ Agar + $FeSO_4$ 6 hrs at 45°C	not blackened	not blackened	not blackened	3+ blackened
16 hrs at 37°C	not blackened	not blackened	not blackened	4+ black

then show that H_2S is not produced by reductase unless active growth accompanies it. Although such growth will not cause H_2S production unless the sulphur compounds are attacked.

It was thought possible that other amino acids might exert a retarding effect upon H_2S production from cystine, either by exerting some inhibiting action on growth, or by being used by the organism in preference to the cystine. That such a retarding effect, occurs when other amino acids are present is shown in plates 1-6. The acids which seemed to have the greatest effect were aspartic acid, glycine and phenylalanine. Creatinine also showed some effect. The appearance of H_2S was delayed from 12 to 24 hours in each case. This is particularly noticeable in the case of B. coli with aspartic acid and cystine, also, in the case of B. aerogenes with phenylalanine and cystine. In the case of phenylalanine the growth was not luxuriant in any of the tubes although it was evident.

The amino acids alanine, leucine and tyrosine seem to have much less effect than the others, although in practically every instance the production is somewhat delayed over that from cystine alone.

That amino acids may be inhibitive to bacterial growth when used in sufficient concentration was shown by Wyon and McLeod (1923) who gave the following percentages showing the inhibitory threshold, as determined

in a 1% to .2% peptone medium.

Histidine 0.18%, tyrosine 0.21%, phenylalanine 0.5%, tryptophane 0.51%, leucine 0.85%, glycine 0.96%, alanine 1.0%, glutamic acid 1.9%, asparagine 2.25%, aspartic acid in concentration of 1.73% was inhibitive. Burrows and Neyman (1917) have called attention to their toxic-like action of tissue cells in culture.

The percentage given by Wyon and McLeod are all higher than those used in the present work, but the fact that they used them in a peptone solution, and that the concentration used in the smaller amounts of peptone were more toxic might indicate a protective action toward the bacteria by the peptone. They also failed to consider the amino acids normally present in the peptone.

GROWTH RATES.

In order to determine whether the delayed H_2S production was caused by inhibition of growth or by selective preference for one or the other of the amino acids in solution, a number of growth rates were run.

Methods.

Media was prepared using the same formula and methods as that used for the study of H_2S production, except for the 1½% agar which was omitted. The cystine

was used in 0.05% concentration. The other amino acids in 0.1% concentration. The media was sterilized in 100 c.c. amounts in 250 c.c. flasks. The reaction was adjusted to P_H 6.8. Innoculations were made by emulsifying a 24 hour agar slant culture of the organism and diluted to 1-100,000. One c.c. of this dilution was innoculated into the flask. This dilution permitted the use of a small number of organisms for innoculation and also eliminated the possibility of any considerable amount of food substance being carried over from the old culture.

Incubation was carried out at 37°C. The cultures were plated out at regular intervals using appropriate dilutions and all plates were made with standard agar and were counted with a lens after 48 hours incubation.

RESULTS.

The plate counts shown in the following tables are expressed in logs of the actual number of colonies counted per c.c. of culture.

A study of the tables 25-30 reveals a marked variation in some cases between the growth rate of cystine used alone, and when another amino acid is present. These differences may also be seen in plates 7-11.

The case of B. aerogenes in the glycine,

TABLE 25

RESULTS SHOWING RATE OF GROWTH WITH CYSTINE ALONE AND TOGETHER WITH ASPARTIC ACID

Time	Hrs in-cu-ba-tion	B.coli F50		B.aerogenes F23		B.paratyphosus B	
		Cystine	Cystine & Aspartic A	Cystine	Cystine & Aspartic A	Cystine	Cystine & Aspartic A
8:15A	0	*2.9540	3.0792	2.7782	2.8451	2.8451	2.7782
9:15A	1	2.7782	2.8451	2.4771	2.6990	2.3010	2.3010
10:15A	2	2.6990	2.6990	2.6021	2.6990	2.9542	2.6990
11:15A	3	2.8451	2.9540	3.0000	2.7782	2.8451	3.1761
12:15P	4	2.4771	2.8451	2.6990	2.6990	2.9542	2.6021
1 :15P	5	3.0792	3.2304	3.1761	3.3802	2.8451	3.0792
2:15P	6	3.3979	3.3979	3.5441	4.0453	2.8451	3.1139
3:15P	7	3.7160	3.9243	4.1038	4.5563	3.1461	3.6232
4:15P	8	4.0719	4.6021	4.3820	5.3010	3.2788	3.9685
5:15P	9	4.4502	5.0414	5.0414	5.7559	3.7709	4.2601
6:15P	10	5.0000	5.2788	5.4314	6.2175	3.9395	4.5250
7:15P	11	5.3222	5.5185	5.8751	6.8062	4.2430	4.9031
8:15P	12	5.7076	5.7853	6.3010	7.2788	4.3927	5.3222
8:15A	24	6.5441	8.2923	8.0414	7.4914		
8:15P	36	7.0000	7.9294	7.5798	6.0000		
8:15A	48	6.4771	7.4472	5.9542	6.7782		

* In this and the following tables the numbers are logs of the actual colony count.

TABLE 26

RESULTS SHOWING RATE OF GROWTH WITH TYROSINE ALONE AND WITH CYSTINE AND TYROSINE

Time	Hrs in-cu-ba-tion	B. coli F 50		B. aerogenes F 23		B. paratyphosus B	
		Cystine & Tyrosine	Tyrosine	Cystine & Tyrosine	Tyrosine	Cystine & Tyrosin	Tyrosine
11:00A	0	2.3222	2.4314	2.4772	2.4772	2.8129	2.7634
2:00P	3	2.7782	2.4771	3.6021	2.9031	3.3979	2.9031
5:00P	6	2.6021	2.6021	5.1614	3.1139	3.0792	2.6990
8:00P	9	lost	lost	8.0170	6.4579	lost	lost
11:00P	12	2.6990	2.9031	8.0000	8.1761	3.4472	3.0792
11:00A	24			11.1761	11.0000	6.3979	4.9138
11:00P	36	7.6021	Less than 4.0000	11.7226	11.2672	10.0000	9.3010
11:00A	48	9.5416	less than 4.0000	11.0792	11.6990	11.9031	9.6990

TABLE 27

RESULTS SHOWING RATE OF GROWTH WITH GLYCINE ALONE AND WITH GLYCINE AND CYSTINE

Time	Hrs incubation	B.Coli F50		B.aerogenes F22		B.paratyphosus B	
		Glycine & Cystine	Glycine	Glycine & Cystine	Glycine	Glycine & Cystine	Glycine
10:40A	0	4.5682	4.6532	4.5442	4.6532	4.5185	4.5441
1:40P	3	4.9085	4.8865	4.7634	4.8324	4.5315	4.3979
4:40P	6	lost	lost	lost	lost	4.6532	4.5441
7:40P	9	7.2430	7.3979		7.7782		
10:40P	12	9.4771	9.3979	9.3010	8.9542	6.2355	4.3010
10:40A	24	11.1761	11.4771	11.3010	11.6990	6.3979	6.4771
10:40P	36	11.7782	11.9031	11.4771	11.9542	6.6902	6.6990

TABLE 28

RESULTS SHOWING RATE OF GROWTH WITH PHENYLALANINE ALONE AND WITH PHENYLALANINE AND CYSTINE

Time	Hrs Incubation	B.coli F 50		B.aerogenes F23		B.paratyphosus B	
		Phenylalanine & Cystine	Phenylalanine	Phenylalanine & Cystine	Phenylalanine	Phenylalanine & Cystine	Phenylalanine
11:30A	0	2.4314	2.4772	2.4314	2.3979	2.8062	2.8195
2:30P	3	2.8451	2.6021	3.0000	3.2041	2.9031	3.1139
5:30P	6	2.6021	3.0414	4.4771	4.3979	3.7559	3.1139
8:30P	9	lost	4.6021	lost	6.0492	4.0000	3.7782
11:30P	12	3.1139	4.6128	8.1761	8.0000	6.1206	4.6990
11:30A	24	2.7782	5.3010	9.7634	10.8633	lost	6.6990
11:30P	36	4.9542	7.6990	9.6990	10.9031	9.4771	9.5441
11:30A	48	less than 4.0000	9.2430	10.1761	11.3010	lost	8.2900

TABLE 29

RESULTS SHOWING RATE OF GROWTH WITH ALANINE ALONE AND WITH ALANINE AND CYSTINE

Time	Hrs incubation	B.coli F 50		B.aerogenes F23		B.paratyphosus B	
		Alanine & Cystine	Alanine	Alanine & Cystine	Alanine	Alanine & Cystine	Alanine
10:00A	0	4.5441	4.6021	4.5441	4.6990	4.5181	4.5441
1:00P	3	4.7482	4.6812	4.6335	4.7993	4.6435	4.4771
4:00P	6	5.3424	5.0000	5.2041	5.1761	4.5682	4.6232
7:00P	9	6.7404	6.7324	7.2041	6.9031		5.7482
10:00P	12	7.4771	7.3522	9.2601	8.2122	6.4771	
10:00A	24	11.6021	11.4771	11.3010	11.4771	6.3979	6.3444
10:00P	36	11.7782	11.9542	11.6990	11.8451	8.2068	8.2672

