7-1941

Utilization of Peptones and Peptone Fractions by Bacteria

Margaret E. McMaster
University of Nebraska-Lincoln

Utilization of Peptones and Peptone Fractions by Bacteria

by

Margaret E. McMaster

A THESIS

Presented to the Faculty of

The Graduate College in the University of Nebraska

in Partial Fulfillment of Requirements for

The Degree of Master of Arts

Department of Bacteriology

Lincoln, Nebraska

July 29, 1941

The author takes pleasure in acknowledging her indebtedness to Dr. Carl E. Georgi for his direction of this problem, and to Dr. George L. Peltier for his interest and criticism of the work.

TABLE OF CONTENTS

INTRODUCTION 1

REVIEW OF LITERATURE 1

Preparation and Chemical Analysis of Peptones 1

Minimum Concentration of Peptones 3

Extraction of Peptones 4

Peptones and Hydrogen Sulfide Production 5

Peptones and Indol Production 8

Peptones and Acetyl Methyl Carbinol Production . . . 9

METHODS AND MATERIALS 10

Cultures 10

Peptones 10

Inoculation. 10

Incubation 17

Cleaning of Glassware. 17

EXPERIMENTAL 18

I The Minimum Concentration of Peptone Necessary for Bacterial Growth. 18

Peptone in Concentration of 0.1, 0.01, and 0.001 per cent 18

Peptone in Concentration of 0.0005, 0.00001 and 0.00005 per cent 25

II Salmonella pullorum Studies 25

Minimum Concentration of Peptones 25

Effect of Nicotinic Acid 29

III The Effect of Extraction on Peptone. 29

IV The Effect of Peptones on End-Products of Bacterial Metabolism 31

Effect on Acetyl Methyl Carbinol Production. . . 31

Effect on Indol Production 32

Effect on Hydrogen Sulfide Production. 34

Lead Acetate Method 34

Bismuth Sulfite Method. 36

Ferric Citrate Method 39

Comparison of Methods 41

DISCUSSION 45

SUMMARY. 49

BIBLIOGRAPHY 50

INTRODUCTION

Proteins are complex compounds composed of amino acids conjugated through the peptide linkage. On hydrolysis, proteins yield metaproteins, proteoses, peptones, polypeptides, peptides and amino acids. These intermediate compounds are not sharply differentiated, but change gradually from one into another. Commercial peptones, therefore, are not made up entirely of any one of these intermediate compounds, but of a mixture of them. The amount of each component will vary according to the original protein digested, the method of hydrolysis, and the length of time the hydrolysis is carried out. Commercial peptones, therefore, will vary from lot to lot and from brand to brand.

In this investigation an attempt was made to study these variations as they influence the growth of organism and the end products produced by these organisms from different lots and brands of commercial peptones.

REVIEW OF LITERATURE

Preparation and Chemical Analysis of Peptones:

A number of investigators have analyzed various peptones. Redfield and Huckle (1915) determined total sulfur in peptone by a modified Liebig-Koch method. Colwell (1915) analyzed four brands of peptones and found that those free from lactose were best suited for culture media. The lack of uniformity of local peptones made identification of the typhoid bacillus and other pathogenic organisms very difficult according to Gorini (1916).

Penau and Simmonet (1922) and Yaoi (1926) studied the cystine content of several peptones. The former found that a peptone prepared by pancreatic digestion of beef and extracted by ethyl ether was deficient in cystine. The addition of pure casein made up this deficiency. Of the group of peptones analyzed for cystine, Yaoi reported that Witte's contained the most and May-Boher's the least.

McAlpine and Brigham (1928) studied and compared Bacto peptone, Bacto proteose, Fairchild and Witte's peptones. The total nitrogen in the four peptones appeared to be equal, but different nitrogen fractions of the various peptones varied greatly. The non-protein nitrogen content of Witte's peptone was comparatively small, consisting of equal amounts of polypeptides and amino acids. A large portion of the total nitrogen was proteose nitrogen. In Bacto proteose-peptone the total nitrogen was divided equally between protein and non-protein types. Only Fairchild's peptone was found to contain appreciable amounts of ammonia. Bacto-peptone was found to be low in proteose-peptone which is the protein necessary for toxin production.

Blanchetiere (1927), Pepin (1932), Topshtein (1941), and Clock and Tanner (1938) all have reported on the composition of commercial peptones. The Difco Manual (1939) contains a table of typical analysis of eight of the peptones marketed by the Digestive Ferments Company. O'Meara and Macsween (1937) found that the copper present in commercial peptones affected the growth of certain pathogens. They reported that all the organisms used by them, except Gram negative bacilli, were adversely affected by the copper present in commercial peptones.

Minimum Concentration of Peptones:

There has been few studies to determine the minimum concentration of peptones permitting bacterial growth.

Penfold and Norris (1912) studied the relation of concentration of food supply to the generation time of bacteria. Using Eberthella typhosa as the test organism, they varied the peptone from 0.0125 to 1.05 per cent in 0.5 per cent sodium chloride broth. The generation time of Eberthella typhosa in 1 per cent peptone was 40 minutes. The rate of generation time was greatly influenced by the concentration of peptone where the peptone was less than 0.4 per cent. Below 0.2 per cent the generation time was inversely proportioned to the concentration of the peptone used. By the addition of 0.175 per cent glucose to a media containing only 0.1 per cent peptone, the generation time was lowered by about 50 per cent. With 1 per cent peptone this effect was less marked.

Day and Walker (1913) measured the effect of varying the amount of peptone in a sugar-free broth on the proteolytic activity of certain organisms, taking the amount of ammonia produced as a measure of the activity. Escherichia coli produced the same amount of ammonia in 1 and 2 per cent peptone while Eberthella typhosa produced more ammonia in the 2 per cent peptone. This, they reasoned, indicated a selective action by Eberthella typhosa for some constituent of the peptone.

According to Levine (1921) the concentration of peptones has a marked influence on the inhibitory effect of dyes in culture media. Escherichia coli would not grow in 0.5 per cent peptone with crystal

violet in a dilution of 1:200,000 or brilliant green in a dilution of 1:1,000,000. Bile salts stimulated the growth of Escherichia coli when the concentration of the peptone was less than 0.5 per cent, but were inhibitory when the concentration was raised to 0.7 per cent to 1.0 per cent.

Williams (1930) found that ratio counts of spores of Bacillus subtilis to vegetative cells in peptone water of various concentrations showed that the per cent of spores in the more dilute broth was greater than in the more concentrated. Direct microscopic counts showed that the absolute number of spores in the more concentrated medium was greater than in the dilute.

Regnier and Lambin (1938) observed that in media containing 0.001 to 20.0 grams peptone per liter, the maximum bacterial count reached was roughly proportional to the peptone concentration.

Extraction of Peptones:

Redfield and Huckle (1915) and Chamot and Redfield (1915) extracted Witte's peptone with alcohol. Koch and Carr had previously observed that lipoid sulfur compounds were soluble in alcohol and that protein sulfur compounds were not. The alcohol soluble portion contained 0.80 per cent of the total sulfur, while the insoluble portion contained 1.08 per cent of the total sulfur. Redfield et al reported that only one half as much hydrogen sulfide was produced from alcohol-insoluble peptone as from the unextracted peptone. Much less hydrogen sulfide was produced from the alcohol-soluble fraction of the peptone than from the alcohol-insoluble fraction.

Able and Geiling (1924) fractionated Witte's peptone by repeated salting out in acid and alkaline media with ammonium sulfate and precipitation with ethyl alcohol. They obtained primary and secondary albumineses by this method. Ciaccio and Trimarchi (1927) also fractionated Witte's peptone. They obtained three fractions by the following methods: (1) By the addition of nine volumes of absolute ethyl alcohol to a concentrated peptone solution. (2) By extraction of the peptone with acid ethyl alcohol, adding sodium hydroxide to pH 5-6, filtering, concentrating under reduced pressure, adding 95 per cent ethyl alcohol, and drying in vacuo. (3) By treating the peptone by the Doisy, Somogyi, Shaffer method.

According to Dubos (1930), there were substances present in commercial peptones which were bacteriostatic for certain organisms. These substances were bacteriostatic in the oxidized but not in the reduced form. He removed the bacteriostatic fraction of a certain peptone solution by precipitation with acid and acetone.

Robbins (1941) found a growth-promoting factor for Phycomyces in neopeptone. He concluded that the peptone was rich in factor "Z_1", and less rich in "Z_2". The "Z" factor had previously been found by him in agar. It could be extracted with methyl alcohol or aqueous pyridine. Factor "Z_1" seemed to belong to the vitamin B complex. It was not identical with biotin, panthothenic acid, vitamin B_2, or thiamin. It was soluble in water and in aqueous alcohol or acetone.

Peptones and Hydrogen Sulfide Production:

Tilley (1923) analyzed peptones for unoxidized, partially oxidized

and oxidized sulfur. Using lead acetate media, he found hydrogen sulfide was not produced when oxidized sulfur is the only source of sulfur, but was produced from partially oxidized or unoxidized sulfur compounds. The production of hydrogen sulfide varied according to the peptone used and, because of variations in their composition, he advocated the addition of known quantities of an unoxidized sulfur in the form of sodium thiosulfate to media containing lead acetate as an indicator. His results also indicated that there were differences in hydrogen sulfide production related to variation in bacterial strains.

Fellers, Shostrom, and Clark (1924) advocated the use of lead acetate impregnated on papers placed at the mouth of the test tube instead of in the medium because of the toxicity of lead. This method gave quantitative as well as qualitative results. Difco-peptone gave good results, but Armour and Witte's peptones were not satisfactory by this method.

Using lead acetate paper as an indicator, Myers (1920) compared several peptones. As a result of his investigations, he found Witte's peptone to be the best, Fairchild next, and Difco-peptone the poorest. He also observed that glucose and sucrose did not influence the rate or amount of hydrogen sulfide produced.

Kahn (1925) studied hydrogen sulfide production by sporeforming anaerobes using lead acetate as an indicator and added sodium thiosulfate. When various peptones without added thiosulfate were employed, the results were not as clear cut and there were more variations.

ZoBell and Feltham (1934) compared lead, bismuth, and iron as indicators of hydrogen sulfide production. From experiments they concluded 0.1 mille mole of ferrous iron, 0.2 mille mole lead, and 0.5

mille mole bismuth were needed to give a positive test in peptone medium. Twice these amounts gave better tests. Lead, iron and bismuth were more toxic to recently isolated strains than to old cultures, but most cultures would develop in the presence of 0.2 mille mole of lead, 4.0 mille moles of ferric or 2.0 mille moles ferrous iron and 1.0 mille mole of bismuth. It was recommended that either 0.05 per cent ferric ammonium citrate or 0.03 per cent ferrous acetate be employed as an indicator for hydrogen sulfide in solid media containing at least 3.0 per cent Bacto-tryptone (Difco). For highly pigmented organisms or for recently isolated cultures they advised the use of lead acetate papers and liquid peptone medium.

Levine, Vaughn, Epstein, and Anderson (1932) determined hydrogen sulfide production employing ferric chloride, nickel, lead, and manganese as indicators. Their results indicated that a medium containing 2.0 per cent proteose-peptone, 0.1 per cent dipotassium phosphate, agar, and 0.05 per cent ferric citrate was very sensitive. Vaughn and Levine (1936) using 2.0, 3.0, and 5.0 per cent proteose-peptone found no difference in hydrogen sulfide production by Escherichia, but after long incubations at high concentrations some Aerogenes gave positive tests. It was pointed out by Vaughn and Levine that the amount of agar present had a marked influence on hydrogen sulfide production. The lower the concentration of agar the higher the per cent positive hydrogen sulfide tests.