TABLE 30

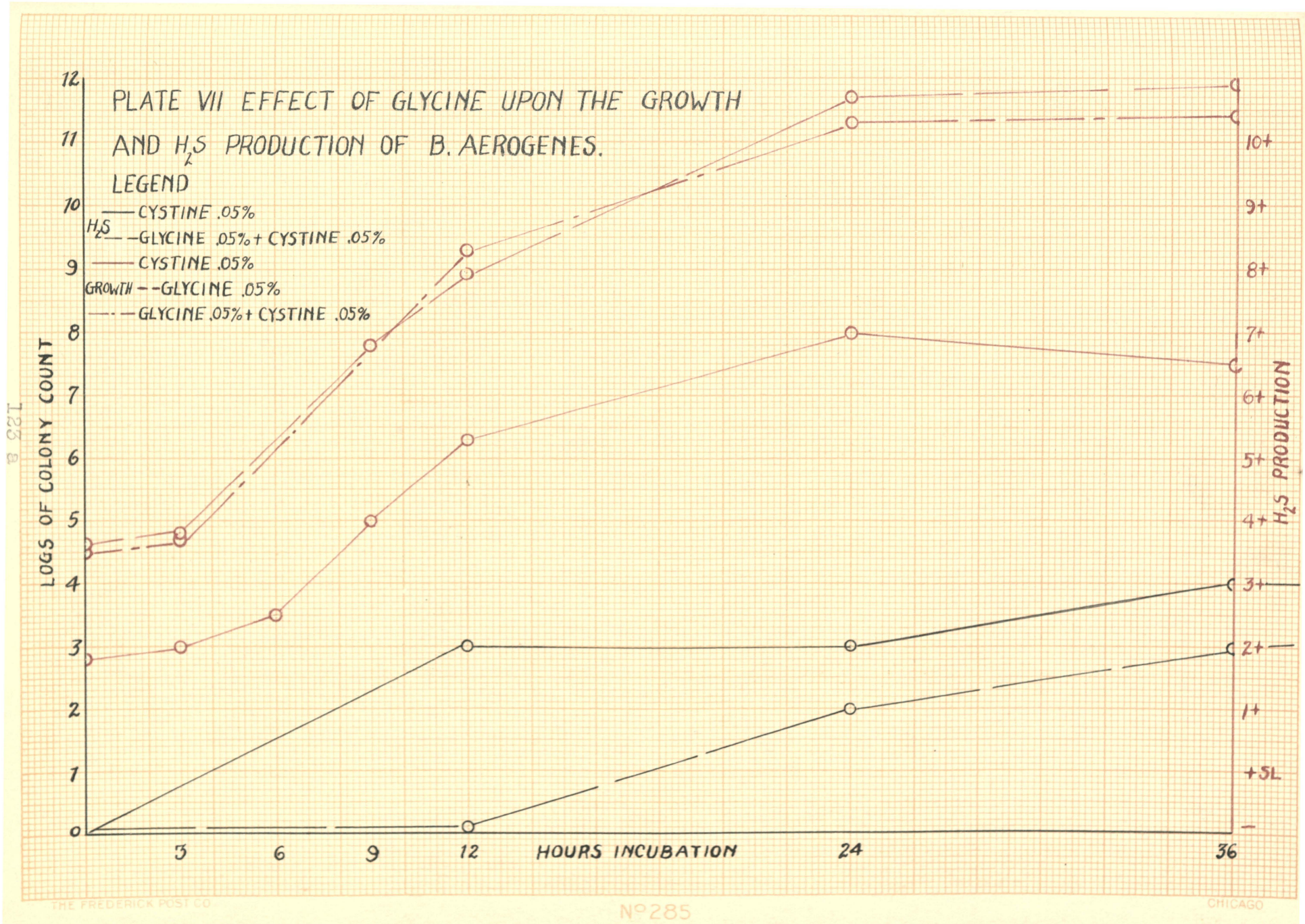
SHOWING GROWTH IN BASE MEDIUM WITHOUT ADDITION OF AMINO ACIDS

Time	Hours Incub.	B. coli F50	B. aerogenes F 23	B. para-typhosus B.
10:00A	0	4.5441	4.6532	4.4150
1:00P	3	4.7782	4.8325	4.6628
4:00P	6	4.3010	4.6021	4.0212
7:00P	9	5.5185	6.8751	3.4914
10:00P	12	5.9912	8.1761	3.1461
10:00A	24	6.3979	9.0000	2.8451
10:00P	36	5.7782	8.7482	Less than 2.0
10:00A	48			

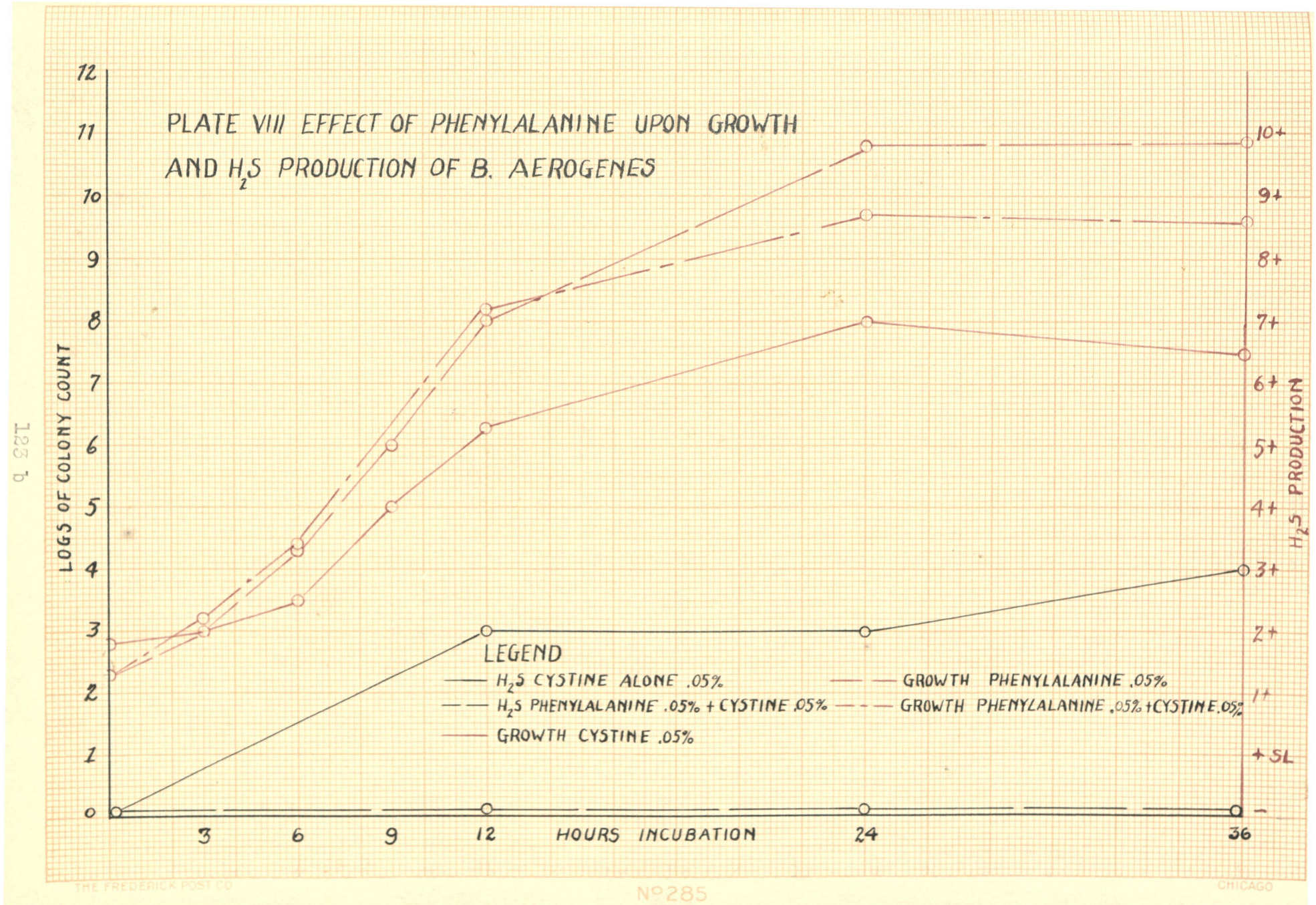
cystine medium may be considered to be an example of one type of reaction (Plate 7). The growth in the media containing glycine is much more luxuriant, and the growth curve much steeper than in the medium containing cystine alone. It is evident that B. aerogenes finds glycine more available than it does cystine. That the cystine is spared when glycine is present is shown by the H_2S production curves on the same plate. When glycine is present, no H_2S is released from the cystine until after 12 hours and only a 2 / reaction after 36 hours while with cystine alone in the medium H_2S production is very evident (2/) in 12 hours.

Plates 8/10 show much the same reaction. This is particularly evident in plate 8, in the case of aerogenes and phenylalanine. This amino acid was found to give a much steeper growth curve with B. aerogenes than cystine while no H_2S production was evident until after 5 days.