Bismuth sulfite medium was found by Hunter and Crecelius (1938) to be far superior to any medium which employed lead or iron

as detectors of hydrogen sulfide in bacterial cultures. The peptone recommended was tryptone with 5.0 per cent skimmed milk added to prevent precipitation of certain fractions of the tryptone.

Almy and James (1928) used five peptones (unnamed), and compared the amounts of hydrogen sulfite produced in liquid medium. All were satisfactory except one labled "C".

Utermohlen and Georgi (1940) using cobalt and nickel as indicators, found Bacto-proteose-peptone superior to tryptone.

According to Tarr (1933-34), the formation of hydrogen sulfide from organic sulfur compounds appears to require the presence of a specific enzyme. Cysteine added to the medium stimulated the formation of the enzyme. He was able to extract the latter from Proteus vulgaris. The enzyme was most active at pH 7.8 - 9.0, and required the presence of an amino acid before it became active.

Andrews (1937-38) found hydrogen sulfide produced by microorganism of the mucosa of the small intestine of the dog from l-cystine, i-cystine, dibenzoylcystine, cystine hydantoin, taurine, methionine, sodium sulfate, sodium sulfite, sodium thiosulfate and free sulfur.

Peptones and Indol Production:

Norton and Sawyer (1921) compared Dunham's peptone solution, River's trypsinized peptone, and Cannon's casein medium as to suitability as media for the production of indol by bacteria. The final results were the same but quicker results were obtained in the trypsinized casein or peptone than in Dunham's solution.

Armour, Witte, and Difco proteose-peptones were found to be best for indol production according to Chamot and Georgia (1925).

Kulp (1925), because of the difference in tryptophane content of various peptones, advised the use of a uniform medium. He found that peptone plus tryptophane casein digest (or any medium containing free tryptophane which will support growth of the organism) can be used for indol formation.

Pierce (1931) found viable yeast, autolyzed yeast, and vitamin B increased indol and ammonia production by Escherichia coli.

Peptones and Acetyl Methyl Carbinol Production:

The reaction of acetyl methyl carbinol, peptone and alcoholic solution to give a red color was first observed by Voges and Proskauer in 1898.

Bedford (1929) found a medium of proteose-peptone, glucose, and dipotassium phosphate satisfactory for production of acetyl methyl carbinol.

Barritt (1936) used Bacto-peptone with good results when he tested for acetyl methyl carbinol production with alpha naphthol and potassium hydroxide.

Standard Methods for Water Analysis (1936) recommends proteose-peptone Difco or Witte's to be used as the peptone in the medium.

METHODS AND MATERIALS

Cultures:

The organisms used in these experiments were obtained from the stock culture collection of the Department of Bacteriology at the University of Nebraska. A list of the organisms and strains used will be found in Table I. The organisms in Section I represent a typical group of aerobes and include pathogenic and non-pathogenic bacteria. Those listed in Section II are various strains of Salmonella pullorum. Section III is made up of hydrogen sulfide producing bacteria. Section IV is composed of various species of the coliform group which are identified partly by the production, or lack of production, of indol and acetyl methyl carbinol. The cultures were carried on nutrient agar slants and transferred at frequent intervals to maintain their viability.

Peptones:

The peptones, with the sources, which were utilized in these experiments are listed in Table II-a. Bacto-peptone was extracted with several solvents. These extracts and extracted residues are included in Table II-b.

Inoculations:

A suspension, in physiological salt solution, was made from a 24 hour culture of each organism. All inoculations were then made from the suspensions employing a straight needle for stabbing into solid and semi-solid media, and a loop when seeding liquid media.

Table I

Code Number, Species and Strain of Organisms Used in These Experiments

Group	Code No.	Species	Strain
I	Pv-1	Proteus vulgaris	Novy
	Pv-2	Proteus vulgaris	McCoy
	Et-R	Eberthella typhosa	Rowlins
	Et-H	Eberthella typhosa	Hopkins
	Ao	Aerobacter oxytocum	
	Aa-1	Aerobacter aerogenes	McCoy
	Aa-2	Aerobacter aerogenes	I.S.C.
	Aa-3	Aerobacter aerogenes	K.S.C.I.
	Sa	Staphylococcus aureus	Reddish
	Sl	Streptococcus lactis	
	Sa-a	Salmonella typhimurium	355-70
	P-L	Salmonella pullorum	L
	Spt	Salmonella paratyhpi	Kessel-3
	Ss	Salmonella schottmülleri	Ames - 37
	Sg-1	Shigella gallinorum	K.S.C.
	Sd-B	Shigella dysenteriae	55B
	Shs	Shigella sonnei	701
	Pf	Pseudomonas fluorescens	
	Pd	Paradysenteriae	Flexner
	Ec-1	Escherichia coli	I.S.C. typ.
	Bs-L	Bacillus subtilis	L

Table I (continued)

Group	Code No.	Species	Strains
	Bs-M	Bacillus subtilis	Marburg
	Bm	Bacillus mesentericus	
	Bc	Bacillus cereus	
II	P-L	Salmonella pullorum	L
	P1	Salmonella pullorum	I.S.C. #1
	P3	Salmonella pullorum	I.S.C. #3
	P4	Salmonella pullorum	I.S.C. #4
	P292	Salmonella pullorum	K.S.C. #292
	P321	Salmonella pullorum	K.S.C. #321
	P336	Salmonella pullorum	K.S.C. #336
	P706	Salmonella pullorum	K.S.C. #706
	P723	Salmonella pullorum	K.S.C. #723
III	Pv-1	Proteus vulgaris	Nevy
	Pv-2	Proteus vulgaris	McCoy
	Et-R	Eberthella typhosa	Rawlins
	Et-H	Eberthella typhosa	Hopkins
	Ao	Aerobacter oxytocum	
	Aa-1	Aerobacter aerogenes	McCoy
	Aa-2	Aerobacter aerogenes	I.S.C.
	Sa-a	Salmonella typhimurium	355-70
	Saal	Salmonella typhimurium	

Table I (continued)

Group	Code No.	Species	Strains
	Ss	Salmonella schottmülleri	Ames - 37
	Ss-1	Salmonella schottmülleri	Yale
	Sg-1	Shigella gallinarum	K.S.C.
	Sg-2	Shigella gallinarum	F6-25
	Shs	Shigella sonnei	701
	Sd-Y	Shigella dysenteriae	Yale
	Sd-B	Shigella dysenteriae	55B
	Sl	Streptococcus lactis	
	Cs	Citrobacter sulfidogenes	
	Pl	Salmonella pullorum	I.S.C. #1
	P292	Salmonella pullorum	K.S.C. #292
IV	Aa-1	Aerobacter aerogenes	McCoy
	Aa-2	Aerobacter aerogenes	I.S.C.
	Aa-3	Aerobacter aerogenes	M.S.C. I
	Aa-4	Aerobacter aerogenes	
	Aa-5	Aerobacter aerogenes	DL
	Aa-6	Aerobacter aerogenes	L
	Aa-7	Aerobacter aerogenes	M.S.C. II
	Ac	Aerobacter cloacae	
	Ao	Aerobacter oxytocum	
	Av	Aerobacter viscosum	
	Ec-1	Escherichia coli	I.S.C. typ.

Table I (continued)

Group	Code No.	Species	Strains
	Ec-2	Escherichia coli	I.S.C.
	Ec-3	Escherichia coli	E.M.
	Ec-4	Escherichia coli	horse
	Ec-5	Escherichia coli	L
	Ec-6	Escherichia coli	#33
	Ec-7	Escherichia coli	Yale (old)

Table II-a

Names and Sources of Peptones Used

Code	Name	Source	Serial No.
P	Peptone	Pfanstiehl Chem. Co.	2303
W	Nutri Peptone	Wilson Laboratories	38996
PD	Bacteriologic Peptone	Parke Davis & Co.	3128288
S	Peptonum Siccum	Frederick Stearns & Co.	3474 K
AS	Peptonum Siccum	Armour & Co.	100858
AP	Peptic Digest of Pork Muscle	Armour & Co.	
AB	Pancreatic Digest of Beef Muscle	Armour & Co.	
AC	Pancreatic Digest Casein	Armour & Co.	
AI	Infusion Peptone	Armour & Co.	
PL	Bacteriological Peptone (Siccum)	Paul Lewis Lab. Inc.	
CHP	Hog Protein Peptone	Cudahy Packing Co.	
CH	Hog peptone	Cudahy Packing Co.	
CB	Beef Peptone	Cudahy Packing Co.	
DT	Bacto-tryptose	Difco Laboratories	312137
D1	Proteose-Peptone	Difco Laboratories	305399
D2	Proteose - Peptone #2	Difco Laboratories	312978
D3	Proteose-Peptone #3	Difco Laboratories	312977
DP	Bacto-Peptone	Difco Laboratories	312823
DY	Bacto-Tryptone	Difco Laboratories	313128
DN	Neopeptone	Difco Laboratories	300250
DPr	Protone	Difco Laboratories	
WT	Peptonum Siccum	Friedr. Witte, Rostock	
DPP	Proteose-Peptone #3	Difco Laboratories	38756

Table II-b

Extracted Bacto-Peptone

No.	Solvent	Hours Extracted	Fraction
M-1	Methyl alcohol	10	Extracted residue
M-2	Methyl alcohol	10	Extract
M-3	Methyl alcohol	60	Extract
M-4	Methyl alcohol	60	Extracted residue
B-1	Benzene	60	Extracted residue
E-1	Diethyl ether	10	Extracted residue
Et-1	Ethyl alcohol	10	Extracted residue
Et-2	Ethyl alcohol	10	Extract
A-1	Acetone	10	Extracted residue
X-1	Xylol	10	Extracted residue

Incubation:

All tubes were incubated at 37° C., except those inoculated with Salmonella pullorum in the hydrogen sulfide experiment, which were, in turn, incubated at 30° C. According to Tittsler (1931), the optimum temperature for the production of hydrogen sulfide by Salmonella pullorum was between 30° - 34° C.

Cleaning of Glassware:

All glassware used in the experiments on the minimum concentration of peptone necessary for bacterial growth, and the studies on the extracts and residues of Bacto-peptone were cleaned in dichromate-sulfuric acid cleaning solution.

EXPERIMENTAL

I The Minimum Concentration of Peptone Necessary for Bacterial Growth

An attempt was made to determine the minimum concentration of the different peptones which are necessary to support the growth of a group of aerobic microorganisms. The medium for this experiment was semi-solid glucose peptone agar, with the peptone being varied in amount and kind. The formula for the basic medium is as follows:

Medium I

Dextrose, C. P. ..... 5.0 g.
K_2HPO_4, Anal. Reagent.. 5.0 g.
Agar, Bacto ..... 3.36 g.

Peptone in Concentrations of 0.1, 0.01, and 0.001 per cent:

The amounts of peptone added to Medium I were varied so that each peptone was made up in 0.1, 0.01, and 0.001 per cent. The media were tubed in 10 cc. portions and sterilized at 15 pounds pressure for 15 minutes. These semi-solid media were inoculated with organisms of Group I by the stab method as near to the center of the tube as possible. The media were clear and growth was readily detectable along the line of inoculation. The tubes were incubated at 37^{0} C., and observations were made at 24, 48, 72, and 120 hours.