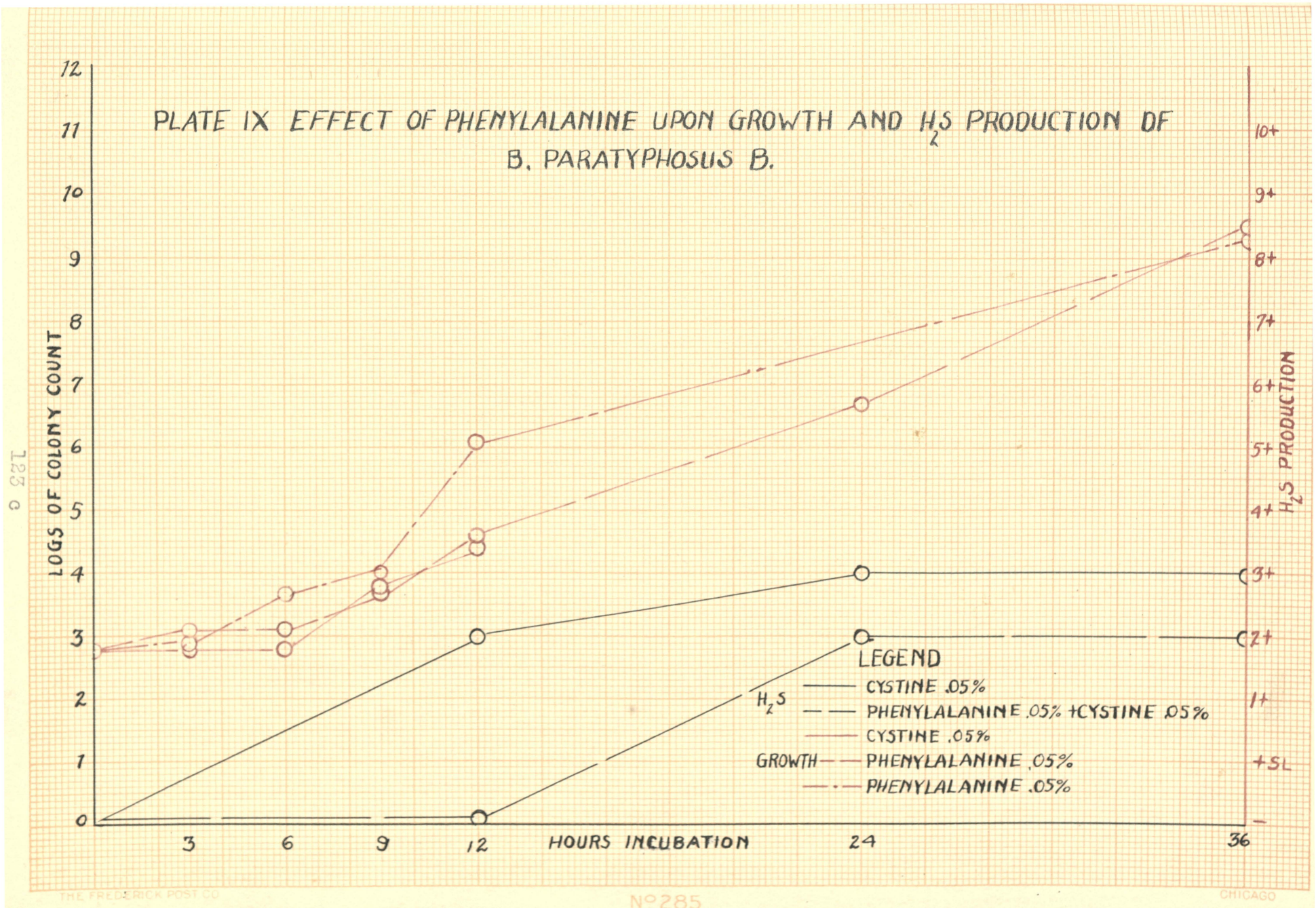
This reaction is not nearly so marked in the case of B. paratyphosus B, shown in Plate 9. The delay in H_2S production here is probably due to the fact that the organism grows poorly in most of the amino acid media and the small amount of H_2S produced is the result of lack of utilization of amino acid rather than a discrimination against cystine. The growth curves show