Tables 3, 4, and 5 indicate the growth of the organisms in the twenty-one peptones in concentrations of 0.1, 0.01, and 0.001 per cent respectively. They show that the minimum concentration of peptone necessary for a particular organism varies a great deal depending upon the peptone used. The minimum concentration of most of the peptones tested necessary for the growth of Shigella dysenteriae was somewhere

Table 3

Growth of Organisms in Media Containing .1% Peptone after Incubation at 37° C. for 5 days

Org.	Peptones																			
	P	W	PD	S	AS	AP	AB	AC	AI	PL	CH	CHP	CB	DT	D1	D2	D3	DP	DY	DN	DPr
Pv-1	+++	++	+++	+++	++	+++	+++	+	++	+++	+++	+	+++	+++	+++	+++	++	½+	+++	++	½+
Pv-2	++++	+++	++++	+++	++++	++++	++++	++	++++	++++	++++	+++	++++	++++	++++	++++	++++	+	++++	++++	+++
Et-R	++	++	+++	+++	+++	++	++	+++	+++	+++	++++	½+	++	+++	+++	+++	++	++	++	+++	+
Et-H	++	+	++++	++	++++	++	++++	++	+++	+++	+++	½+	++++	+++	++++	+++	++	+	++	+++	++
Ao	+++	++++	++++	+++	+++	+++	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++	++++	++++	++++	+++
Aa-1	++++	++++	++++	+++	+++	++++	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++	++++	++++	++++	+++
Aa-2	+++	++++	++++	+++	++++	+++	+++	++++	+++	++++	+++	+++	+++	++++	++++	++++	+++	+++	++	++++	++
Aa-3	++++	++++	++++	+++	++++	++++	++++	++++	++++	+++	++++	+++	++++	++++	++++	++++	++++	++++	++++	++++	+++
Sa	++++	++++	+++	+++	+++	+++	++	+	+++	+++	+++	++	+++	+++	+++	+++	+++	+++	++	++	½+
Sl	+	½+	++	+	++	+	+++	++	+++	++	+	+	+	++	++	++	++	+	½+	+	½+
Sa-a	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++	++++	++++	++++	++++	++++	++++	++++	++++	+++
P-L	++	++	+++	½+	½+	+++	+½+	½+	+++	+++	+++	-	+++	++	++	+++	+++	½+	½+	½+	-
Spt	+++	+++	++++	½+	+++	++++	++++	++++	+++	+++	+++	-	++++	++++	++++	++++	++++	++++	+++	++++	+++
Ss	++	++	++++	+	+++	++++	+++	++++	+++	+++	++++	-	++++	++++	++++	++++	++++	++++	+++	++++	++

Table 3 (continued)

Org.	Peptones																			
	P	W	PD	S	AS	AP	AB	AC	AI	PL	CH	CHP	CB	DT	Dl	D2	D3	DP	DY	DN	DPr
Sg-1	++	++	++	++	+++	+++	++	++	++	++	++	++	+	++	+	++	+++	++	++	++	+
Sd-3	++	++	++	½+	+	++	+½+	++	++	++	++	-	++	++	++	++	++	++	++	++	tr
Shs	++	++	+++	++	++	+++	+++	++	++	++	++	++	++	++	++	++	+++	+++	+++	++	++
Pf	++	+½+	+	++	++	+	++	+½+	++	++	++	++	+½+	++	+	++	+½+	++	++	++	++
Pd	+++	+++	+++	++	++	+++	++++	++	+	++	++	++	++	++	++	+++	++	+++	+++	+++	++
Ec-1	++++	+++	++++	++++	++++	++	++++	+++	+++	+++	+++	++	+++	++++	++++	++++	++++	++++	++++	++++	++
Bs-L	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	++	+++	+++	+++	+++	+++	++
Bs-M	+	+	+++	+++	+++	++	++	++	++	++	+++	+++	++	+++	++	+++	++	++	+++	++	++
Bm	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	++++	++++	+++	++++	++
Bc	++++	++++	++++	++++	+++	++++	+++	++++	+++	++++	++++	+++	++++	++++	++++	++++	++++	++++	+++	++++	++

Legend for Table 3 through 9

tr. = very slight growth
½+ = slight growth
\+ = fair growth
++ = abundant growth
+++ = good growth
++++ = very good growth

Table 4

Growth of Organisms in Media Containing .01% Peptone after Incubation at 37° C. for 5 days

Org.	Peptones																			
	P	W	PD	S	AS	AP	AB	AC	AI	PL	CH	CHP	CB	DT	D1	D2	D3	DP	DY	DN	DPr
Pv-1	½+	½+	+	½+	½+	-	+	½+	-	½+	½+	-	+	½+	½+	½+	+	-	tr	½+	tr
Pv-2	+++	+++	++	½+	+	+	++++	½+	++	++	½+	+	+++	+	++	++	+++	½+	+++	++	½+
Et-R	+	+	½+	-	+	½+	½+	½+	+	+	++	-	+	+	++	+	++	½+	+	++	tr
Et-H	++	++	+	tr	+	½+	+	+	tr	½+	+	-	+	+	+	+	+	tr	+	½+	tr
Ao	++	++	+++	++	++	+++	+++	++	+++	+++	++	++	+++	++	+++	+++	+++	++	++	++	++
Aa-1	++	++	++	++	++	++	+++	++	+++	++	++	++	++	++	+½+	+++	+++	++	++	++	++
Aa-2	++	++	++	++	++	++	++	++	+++	++	++	+	++	++	++	+++	++	++	++	+++	++
Aa-3	+++	+++	+++	++	+++	++	+++	++	+++	++	++	++	+++	+++	++	+++	+++	++	+++	++	++
Sa	++	½+	½+	-	tr	½+	½+	½+	½+	½+	½+	tr	+½+	½+	½+	++	½+	½+	½+	½+	tr
Sl	tr	tr	tr	-	tr	½+	+	tr	½+	½+	½+	-	½+	+	+	+	½+	tr	tr	tr	-
Sa-a	++	++	½+	½+	½+	++	++	++	++	++	++	+	++	++	++	++	++	++	++	++	+
P-L	+	½+	½+	-	-	-	-	-	tr	tr	tr	-	+	tr	½+	½+	+	-	-	-	-
Spt	½+	+½+	++	-	½+	+	tr	½+	tr	+	+	-	++	½+	+	++	tr	+	+	+	-
Ss	+	+	+	tr	½+	+	½+	½+	½+	½+	½+	-	++	½+	+	++	tr	½+	+	+	½+

Table 4 (continued)