123 a



123 b

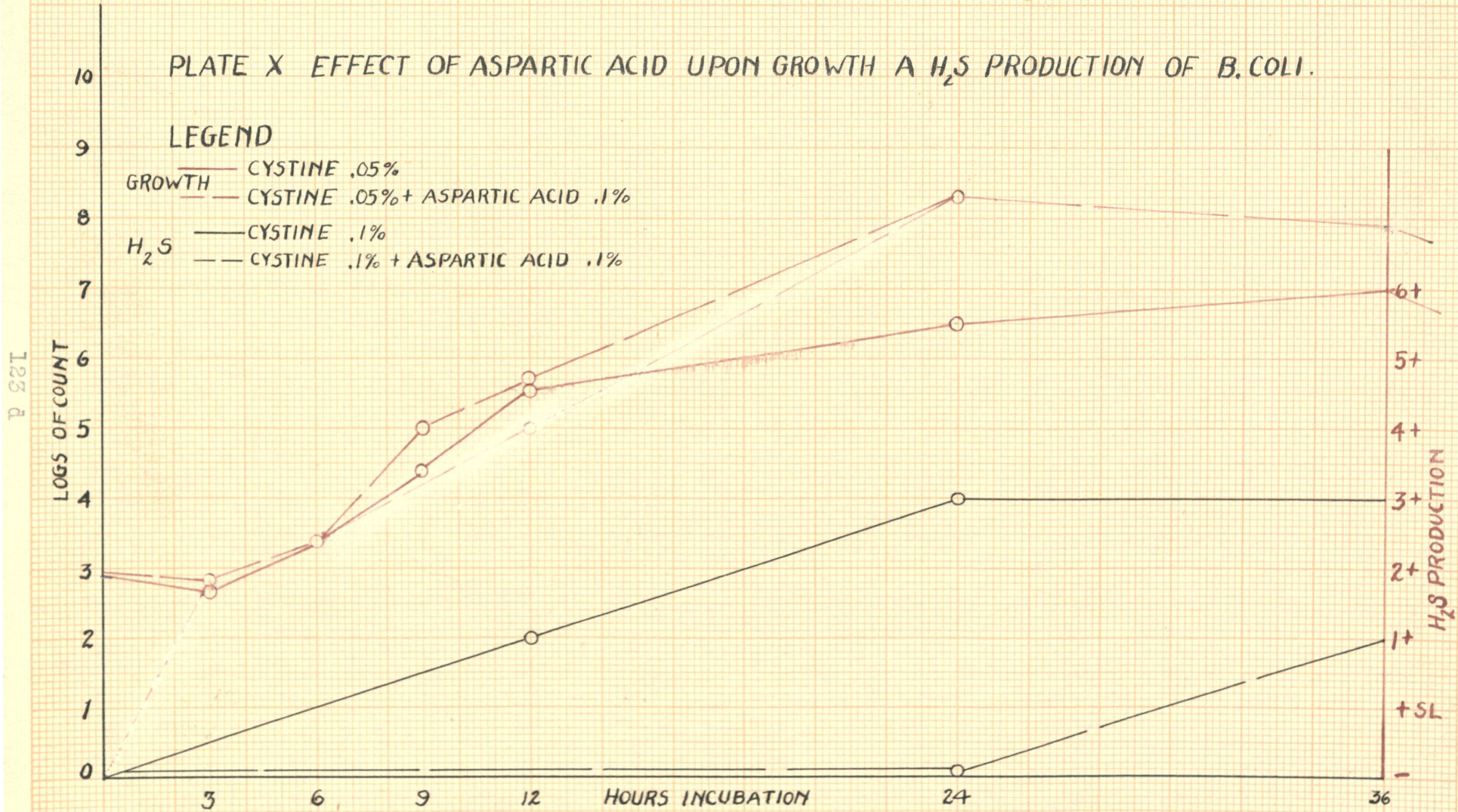


123 c

THE FREDERICK POST CO

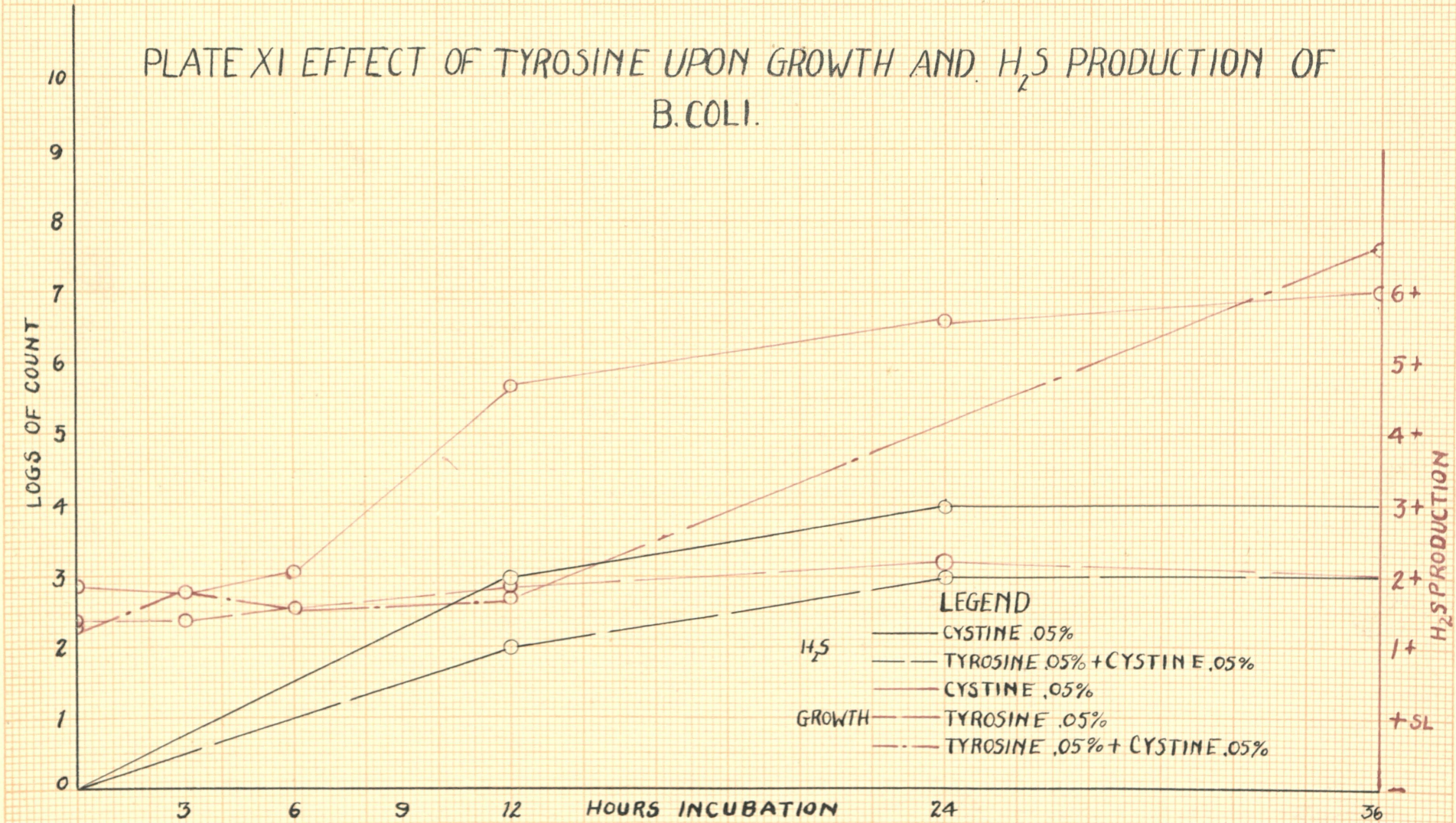
Nº285

CHICAGO



123 d

PLATE XI EFFECT OF TYROSINE UPON GROWTH AND H_2S PRODUCTION OF B. COLI.



that the rate and extent of multiplication varies but little with phenylalanine or cystine in the medium. It will be remembered that B. paratyphosus B. was one of the organisms that usually produced H_2S abundantly from peptone in 24 hours.

The reaction of B. coli with tyrosine shown in plate 11 was evidently of a different type. As shown by the growth curves practically no multiplication took place when tyrosine was alone as a source of nitrogen. Not only did tyrosine fail to furnish proper nourishment for B. coli but it also seemed to interfere in some way with the utilization of cystine as shown in a comparison of the cystine curve with that of the cystine + tyrosine. The slight decrease in H_2S production when tyrosine was present was probably caused by this interference with growth. This interference might be due to phenol groups split off from the tyrosine. If this is true, then B. aerogenes must break down the tyrosine molecule in a different manner since this organism is not apparently inhibited. This possibility might be investigated by quantitatively determining the phenol groups split off. Koser (1919) using visible turbidity in the culture tube as an index of utilization, found no development by B. coli in tyrosine media.

The results given above would seem to show a

marked variation in the availability of amino acids for bacterial use. Also that this variation may be demonstrated by comparing the amino acid in question to cystine, and using H_2S production as an index of the utilization of the cystine.

Explaining the delayed H_2S productionffrom peptone on this basis, it is seen that the delay is caused by the organisms finding, in the peptone, amino acids more readily utilized than cystine. The cystine is therefore spared, at the expense of these amino acids.

NITROGEN DETERMINATIONS

Early in the course of the present work it was thought that determination of the nitrogen contained in the different fractions of cultures grown in the 2% Difco peptone medium, might throw some light upon the type of reaction produced by B. coli and B. aerogenes.

These fractions as suggested by Kendall (1922) consist of (1), the total nitrogen, (2) non-protein nitrogen, (3) protein nitrogen, (4) polypeptide nitrogen, (5) amino acid nitrogen, (6) ammonia nitrogen.

METHODS

The methods used for these determinations were largely those used by Kendall in his many studies.

Total nitrogen was determined by the Folin and Farmer micro method (1912) except that the digestion mixture was similar to that used for the non-protein nitrogen. It consisted of equal parts of the phosphoric sulphuric acid mixture and of concentrated sulphuric acid. Direct nesslerization was used, employing the Nessler's solution as modified by Folin and Wu (1919).

The non-protein nitrogen was determined by the Folin and Wu method.

The protein nitrogen was obtained by calculating the difference between the total nitrogen and the

non-protein nitrogen.

The amino nitrogen was determined by the Formol tritration method of Henriques and Sörensen (1910), correction being made for ammonia.

The "polypeptid"hnitrogen, so-called by Kendall, was considered to be the non-protein nitrogen not accounted for by the amino nitrogen and ammonia. It was determined by calculation.

The ammonia nitrogen was determined by the Folin and McCallum (1912) modification of the Folin aeration method.

RESULTS.

A number of each determinations were made using 7 day cultures of the organisms, B. aerogenes and B. coli.

A protocol of one of such series of determinations is given in Table #31.

The results show a slight reduction of the protein nitrogen. This seems to have been broken down to non-protein nitrogen of the "polypeptid" type, since this showed some increase. The amino N_2 is decreased by both organisms, and the coincident increase in ammonia N_2 would indicate that some of the amino acids had been deamidized, with the production of ammonia. These results are similar to those produced by other members of the intestinal group of bacteria as shown by Kendall

TABLE 31

RESULTS OF FRACTIONAL NITROGEN DETERMINATIONS UPON 2% DIFCO PEPTONE 10% GELATINE CULTURE. INCUBATION, 7 DAYS AT 37°C. EXPRESSED IN MILLIMETERS NITROGEN PER 100 cc MEDIUM

	Control		B. aerogenes		B. coli	
	Mgr N_2	% Total N_2	Mgr N_2	% Total N_2	Mgr N_2	% Total N_2
Total N_2	1177.77		1797.75		1739.13	
Protein N_2	1519.71	85.48	1471.22	81.83	1412.60	81.22
Non Protein N_2	258.06	14.52	326.53	18.17	326.53	18.78
Polypeptid N_2	172.96	9.72	240.82	13.40	246.97	14.20
Amino N_2	78.03	4.38	63.81	3.55	70.39	4.04
Ammonia N_2	7.07	0.39	11.90	1.22	9.17	0.54

(1922). While there were some quantitative differences in nitrogen fractions produced by growth of the two organisms, it was believed they were too small to be significant. This line of investigation, therefore, was not pursued further.

GENERAL DISCUSSION

A rather thorough discussion has already been given immediately following the results obtained in each phase of the problem.

It, therefore, seemed desirable in this General Discussion to call attention only to the correlations which may be drawn from the results obtained in the various studies.

Evidence has been presented in the foregoing paper to show that there is a fundamental difference in the protein metabolism of B. coli and B. aerogenes. This evidence is based on the following points; (1) Differences in the CO_2/H_2 ratio of the gas produced from Difco peptone; (2) Differences in the utilization of certain amino acids; and (3) Differences in the degree of resistence to brilliant green.

These differences would seem to be fundamental in nature since they are based, (1) upon the end products obtained from the decomposition of what appears to be the same substance in the peptone and would indicate a difference in the manner of breaking down that substance, and (2) upon the fact that these organisms cannot utilize the same, simple nitrogen compounds.

This lack of utilization would indicate a difference in the enzymes produced by the two organisms. (3) The difference in the resistence of these organisms to a dye such as brilliant green, might be taken to indicate a difference in the character of the protoplasm of the cell, since it is believed that the selective action of such dyes depends to a great extent upon difference in the penetration of the dye and its chemical reaction with the proteins of the cell.

Such observations upon the difference in the proteolytic properties of the two organisms are of significance since classification within this group in the past has been based almost entirely upon differences in carbohydrate metabolism.

A consideration of the normal habitat of these organisms would be of interest since it is upon such a basis that the interpretation of their presence in water and soil, etc., is made. B. coli is found in greater numbers in the intestinal tract, where it has been able to adapt itself and has become to a certain extent parasitic. B. aerogenes, on the other hand, is found in greater numbers in the soil and on grains, and is more saprophytic in character.

According to Kligler (1917), and Winslow, Kligler and Rothberg (1919 in their studies on the

relationship of bacteria, B. aerogenes is considered to be a rather primitive type in the evolutionary development of the bacterial species. From which type there has developed the various members of the colon-typhoid group of bacteria. If this is true, then B. coli might be considered to be a type of B. aerogenes which has adapted itself to a more or less parasitic existence in the intestinal tract and whose characteristics have accordingly been changed to suit this envoirment.

It has been seen that the normal environment of the two organisms is considerably different. It is of interest to consider what the fate of one type would be if placed in the environment of the other. Certainly, B. aerogenes must have ample opportunity to enter the intestinal tract. The fact that this organism is found in very small numbers in the feces would indicate that it does not find suitable conditions in the lower intestine for its development. These organisms then, must either be destroyed in their passage through the intestines or their characteristics be so changed that they no longer resemble the typical B. aerogenes If this evolution toward the B. coli type occurs, there would be found in the feces, organisms with characteristics varying from the typical B. aerogenes through many steps to the typical B. coli.

These intermediate types have been demonstrated from feces by the use of brilliant green dextrose broth as an enrichment medium as shown by results given in Part I. They comprised a considerable percent of the organisms isolated from normal stools by this means.

Organisms showing identical characteristics are found quite commonly in water. These are usually referred to as "soil coli" and are interpreted as indicating remote pollution. That such organisms might be present in water due to direct fecal pollution is shown by results obtained in this study. The relative importance of such pollution can be determined only by a more comprehensive study of these atypical strains. The methods of study used in this work, namely, the gas production from peptone, and the relative resistence to brilliant green has been shown to be particularily adapted for such a study.