Org.	Peptones																			
	P	W	PD	S	AS	AP	AB	AC	AI	PL	CH	CHP	CB	DT	D1	D2	D3	DP	DY	DN	DR
Sg-1	+	+	+	+	++	+	½+	½+	½+	+	+	+	+	+	+	½+	½+	½+	+	½+	½+
Sd-B	½+	½+	-	-	-	½+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shs	++	++	++	++	++	+	++	+	½+	+	+	+	++	+½+	++	++	++	½+	+	+½+	++
Pf	+	+ ½+	½+	+	+½+	+	+	½+	½+	+	+	½+	+	+	+	½+	½+	½+	½+	+	½+
Pd	++	++	++	½+	½+	+	+	+	+	+	½+	tr	++	+	++	++	+	½+	+	+½+	½+
Ec-1	+	++	++	½+	½+	+	½+	½+	½+	½+	tr	tr	+	+	½+	½+	½+	tr	+½+	½+	tr
Bs-L	++	++	++	++	++	+++	++	+++	++	++	++	++	++	++	++	++	++	+++	+++	+++	++
Br-M	+	+	++	++	++	+	+	+	+	+	++	++	++	++	+	+	++	++	++	+	++
Em	++	+	++	+½+	+++	++	+	++	+ ½+	+	+++	½+	++	½+	++	+++	++	++	++	+½+	+++
\Bo	++	+	+++	+½+	++	+++	+	++	++	++	+++	½+	++	++	+++	+++	++	++	+++	++	+++

Table 5

Growth of Organisms in Media Containing 0.001% Peptone after Incubation at 37° C. for 5 days

Org.	Peptones																			
	P	W	PD	S	AS	AP	AB	AC	AI	PL	CH	CHP	CB	DT	D1	D2	D3	DP	DY	DN	DPr
Pv-1	-	-	-	-	-	-	½+	-	-	-	tr	-	-	-	-	-	-	-	-	-	-
Pv-2	½+	½+	tr	tr	-	½+	½+	½+	½+	+	½+	½+	½+	½+	½	½+	+	½+	½+	-	tr
Et-R	-	tr	-	-	-	tr	-	-	-	½+	½+	-	-	tr	tr	tr	tr	½+	-	-	-
Et-H	-	tr	tr	-	tr	tr	½+	tr	-	tr	-	-	tr	-	tr	tr	tr	tr	-	-	-
Ao	++	++	+	++	++	++	++	++	++	++	++	+	++	+	+½+	++	++	++	++	½+	++
Aa-1	++	++	+	+	+	++	++	++	+	+	+	+	+	+	+½+	+	+	++	++	++	+½
Aa-2	++	++	+½+	+	+	+	++	+	+	+	+	½+	+	+	+	+	+	++	+	+	+
Aa-3	++	++	+	+	+	+	++	+	+	+	+	+	+	+	+	+	++	++	++	++	++
Sa	-	tr	-	-	-	tr	-	-	-	-		tr		tr	tr	tr	tr	-	tr	tr	-
Sl	-	-	-	-	-	tr	-	-	-	-	-	-	tr	-	tr	-	tr	-	-	-	-
Sa-a	½+	-	tr	-	-	-	tr	-	-	+	tr	tr	tr	-	+	+	+	+	+	+	+
P-L	½+	tr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spt	tr	tr	½+	-	tr	-	tr	tr	tr	tr	tr	-	tr	½+	tr	tr	tr	tr	tr	-	-
Ss	tr	tr	½+	-	tr	tr	½+	tr	tr	tr	tr	-	½+	½+	tr	tr	tr	tr	tr	tr	tr

Table 5 (continued)

Org.	Peptones																			
	P	W	PD	S	AS	AP	AB	AC	AI	PL	CH	CHP	CB	DT	D1	D2	D3	DP	DY	DN	DR
Sg-1	+	+	+	½+	+	½+	½+	½+	½+	½+	½+	½+	½+	½+	½+	½+	tr	tr	tr	tr	tr
Sd-B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shs	+	½+	+	½+	+	+	+	+	½+	+	+	½+	½+	+	½+	+	½+	½+	½+	+	+
Pf	+	½+	½+	+	½+	½+	½+	½+	½+	+	½+	½+	½+	½+	½+	½+	tr	½+	½+	½+	½+
Pd	+	tr	½+	-	tr	½+	½+	½+	½+	tr	tr	tr	tr	½+	½+	½+	tr	tr	tr	½+	tr
Ec-1	tr	tr	tr	-	½+	-	tr	tr	-	-	-	-	tr	-	-	-	tr	tr	-	-	tr
Bs-L	++	+ ½+		+	½+	+	+	+	+	½+	+	+	+	+	½+	+	+	+	++	+	½+
Bs-M	½+	½+	+	+	+	½+	½+	½+	½+	½+	+	+	+	½+	½+	½+	-	½+	½+	½+	½+
Bm	tr	½+	½+	½+	½+	½+	½+	½+	½+	½+	½+	-	+	tr	½+	½+	tr	tr	½+	tr	tr
Bc	+½+	½+	½+	½+	½+	½+	½+	½+	½+	½+	½+	tr	½+	tr	½+	½+	tr	½+	½+	½+	tr

between 0.1 and 0.01 per cent. The minimum concentration of most of the peptones for Proteus vulgaris, Streptococcus lactis, and Salmonella pullorum was somewhere between 0.01 and 0.001 per cent. Many of the remaining organisms showed at least traces of growth in most of the peptones in 0.001 per cent concentration.

Peptone in Concentration of 0.0005, 0.0001 and 0.00005 per cent:

Several of the organisms showed good growth in media containing but 0.001 per cent peptone. These organisms and the five peptones in which they grew especially well were used in another set of experiments, in which the peptone content of the media was 0.0005, 0.0001, and 0.00005 per cent.

The results summarized in Tables 6, 7, and 8 show that the minimum concentration of most of the five peptones for the organisms used was below 0.00005 per cent.

II Salmonella pullorum Studies

Minimum Concentration of Peptones:

Table 4 shows that Salmonella pullorum generally grew very poorly in media containing 0.01 per cent peptone. In order to determine whether this was due to the particular strain of the organism or was generally true of the different strains, eight additional strains were used. The peptones employed were those in which Salmonella pullorum "L" had shown particulary poor growth. The media were made up with 0.1, 0.01, and 0.001 per cent peptone. The media were inoculated and after 72 hours of incubation at 37° C., transfers were made to duplicate tubes of the same media. Transfers were made again after another 72 hours.

Table 6

Growth of Organisms in Media Containing 0.0005% Peptone after Incubation at 37° C. for 5 days

Organism	Peptones				
	P	DP	D3	PL	W
Pv-2	tr.	tr.	tr.	tr.	tr.
Ao	+	+	+½+	++	+
Aa-1	+	+	+	+	½+
Aa-2	½+	+	½+	½+	-
Aa-3	+	½+	+	+	½+
Sa-a	tr.	tr.	-	½+	tr.
Sh	½+	tr.	½+	½+	-
Pf	tr.	tr.	tr.	½+	tr.
Bs-L	+	½+	½+	½+	½+
Bs-M	½+	½+	½+	½+	½+

Table 7

Growth of Organisms in Media Containing 0.0001% Peptone after Incubation at 37° C. for 5 days

Organism	Peptones				
	P	DP	DS	PL	W
Pv-2	-	½+	½+	tr.	tr.
Ao	+	+	½+	+	+
Aa-1	+	+	+	+	+
Aa-2	-	+	-	½+	+
Aa-3	+	+	+	+	+
Sa-a	½+	tr.	tr.	tr.	tr.
Shs	½+	tr.	tr.	tr.	tr.
Pf	tr.	tr.	tr.	tr.	tr.
Bs-L	+	½+	½+	+	½+
Bs-M	½+	½+	½+	½+	½+

Table 8

Growth of Organisms in Media Containing 0.00005% Peptone after Incubation at 37° C. for 5 days

Organism	Peptones				
	P	DP	D3	PL	W
Pv-2	-	-	tr.	tr.	-
Ao	+	+	++	+	½+
Aa-1	+	+	+	+	½+
Aa-2	-	+	-	½+	-
Aa-3	+	-	+	+	½+
Sa-a	½+	++	½+	tr.	tr.
Sh-s	+	tr.	tr.	tr.	tr.
Pf	tr.	tr.	tr.	tr.	tr.
Bs-L	+	½+	½+	½+	½+
Bs-M	½+	½+	½+	½+	½+

Table 9 shows the results after the third transfer in 0.1 per cent peptone. All the strains of Salmonella pullorum used failed to grow in media containing 0.01 per cent peptone. From these results it was concluded that it lacked an essential substance or contained a toxic substance.

Effect of Nicotinic Acid:

The preceding results indicate, the presence of a toxic substance in the media, the absence of an essential growth substance, or the lack of a specific growth promoting factor. According to Koser et al (1938) nicotinic acid (or its amide) is an essential growth substance for the dysentery bacilli.

To basic Medium I containing 0.1 and 0.1 per cent Bacto-peptone, nicotinic acid was added at the rate of 0.1 microgram per cc. of medium.

There were no differences in growth of Salmonella pullorum in media which originally failed to support growth and that to which nicotinic acid had been added. Therefore nicotinic acid is not a factor in the failure of Salmonella pullorum to grow well in 0.01 per cent peptone media.

III The Effect of Extraction on Peptone

One peptone, Bacto-peptone, was extracted with several solvents. It was hoped that a growth stimulating, a toxic substance, or certain amino acids might be extracted and the extract and the extracted residue would exhibit different growth producing properties than the unextracted peptone.

The peptone was extracted in a Soxhlet extractor. About 30.0 grams of peptone were added in the thimble and the extraction with a

Table 9

Growth of 9 Strains of *Salmonella pullorum* in 0.1% Peptone Media after 5 days Incubation at 37° C.

Strain	Peptone					
	S	AB	AS	DN	CHP	DP
P1	-	-	++	+	-	++
P3	++	++	++	+	-	++
P4	-	++	++	+	-	++
P292	++	+++	++	+	-	++
P321	++	++	++	+++	+	++
P336	-	-	-	-	-	+++
P706	-	++	++	+	-	++
P723	++	++	++	+	-	++
P-L	++	-	++	++	-	+++

given solvent was carried on. At the end of the extraction period the peptone was removed from the extract (under reduced pressure if the boiling point of the solvent was above 100° C.). A new portion of peptone was then extracted with another solvent.

A list of the solvents used and time of extraction is found in Table 2-b. Absolute methyl alcohol and absolute ethyl alcohol were the only solvents which extracted any appreciable amounts of substance from the peptone.

Each extract and extracted residue was added to Medium I in concentration of 0.1, 0.01 and 0.001 per cent. The media were inoculated with organisms in Groups I and II.

Observations at the end of five days failed to show any differences in growth between the unextracted peptone used as a control, the extracts, or the extracted residues.

It was suggested that the extraction time was not long enough. Bacto-peptone was extracted for 60 hours with absolute methyl alcohol and benzene. These extracts and extracted residues also failed to show any differences in growth from the control.

The extracts and residues were also used in the following experiments. The results being tabulated with the other peptones. (See Tables 10, 11, 12, 13, 14, and 15).

IV The Effect of Peptones on End-Products of Bacterial Metabolism

Effect on Acetyl Methyl Carbinol Production:

In order to test the effect of peptones on the production of acetyl methyl carbinol, 5.0 grams of the peptone to be tested were

added to one liter of Medium II.

Medium II

Dextrose, C. P. ..... 5.0 g.
K_2HPO_4, Anal. Reagent..... 5.0 g.
Distilled Water..... 1000.0 cc.

The media was tubed in 5 cc. portions and inoculated with the coliform bacteria, Group IV. One cc. was removed and tested for acetyl methyl carbinol after incubation of 24 hours. Barritt's reagents (Barritt-1936) were used to make the test. Acetyl methyl carbinol was again tested for after 72 hours because, according to Paine (1927), Williams and Marrow (1928), Tittsler (1938) and Segal (1940), some organisms utilize the acetyl methyl carbinol produced.