The above discussion serves to emphasize the fact that although the colon-aerogenes group has been one of the most thoroughly studied, of all the groups of bacteria, our knowledge is still very incomplete.

SUMMARY

Part I

Two hundred and sixteen strains of the colon-aerogenes group were isolated from the following sources: feces, 109 strains; soil (stored), 6 strains; flour sacks, 6 strains; milk and cream, 14 strains; oysters and meat, 45 strains,; and surface water, 37 strains.

Preliminary enrichment in brilliant green broth was applied successfully to the separation and isolation of the various colon-aerogenes types found in feces.

These organisms were studied as to their cultural characteristics. The following tests were used: the fermentation of dextrose, lactose, saccharose, dulcitol and adonitol; the methyl red test; the Voges and Proskauer reaction; production of indol; production of H_2S; utilization of sodium citrate and uric acid; the liquefaction of gelatine and the production of gas from a 2% Difco peptone gelatine medium. Correlations were made between the different cultural characteristics and between these and the source of the organisms.

Part II

The metabolic studies consisted of a study of the conditions under which gas was produced from Difco peptone, with a comparative study of a number of other commercial peptones such as Witte's, Park-Davis, and Armour's.

Analyses of the gas produced from Difco peptone by B. aerogenes and B. coli were made and the CO_2/H_2 ratio determined. The micro method of Brown was successfully applied to the study of this problem.

A comparative study of the H_2S production by a number of organisms was made upon Witte's peptone, Difco peptone, cystine, and sodium thiosulphate.

A method using $FeSO_4$ in a solid medium was devised as a qualitative test and for roughly measuring the H_2S produced.

By the use of the $FeSO_4$ agar method, the effect of various amino acids upon H_2S production from cystine was studied for a number of organisms.

The cause of the delayed production of H_2S when certain other amino acids were present, was found by a study of comparative growth rates upon these amino acids in a synthetic medium.

The selective utilization of amino acids by bacteria was demonstrated by means of growth rates, and by using H_2S production from cystine as an indicator.

In this way the following amino acids were studied: glycine, alanine, leucine, tyrosine aspartic acid, phenylalanine and the extractive creatinine.

A study of the nitrogen metabolism of B. coli and B. aerogenes was made in Difco peptone gelatine, and the nitrogen changes occurring in the various fractions were considered. The methods used were largely those applied by Kendall.

The results obtained from the studies indicated in the above Summary would seem to justify the following conclusions.

C O N C L U S I O N S

1. Two percent peptone gelatine medium may be used as a means of demonstrating gas production from peptone. This medium serves a double purpose in that it also can be used for noting gelatine liquefaction.
2. The gas ratio, CO_2/H_2, as obtained from Difco peptone by the action of B. coli and B. aerogenes is characteristic for each type of organism. This may be used as a criterion in differentiating these organisms.
3. The gas produced from Difco peptone by B. aerogenes and B. coli has its origin from the amino acids present and is not produced from a carbohydrate radicle.
4. B. aerogenes produces visible gas from Difco and from Park-Davis peptones but not from Witte's or from Armour's peptones.
5. The vaseline tube and syringe method for micro gas analysis may be successfully used in the routine study of gas production from peptones.
6. B. coli and B. aerogenes both produce H_2S from pept This reaction is a delayed one, only 2 strai

B. coli out of 109 studied, and none of the B. aerogenes produced H_2S within 48 hours. All of the strains tried were positive after 5 to 7 days.

7. B. coli and B. aerogenes produce H_2S from cystine in 12 to 24 hours, when cystine is alone as a source of nitrogen. None of the strains studied produced H_2S from sodium thiosulphate.

8. The production of H_2S from cystine by bacteria is dependent upon its utilization by the organisms. This is shown by the correlation of growth rates and H_2S production and by the fact that the reductases produced by the organisms studied are not in themselves sufficient to release H_2S from cystine.

9. Hydrogen sulphide production as shown by the blackening of $FeSO_4$ agar may be used to measure cystine utilization.

10. Ferrous sulphate agar, as prepared by the author, is superior to lead acetate agar, as an indicator of H_2S production.

11. Bacteria show a selective preference for certain amino acids. This was shown by a comparison of growth rates upon amino acids in a synthetic medium, and by a comparison of their utilization

to that of cystine, using H_2S production as an index of cystine utilization.

12. Certain amino acids may selectively inhibit the the growth of bacteria, as shown in the present study of the inhibiting effect of tyrosine on the growth of B. coli, while it has practically no effect on that of B. aerogenes and B. paratyphosus B.

13. B. coli may exist in the soil for a period of 8 years, under the conditions found in a sealed mason fruit jar.

14. Brilliant green dextrose broth is of great value in the isolation and study of B. aerogenes and of certain atypical B. coli strains found in feces.

15. B. aerogenes strains which fail to ferment adonitol are found in the feces. Six of the fourteen strains isolated from feces by brilliant green broth were of this type. The possibility of the effect of the brilliant green upon adonitol fermentation was ruled out.

16. Atypical B. coli strains may be isolated from feces in comparatively large numbers, by the use of brilliant green enrichment. These strains are

similar in every way to those found in water.

17. Atypical B. coli strains found in feces may be considered to be organisms which are in the process of an evolutional change from the primitive B. aerogenes type to the more parasitic B. coli type.

18. Direct fecal pollution must be considered as a possible source of the atypical B. coli types found in water.

BIBLIOGRAPHY

Anderson, Belle G., (1924), Gaseous Metabolism of Some Anaerobic Bacteria. Jour.Inf. Dis. 35, #3, 213-243 and 244-281.

Ayers, S.H., Rupp, Philip, and Mudge, Conriland, (1921), The Production of Ammonia and CO_2 by Streptococci. Jour.Inf.Dis. 29, 235-260.

Ayers, S.H., and Rupp, Henry, (1918), Simultaneous Acid and Alkaline Bacterial Fermentations from Dextrose and the Salts of Organic Acids Respectively. Jour. Inf. Dis. 23, 188-216.

Ayers, S.H., and Rupp, Philip (1918), A Synthetic Medium for the Direct Enumeration of Organisms of the Colon Aerogenes Group, Jour. Bact. 3, 433-436.

Ayers, S.H.,& Mudge, C.S. (1922), The Relation of Vitamines to the Growth of a Streptococcus. Jour. Bact. 7, 449-463.

Ayers, S.H. & Rupp, Philip, (1920), Extracts of Pure Dry Yeast for Culture Media. Jour.Bact. 5, 89-98.

Bailey, Sadie F., (1925) "Hormone" Mediums. Jour. Inf.Dis. 36, 340-342.

Bainbridge, F.A., (1911), The Action of Certain Bacteria on Proteins. Jour. Hygiene 11, 341.

Benton, Anna G. (1919), Studies in the Nitrogen Metabolism of Bacteria. Jour.Inf. Dis. 25, #3, 231-247.

Bergeys' Manual of Determinative Bacteriology, (1923), Williams & Wilkins Company, Baltimore.

Berthelot, M. Albert, (1917), Recherches sur la production du phenol par les microbes. Compt. rend. Acad. de Sc. Par. 164, 196-199.

Broadhurst, Jean, (1912), A Biometrical Study of Milk Streptococci. Jour.Inf.Dis. 10, 272-284.

Bondo, Erik, (1922) Influence des hydrates de carbone sur la formation de l'indol dans les cultures de coli. Compt. rend. Soc. de Biol. 87, 472.

Brown, J. Howard, (1922) The Vaseline Tube and Syringe Method of Micro Gas Analysis of Bacterial Cultures. Jour. Expr. Med., 35, 667-684.

Brown, H.C., Duncan, J.T., and Henry, T.A. (1924), The Fermentation of salts of Organic Acids as an aid to the Differentiation of Bacterial Types. Jour.Hygiene, 23, 1-22.

Browne,Wm.W., (1914), The Production of Acid by the B.coli Group. Jour.Inf. Dis. 15, 580-604.

Browne, Wm. W., (1916), A Comparison of the Acid Production of the B. Coli Group Isolated from Various Sources. Am.Jour.Pub.Health, 6, 39-48.

Burrows, M.T., and Neymann, C.A., (1917), Studies on the Metabolism of Cells in Vitro. 1. The Toxicity of the A-Amino Acids for Embryonic Chicken Cells. Jour. Exp. Med. 25, 93-108.

Burton, L.V., and Rettger, L.F. (1917) Correlations in the Colon Aerogenes Group. Jour. Inf. Dis. 21, 162-195.

Bushnell, L.D., (1922) Quantitative Determinations of Some of the Biochemical Changes Produced by a Saprophytic Anaerobe. Jour. Bact. 7, #4, 373-403.

Castellani, A, and Chambers, A.J., (1920), Sur la classification de certains groups de bacilles afrobes de l'intestin humain. Ann. de l'Inst. Pasteur, 34, 600-621.

Churchman, John W., (1913), The Selective Bactericidal Action of Stains Closely Allied to Gentian Violet. Jour. Exp.Med., 17, 373-378, plates 62-65.

Churchman, John W., (1912). The Selective Bactericidal Action of Gentian Violet, 221-247, Plates 21-31.

Churchman, J.W., and Michael, W.Howard, Selective Action of Gentian Violet on Closely Related Bacterial Strains. Jour. Exp.Med. 16, 221-and 822-830.Plates 85.

Chen, Chong Chen, & Rettger, L.F.(1920), A correlation Study of the Colon-aerogenes Group of Bacteria, with Special Reference to the Organisms Occuring in the Soil. Jour.Bact. 5, 253-298.

Clark, Wm.m. & Lubs,H.A.(1917), Improved Chemical Methods for Differentiating Bacteria of the Coli-Aerogenes Family. Jour.Biol.Chem.30, 209-234.

Clark, Wm.M. and Lubs, H.A., (1915), TheDifferentiation of Bacteria of the Colon Aerogenes Family by the Use of Indicators. Jour. Inf. Dis. 17, 160-173

Coulter, Calvin B., (1917) The Biological Identity of the Friedlander Bacillus. Jr. Expr. Med. 26, 763-768.

Dalimer, R.,et Laucereaux, Edg. (1913) (a) Le Milieu de l'acides amines complets pour les microorganismes. Compt. Rend. Soc. de Biol. 74, 1081-1082.

Dalimer, R.,et Laucereaux, Edg. (1913) (b) Le Products de la Proteolyse naturelle et totale comme milieux de culture pour les micro-organismes, Arch. de. Med. Exp. et d'Anat pathol. 25, 449. Cited by Frouin, Albert, Compt. Rend. Soc. de Biol. 74, 1038-1040.

Darling, Chester A., (1910). Characteristics of theColon Type of Bacteria Found in Feces. Am.Jour.Pub.Health 9,#11, 844-845.

Davis, Lewis, & Ferry,N.S., (1919). Studies on Diphtheria Toxin II, The Role of the Animo Acids in the Metabolism of the Bacterium Diphtheria. Jour.Bact. 4, 217-241.

DeBord, Geo., (1923) Certain Phases of Nitrogen Metabolism in Bacterial Cultures. Jour. Bact. 8, 7-45.

de Megalhaes, A., (1924). 1290 Souches de coli bacilles de l' intestine de l'Homme. Comp.Rend.Soc. de Biol.91,234-236.

Diehl, H.S., (1919) The Specificity of Bacterial Proteolytic Enzymes and Their Formation.Jour.Inf.Dis.24,347-361.

Dolt, M.L., (1908) Simple Synthetic Media for the Growth of B.coli and for its Isolation from Water. Jour.Inf.Dis. 5, 616-626.

Doryland, C.J.T.(1916) Preliminary Report on Synthetic Media. Jour.Bact. 1, 135-152.

Downs, Cora M. (1924) Antigenic and Metabolic Studies of Bacillus Typhosus. Doctorate Thesis,University of Kansas.

Dozier, C.C., Wagner, E., Meyer, K.F., (1924) Effect of Glucose on Biochemical Activities, including Growth andToxin Production of B. botulinus. Jour.Inf.Dis. 24, 85-102.

Dudgeon, L.S., Wordley, E., Bawtree, F., (1921). On Bacillus Coli Infections of the Urinary Tract, Especially in Relation to Haemolytic Organisms. Jour. Hyg. 20, #2, 137-164.

Durham, Herbert E., (1898) A Simple Method for Demonstrating the Production of Gas by Bacteria. Brit. Med. Jour. 1,1387.

Durham, Herbert E., (1900), Some Theoretical Considerations upon the Natura of Agglutinins together with Further Observations upon B. tyohi abdomnalis, B. enteritidis, B.coli communis B.lactis aerogenes and some other Bacilli of Allied Character. Jour. Expr. Med. 5, 353-388.

Eldridge, E.E., and Rogers,L.A., (1914) The Bacteriology of Cheese of the EmmenthalerType. Cent. f. Bakt. II, 40, 5-20.

Fellers,-Shostrom-Clark, (1924) Hydrogen Sulphide Determination in Bacteria Cultures. (technic). Jour.Bact.9, 235-249

Fitzgerald, J.G., (1914) A Biometrical Study of the Mucosus Capsulatus Group. Jour.Inf. Dis. 14, 268-278.

Folin, Otto and McCallum, A.B., (1912). On the Determination of Ammonia in Urine. Jour.Bio-chem. 11, 523-525.

Folin, Otto and Farmer, A.B., (1912). A new Method for the Determination of Total Nitrogen in Urine. Jour. Bio-chem. 11, 493-501.

Folin, Otto, and Wu, Hsien., (1919). A system of Blood Analysis. Jour. Bio-chem. 38, 81.

Frances, Edward (1923). The Amino Acid Cysteine in the Cultivation of Bact. tularense. Pub. Health Reports, Washington. No. 38, 1396-1404.

Fränkel, C., (1894). Bietrage zur Kenntuis des Bakterienwachstumes auf eiweissfrien Nährböden. Hygienische Rundshau 4, 769, from Cent. für Bakt. Ref.(1895) abt. 2, Bd.I. p 252-253.

Frieber, Walther, (1913). Die bedeutung der gasabsorbtion in der bacteriologie. Cent. f. Bact. Abt. I, Orig. 69,437-464.

Galimard, J., and Lacomme, L. (1907). Les acides amines comme nouveaux milieux de culture chimiquement definis pour l'etude des microbes. Jour. de Physiol. et. Path. Gen. 9, 481-486.

Gernez, C.H., and Duthoit, A. (1923) Les reactions biologiques dans la classification des Bacterium coli. Comp. rend. Soc. de Biol. Par. 88, 527-529.

Gordon, J., (1924) Cystine in Bacterial Metabolism. Jour. Path. and Bact. 27, 123.

Gordon, M.H., (1917), On the Nitrogenous Food Requirements of some of the Commoner Pathogenic Bacteria. Jour. Roy. Army Med. Corps. 28, 371-376.

Greenfield, Myrtle, (1916). The Presence of Soil and Fecal Strains or Organisms of the Colon-aerogenes in the Waters of Kansas. Jour. Inf.Dis. 19, 647-651.

Grimpert, L. et Legros G., (1900) De l'identite du bacille lactique aerogene et du pneumo-bacille de Friedlander. Annales de l'Inst. Past. 14, 479-486.

Hall, I.C., Ellefsen, H.J., (1919), Further Studies on Gentian Violet as a means of Eliminating Spurious Presumptive Tests for B. coli in Water. Jour. Am. Water Works Assn. 6, 67-77.

Hall, Ivan C., (1921) The Production of Tyrosine by a Putrifactive Anaerobe. Abstrs. Bact. Vol. 6,p 6,#15.

Harden, A., (1906). On Voges and Proskauer's Reaction for Certain Bacteria. Proc. Roy. Soc. London,B. 77, 424-425.

Harden, A; and Walpole, G.B., (1906) Chemical Action of Bacillus Lactis Aerogenes on Glucose and Mannitol: Production of 2:3 butyleneglycol and acetylmethylcarbonol. Proc. Roy.Soc. London, B. 77,399-405.

Herter, C.A., and Ten Broeck, Carl., (1911) A Biochemical Study of Proteus Vulgaris, Hauser. Jour.Bio-Chem. 9,491-511.

Herter, C.A., (1907) Infections of the Digestive Tract. P229. The MacMillan Co., New York.

Henriques, V., and Sörensen, S.P.L., (1910). Ztschr. für Physiol. Chem. 64, 120-143. Uber die quantitative bestimmung der aminosäuren polypeptide und der hippursäure im harne durch formoltitration.

Holman, W.L.,(1916). Classification of Streptococci. Jour. Med. Res. 34, 377-443.

Holt-Harris, J.E. and Teague, Oscar, (1916). A New Medium for the Isolation of B.typhosus from Stools. Jour. Inf. Dis. 18, 596-600.

Homer, Annie, (1916) A Suggestion as to the Cause of the Lessened Production of Indol in Media Containing Glucose. Jour. Hyg. 15, 401.

Hulton, Florence, (1916). On Voges and Proskauer's Reaction for Certain Bacteria. Proc. Roy. Soc. London, B.77, 424-425.

Hulton-Frankel, Florence, Barber, Helene, and Pyle, Eleanor, (1919). Studies on Synthetic Mediums.
(a) I. Study of the characteristics of some bacteria on a simple synthetic medium. Jour. Inf.Dis. 24, 9-18.
(b) II. Sugar fermentations in synthetic mediums. Ibid. 17-18.
(c) III. Some animal experiments with organisms grown on synthetic medium. ibid. 19-21.

Huntoon, F.M., (1918) "Hormone" medium, a Simple Medium Employable as a Substitute for Serum Medium. Jour. Inf. Dis. 23, 169-182.

Janney, N.W., (1915) The Metabolic Relationship of the Proteins to Glucose. Jour. Bio-chem. 20, 321.

Jensen, Orla, (1909), Die Hauptlinien des natürlichen Bakteriensystems. Centr. für Bakt. II 22, 305-346

Johnson, B.R., (1916) Coli-like Organisms of the Soil. Jour.Bact. 1, 96.

Jones, H.M., (1916) The Protein Sparing Action of Utilizable Carbohydrates in Cultures of Certain Sugar Fermenting Organisms. Jour.Inf.Dis. 19, 33-45.

Jones, H.M., (1920. Effect of Carbohydrate on Amino Acid Utilization of Certain Bacteria. Jour.Inf.Dis. 27,169.

Jordan, E.O., and Victorson, Ruth, (1917) Differentiation of the Paratyphoid Enteritidis Group. Jour. Inf.Dis. 21, 554-555.

Jordan, E.O., (1899) Bacillus pyocyaneus and its Pigments. Jour.Exp.Med. 4, 627-647.

Kendall, A.I., and Farmer, Chester J., (1912) Studies in Bacterial Metabolism, I. Jour. Bio-chem, 12,12.

Kendall, A.I., (1922) The Significance and Quantitative measurement of the nitrogenous metabolism of bacteria. Studies LVII. Jour.Inf.Dis. 30, 211-224.

Kendall, A.I., and Walker, A.W., (1915) Observations on the Proteolytic Enzyme of B. proteins. Jour.Inf.Dis. 17,442-453.

Kendall, A.I., and Mitzuteru Ishikawa, (1925), Effect of Insulin on Bacterial Metabolism. Studies in Bacterial Metabolism LXXV. Jour.Inf.Dis. 37, 337-339.

Kendall, A.I., (1925) Effect of Insulin on Cultures of B. bulgarisus and B. acidophilus. Studies on Bacterial Metabolism LXXIV. Jour.Inf. Dis. 37, 333-336.

Kendall, A.I., (1925) Non-glucose fermenting Bacteria and Insulin. Studies in Bacterial Metabolism LXXIII. Jour. Inf.Dis. 37, 329-332.

Kendall, A.I., and Hamer, R.C., (1922) The Nitrogenous Metabolism of B. dysenteriae Shiga, B. typhosus, B.apartyphosus A and B. paratyphosus B. Jour. Inf.Dis. 30, 225-238.

Kendall, A.I., Day, A.A., and Walker, A.W. (1913), Studies in Bacterial Metabolism XIII to XXX. Jour.Amer. Chem.Soc. 35, 1201-1249.

Keyes, The Gas Production of B. coli. Jour. Med. Res. 21, #1, 69.

Kligler, I.J., (1916) Some Regulating Factors in Bacterial Metabolism, Jour. Bact. 1, 663-671.

Kligler, I.J. (1919) Yeast Antolysate as a Culture Medium for Bacteria. Jour.Bact. 4, 183-188.

Kligler, I.J. (1914) Classification of Colon Group. Jour. Inf. Dis. 15, 187-204.

Kligler, I.J., (1917) The Evolution and Relationships of the Great Groups of Bacteria. Jour.Bact. 2, 165-176.

Koser, S.A., (1924) Differential Tests for Colon-Aerogenes Group in Relation to Sanitary Quality of Water. Jour.Inf.Dis. 35, 14-22.

Kligler, I.J., (1917) A Simple Medium for the Differentiation of Members of Typhoid, Paratyphoid Groups. Jour. Am. Pub. Health. 7, #12, 1042-1044.

Koser, S.A. and Rettger, L.F.(1919) Studies on Bacterial Nutrition. Jour.Inf. Dis. 24, 301-321.

Koser, S.A., (1924) Correlation of Citrate Utilization by Members of the Colon-Aerogenes Group with Other Differential Characteristics and with Habitat. Jour. Bact. 9, 59-77.

Koser, S.A., (1923) Utilization of the Salts of Organic Acids by the Colon-Aerogenes Group. Jour. Bact. 8, 493-520.

Krumweide, Chas., Pratt, Josephine S., and McWilliams, Helen, (1916) The use of Brilliant Green for the Isolation of Typhoid and Paratyphoid Bacilli from Feces. Jour.Inf. Dis. 18, 1-13.

Lampitt, L.H., (1919) Nitrogen Metabolism in Saccharosmyces Cerevisiae. Bio-chem Jour. 13, 459-486.

Laybourne, R.L. (1920) The Fermentation of Polysaccharides by B.aerogenes. Jour.Inf. Dis. 26, 418-423.

Lederer, A. (1913) Some Observations on the Formation of Hydrogen Sulphide in Sewage. Am. Jour. Pub.Health. 3, 552-551.

Lepierre, C. (1903) Les Glucoproteàns Comme Noveaux Milieux de Culture Chimiquement Definis pour l'etude des Microbes. Jour. de Physiol. et de Pathol. 5, 323-330.

Levine, Max, (1916) On theSignificance of the Voges-Proskauer Reaction. Jour. Bact. 1, 153-164.

Levine, Max, and Linton, C.S. (1924) Differentiation of Human and Soil Strains ofAerogenes Section of Colon Group. Am. Jour. Pub.Health 14, 95-99.

Lewis, F.C. (1915) On the Detection and Estimation of Bacterial Indol and Observations on Intercurrent Phenomena. Jour. of Path. and Bact. 19, 429-442.

Lloyd, D.J.(1916), On Vitamines, Amino Acids and Other Chemical Factors Involved in the Growth of the Meningococcus. Jour. Bact. & Pathol. 21, 113-130.

Lockemann, George (1919). Welche nährstoffe sind für das Wachstum der tuberkelbazillen unbedingt notwendig. Cent. für Bakt.83, 420-425.

Logie, W.J. (1920) On the Synthesis of Tryptophane by Certain bacteria and on the Nature of Indole Formation. Jour.Path. and Bact. 23, 224-229.

Long, E.R. (1919).
American Review Tuberculosis, 3,86.

Löwenstein, E. (1913) Beitrage zur cheime des tuberkel Bacillus. Cent. für Bakt. 68, abt.1, Originale, 591-593.

McBeth, I.G. and Scales, F.M. (1913) The Destruction of Cellulose by Bacteria and Filamentous fungi. U. S.Dept. of Agric. Bureau of Plant Ind. Bull 266.

McQuire, Grace,and Falk, K.G. (1924) The Influence of Insulin in the Glucose Fermenting Action of B. coli. Jour. Bio-chem. 60, 489-490.

Mackie, T.J. (1913) Immunity Reactions of the Coil Group. Jour. Path. and Bact. 18, 137-144.

Mathews, A.P. (1920) Physiological Chemistry, 3rd Ed. William Wood & Co., New York.

Mathews, A.P., and Walker, Sydney, (1909) The Action of Cyanides and Nitrides on the Spontaneous Oxidation of Cystèine. Jour. Bio-chem. 6, 29-37.

Mathews, A.P., and Walker, Sydney, (1909) The Action of Metals and Strong Salt Solutions on the Spontaneous Oxidation of Cystein. Jour. Bio-chem. 6,299.

Mathews, A.P., and Walker, Sydney, (1909) The Spontaneous Oxidation of Cystein. Jour. Bio-chem. 6-21-28.

Mecklenburg and Rosenkränger, (1914) Uber eine Methode Zur Kolorimetischen, Besteiminung Kleiner Schwefel-wasser stoffmengen. Zeit. f. Anorgen Chemic. 86, #2 143-153.

Morrison, Lethe E., and Tanner, Fred W. (1922) Studies on Thermophillic Bacteria. I. Aerobic Thermophilie Bacteria from Water. Jour. Bact. 7, 343-366.

Morris, J.L. and Ecker, E.E., (1924) Destruction of Uric Acid by Bacteria and Molds. Jour. Inf. Dis. 34, 592-598.

Mueller, J.Howard (1922) [a] Studies on Cultural Requirements of Bacteria. Jour.Bact. 7, 309-324.

Mueller, J.Howard (1922) [b] Studies on Cultural Requirements of Bacteria II. Jour. Bact. 7, 325-338.

Myers, J.T. (1920) The Production of Hydrogen Sulphide by Bacteria. Jour. Bact. 5, 231-252.

Novy, F.G., Roehm, H.R., and Soule, M.H., (1925) Microbic Respiration, I. The Compensation Manometer and Other Means for the Study of Microbic Respiration. Jour. Inf. Dis. 36, 109-167.

Novy, F.G., and Soule, M.H., (1925) II. Respiration of the Tubercule Bacillus. JJur. Inf. Dis. 36,168-232.

Noyes, Arthur A, and Estill, H.W., (1924) Effect of Insulin on the Lactic Fermentation. Proc. Nat'l.Acad. Sc. 10, 415-418.

Otsuka, Ichiro (1916) Bacterial Decomposition of Polypeptides. Octa. Scholae Med. Univ. Imp. Kiotæ I. 199-214. Abs. Bact. I. No. 4, 299. Physiol.Abstr. Lond. 1917, 2, 15.

Patty, A.F., (1921) The Production of Hydrocyanic Acid by B. Pyocyaneus. Jour. Inf. Dis. 29, 73-77.

Peckham, Adelaide Ward, (1897) The Influence of Environment upon the Biological Processes of the various Members of the Colon Group of Bacilli. Jour. Expr.Med. 2, 549-591.

Pere',M.A. (1892) Contribution a la biologie du Bacterium coli Commune et du bacilli typhique. Annales de Inst. Pasteur. 6, 512-537.

Perry, Margaret C. and Montfort, W.F. (1921) Some Atypical Colon-Aerogenes Forms Isolated from Natural Waters. Jour. Bact. 6, 53-69.

Pietra, Paolo, (1922) On the Differentiation of Various Micro-organisms in Culture Media with Acetate of Lead. Igiene Mod. 15, 75 (International Medical andSurgical Survey,Vol.14,#1, Abs. 2.)

Plimmer, R.H.A. (1912) The Chemical Constitution of the Proteins. (monograph on biochemistry) 2nd Edition. Longsman, Green and Co., London.

Putnam, J.J., and Gay, Douglas M. (1920), Behavior of the Influenza Bacillus in Mixed Culture on Hemoglobin free media. Jour. Med. Res. 42, 1-7.

Raistrich, Harold, (1919) A quantitative Study of theAerobic Decomposition of Histidine by Bacteria. Bio-chem. Jour. 13, 446-458.

Raistrich, H., and Clark, A.B. (1920) A Quantitative Study of the Aerobic Decomposition of Tryptophan and Tyrosin by Bacteria. Bio.Chem. Jour. 15, 76-82.

Remy, L., (1900) A l'etude de la fievre typhoide de son bacille. Annl. de l'Inst. Past. 14, 705-722.

Rettger, Leo F., Berman, Nathan & Sturges, Wm. S., (1916) Further Studies on Bacterial Nutrition; The Utilization of Proteid and non-proteid Nitrogen. Jour. Bact. 1, 15-33.

Rettger, L.F., (1903). An Experimental Study of the Chemical Products of B. Coli Communis and B. Lactis Aerogenes. Am. Jour. Physiol. 8, 284-294.

Rettger, L.F., (1906) Studies on Putrefaction. Jour. Bio-Chem. 2, 71-86.

Revis, Cecil, (1912) Note on the Artificial Production of a Permanently Atypical B. Coli. Cent. f.Bakt. II, Abt. 31, 1-4.

Robertson, R.C. (1924) Food Accessory Factors (vitamines) in Bacterial Growth. VIII. Relation of Substances Formed by B. coli to the Growth of Yeast. Jour. Inf. Dis. 34, 395-399.

Robinson, Harold C. and Rettger, L.F. (1918) The Growth of Bacteria in Protein Free Enzyme and Acid Digestion Products. Jour. Bact. 3, 209-229.

Robinson, H.C. & Rettger, L.F. (1916) Studies on the Use of Brilliant Green and a Modified Endo's Medium in the Isolation of B. Typhosus from Feces. Jour. Med. Research 34, 363.

Rogers, L.A., Clark, Wm. Mansfield, & Lubo, H.A. (1918) The Characteristics of Bacteria of the Colon Type Occuring in Human Feces. Jour.Bact. 3, 231-252.

Rogers, L. A., Clark, Wm. M. and Evans, Alice C. (1914) The Characteristics of Bacteria of the Colon Type Found in Bovine Feces. Jour. Inf. Dis. 15, 99-123.

Rogers, L.A., Clark, Wm.M. and Davis, B.J. (1914) The Colon Group of Bacteria. Jour. Inf. Dis. 14, 411-475.

Ruediger, G.F. (1906* The Cause of Green Coloration of Bacterial Colonies in Blood Agar Plates. Jour.Inf. Dis. 3, 663-665.

Sasaki, Takaoki, (1912) (a) Über den abbau einiger Polypeptide durch Bakterien. I.Mitteilung unter suchung mit Bact. coli commune. Biochem. Zeit. 41,174-179.

Sasaki, Takaoki, (1912) (b) Über den abbau einiger Polypeptide durch Bakterien II Mitteilung unter suchenger mit nicht verflüssigenden Bakterien. Biochem. Zeit. 462-471.

Sasaki, Takaoki, (1912) (c) III Mittielung unter suchengen mit verflüssigenden Bakterien. Biochem. Zeit. 47,472

Sasaki, Takaoki and Otsuka, Ichino, (1912) Experimentelle Untersuchunger über die schmefelwasserstoffentwicklung der Bakterien aus Cystine und Sonstigen. Biochem. Zeit. 39, 208-215.

Sears, H.J., (1916) Studies in the Nitrogen Metabolism of Bacteria. Jour.Inf.Dis. 19, 105-137.

Sherwood, Noble P. (1926) Studies on Bacillus Pyocyaneus. Kansas University Science Bull. (in Press).

Siebakowski, S., Modrezewska, Z, Rabinowicz, H. et Salamon, E, (1923) Rfcherches sur la milieux de culture; influence de differents agents sur la croissance des bacteries pathogenes dous les milieux glosés. Com. rend. Soc. de Biol. par 89, 1386-1389.

Simond, J.P. (1915) The Effect of Bacterial Metabolism on the Antigenic Properties and Serologic Reactions of Bacteria. Jour. Inf.Dis. 17, 500-509.

Skinner, C.E., and Murray, T.J. (1924) Media for Inhibition of Spreaders and Differentiation of B. coli and B. Aerogenes. Jour.Inf.Dis. 34, 585-591.

Smith, Theobald, (1899) The Relation of Dextrose to Production of ~~the~~ Toxin in Bouillon Cultures of the Dyphtheria Bacillus. Jour. Expr. Med. 4, 373-397.

Smith, Theobald, (1895) Über den Nachweis des bacillus coli communis im Wasser. Cent. f. Bakt. 18,494-495

Smith, Theobald (1897) A Modificatinn of the Method for Determining the Production of Indol by Bacteria. Jour. Exp. Med. 2, 543-547.

Sperry, Joel A. and Rettger, L.F. (1915) The Behavior of Bacteria Toward Purified Animal and Vegetable Proteins. Jour.Biol.Chem. 20, 445-459.

Standard Methods of Water Analysis (1923) 5th Ed. American Public Health Assoc. N.Y.

Stevens, F.A., and Koser, S.A. (1919) Streptolysin Production in Carbohydrate Media. Jour. Expr. Med. 30, 539-554.

Stickel, J.E. and Meyer, K.F. (1918). Peptic and Tryptic Digestion Products as Inexpensive Culture Media for Routine Bacteriological Work. Jour.Inf.Dis. 23, 68-81.

Sturges, Wm. S., and Rettger, Leo.F. (1922) Bacterial Autolysis. Jour. Bact. 7, 551-577.

Sullivan (1905-1906) Synthetic Culture Media and the Biochemistry of Bacterial Pigments. Jour. Med. Research. 14, 109-160.

Taylor, A.E., (1902) Ueber Eiweisspaltung durch Bakterien. Zeit. f. Physiol. Chem. 36, 487.492

Thompson, L.S. (1921) The Group of Hydrogen Sulphide Producing Bacteria. Jour. Med. Res. 42, 363-389.

Tilley, F.W., (1923) Variations in H2S Production by Bacteria. Jour. Bact. 8, #2, 115-120.

Tilley, F.W. The Relation Between Chemical Composition of Peptones and H2S Production by Bacteria. Jour. Bact 8,287-295.

Treece, E. Lee (1920) A Substitute for Adonite in the Determination of Fecal and Non-fecal Strains of the Colon Aerogenes Group: Abst. Bact. 4, #1, 9 abs. 27.

Uschinsky, (1893) Ueber eine Eiweissfreie Nährlösung für pathogene Bacterien Nebst Einigen Bermerkungen über Tetannsgift. Central für Bakt. I. 14, 316.

Van Slyke, L.L., and Hart, E.B. (1903) The Relation of Carbon Dioxide to Proteolysis in the Ripening of Cheddar Cheese.N.Y. Agri. Expt. Sta. Bul. 231.

Waksman, S.A. (1917) The Influence of Available Carbohydrate Upon Ammonia Accumulation by Micro-Organisms. Jour. Am. Chem. Soc. 39, 1503.

Waksman, S.A. (1918) Studies on Proteolytic Activities of Soil Organisms, with special reference to Fungi. Jour. Bact. 3, 475-492.

Wells, H.G., DeWitt, Lydia M., and Long, E.R. (1923) The Chemistry of Tuberculosis. Williams & Wilkins, Baltimore, P.55.

Whitehead, H.R. (1924) Studies in Bacterial Nutrition II. Biol.Chem. Jour. 18, 829-832.

Williams, Anna W., and Povitsky, Olga, R. (1921) Growth of B. Influenza Without the Presence of Hemoglobin. Jour. Med. Res. 42, 405-417.

Wilson, W. James (1923) Reduction of Sulphites by Certain Bacteria in Media Containing a Fermentable Carbohydrate and Metalic Salts. Jour.Hyg.21,#4,393-398.

Winslow, C.E.A. (1912) TheClassification of the Streptococci by the Action upon Carbohydrates and Related Organic Media. Jour.Inf.Dis. 10, 285-293.

Winslow, C.E.A., Kligler I.J. and Rothberg,W. (1919). Studies on the Classification of the Colon-typhoid Group of Bacteria with Special Reference to Their Fermentative Reactions. Jour. Bact. 4, 429-503.

Winslow, C.E.A., and Dolloff, A.F. (1922) The Relative Effect of Certain Triphenylmethane Dyes Upon the Growthof Bacilli of the Colon Group in Lactose Broth and Lactose Bile. Jour. Inf. Dis. 31, 302-304.

Winslow, C.E.A. &Cohen, Barnett (1918) The Distribution of B. coli and B. aerogenes Types in Polluted and Unpolluted Water. Jour. Inf. Dis. 23, 90-101.

Winslow, C.E.A., Kligler, I.J. and Rothberg, W., (1919) Studies on the Classification of the Colon-Typhoid Group of Bacteria with Special Reference to their Fermentation Reactions. Jour. Bact. 4, 429-503.

Wolfe, Chas. G.L., (1919) Contributions to the Biochemistry of the Pathogenic Anaerobes. VI. The Proteolytic Action of Bacillus Sporogenes and B. Welchii. Jour. Bath. & Bact. 22. 270-288.

Wollman, E. (1919) B. Coli Comme Indicateur de la Proteolyse. Compt. rend. Soc. de Biol. par. 82, 1263.

Wyeth, F.J.S. (1919) The Effects of Acids, Alkalines and Sugars on the Growth and Indole Formation of Bacillus Coli. The Biochem. Jour. 13, 10-24.

Wyon, G.A., and McLeod, J.W., (1923) Preliminary Note on Inhibition of Bacterial Growth by Amino Acids. Jour. Hygiene, 21, No. 4, 376-385.

Young, C.C., andGreenfield, Myrtle, (1923) Observations on the Viability of the Bacterium Coli Group under Natural and Artificial Conditions. Amer. Jour. Pub. Health 13, 270-273.

Zunz, Edg. , and György, Paul, (1916) Observations sur l'influence chimique des milieux de culture sur le developpement et la production de l'indol par les coli-bacilles et par les bacilles typhiques. Jour. Bact. 1, 627-661.