There were very little difference in acetyl methyl carbinol production in the different media as can be seen in Table 10. Stearns, Armour, Armour peptic digest of pork muscle, Armour pancreatic digest of beef muscle, Armour pancreatic digest of casein, Cudahy beef peptone, Bacto-tryptose, proteose-peptone, and protone peptones gave good clear, easily read tests. Paul Lewis' peptone gave poor results. The results after 72 hours were very little different, and were omitted from the table. None of the strains of Escherichia used produced acetyl methyl carbinol, and were also omitted.

Effect on Indol Production:

It has been shown by Herzfeldt and Klinger, Tilley (1921), Woods (1935) and others that bacteria produce indol entirely from the breakdown of tryptophane. Whether a peptone can be used to show indol production depends primarily on the content of this particular amino acid.

Table 10

Acetyl Methyl Carbinol Production Tested After 24 Hours

Org.	Peptones																						
	P	W	PD	S	AS	AP	AB	AC	AI	PL	CP	CH	CB	DT	Dl	DP	DY	DN	DPr	WT	DPP	B-1	M-4
Aa-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	sl
Aa-2	-	-	-	-	-	-	+	-	-	-	-	sl	-	-	-	-	-	-	-	-	-	-	-
Aa-3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aa-4	-	-	-	-	-	-	+	-	-	-	-	sl	-	sl	+	-	-	-	-	-	-	-	-
Aa-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aa-6	-	-	-	-	-	-	+	-	-	sl	-	sl	-	sl	-	-	-	-	-	-	-	-	-
Aa-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ac	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ao	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	sl	+	+	+	+	sl	sl	+
Av	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

- = no acetyl methyl carbinol
sl = slight amount of acetyl methyl carbinol
+ = acetyl methyl carbinol produced

The medium for this experiment was 10 grams of the peptone dissolved in one liter of distilled water. The media was tube in 5 cc. portions and inoculated with organism in Group IV.

Observations were made after incubation at 37° C. for 24 hours according to the procedure in Standard Methods of Water Analysis (1936). Kovac's reagent made up according to Levine (1937) was the test solution.

To 5 cc. of the media to be tested 0.2 to 0.4 cc. of the reagent was added. A deep red color on the surface indicated a positive test.

According to the results summarized in Table II, there are several peptones which can be utilized as well as tryptone to demonstrate indol production. Armour pancreatic digest of casein gave the clearest positive tests. Wilson, Paul Lewis, Bacto-tryptose, and Bacto-tryptone gave good results. The benzene, methyl alcohol and ether extracted residues also gave good results. Stearns, Cudahy hog protein and Bacto-protone-peptones did not contain enough tryptophane to give any positive tests with the organisms used. The strains of Aerobacter which did not produce indol were omitted from Table 12.

Effect on Hydrogen Sulfide Production:

Lead Acetate Method

The formula of the basic medium used in this experiment is as follows:

Medium III

Dextrose, C. P.	1.0 g.
Lead Acetate	0.2 g.
Agar, Bacto	10.0 g.
Distilled Water....	1000.0 cc.

Table 11

Production of Indol from the Various Peptones

Org.	Peptones																							
	P	W	PD	S	AS	AP	AB	AC	AI	PL	CHP	CH	CB	DT	Dl	DP	DY	DN	DPr	WT	DPP	B-1	M-4	Et-1
Ao	½+	+	+	-	+	½+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
Av	½+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
Ec-1	½+	+	½+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
Ec-2	½+	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
Ec-3	½+	+	+	-	+	+	½+	+	+	+	-	+	+	+	+	½+	+	+	-	+	+	+	+	+
Ec-4	½+	+	½+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
Ec-5	½+	+	+	-	+	+	½+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
Ec-6	½+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	½+	+	+	-	+	+	+	+	+
Ec-7	½+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	½+	+	+	-	+	+	+	+	+

- = no indol produced
½+ = slight amount of indol produced
\+ = indol produced

To this Medium III, 20.0 grams of the peptone to be tested were added. The pH was adjusted, and the media tubed, plugged, and sterilized at 15 pounds pressure for 15 minutes. Armour infusion, neopeptone and Witte's peptones gave dark colored and cloudy media which made observation difficult.

Duplicate tubes were inoculated with the organisms in Group III. The puncture was made close to the side of the tube so if blackening of the media occurred it could easily be seen. All the tubes were incubated at 37° except those of Salmonella pullorum which were incubated at 30° C. Observations were made at 2, 4, and 7 days.

The results are summarized in Table 12. Wilson, Parke Davis, Paul Lewis, and Stearns peptones gave very satisfactory results when employed in lead acetate media. None of the organisms grew in Bacto-protone or Witte's peptone or produced hydrogen sulfide from Armour infusion peptone so these peptones were omitted from the table.

Bismuth Sulfite Method

The basic medium was prepared according to the method of Hunter and Crecelius (1938).

Medium IV

K_2HPO_4, Anal. Reagent .. 0.3 g.
Agar, Bacto ..... 5.0 g.
Distilled Water ..... 940.0 cc.

Dissolve and filter, then add:

Na_2SO_3, (20% solution) ..... 10.0 cc.
Bismuth and Ammonia citrate, Merck .. 0.16 g.
Mannitol, C. P. ..... 5.0 g.
Milk, skimmed .....50.0 cc.

Table 12

Hydrogen Sulfide Production from the Various Peptones Using Lead Acetate as Indicator

Org.	Peptones																		
	P	W	PD	S	AS	AP	AB	AC	PL	CHP	CH	CB	DT	Dl	DP	DY	DN	DPP	M-4	B-l
Pv-1	*	+++	(-)	+++	+	++	+	+	**	tr.	++	***	**	**	++	+	(-)	***	(-)	tr.
Pv-2	-	+	*	+	½+	-	-	+	*	tr.	+	½+	+	+	+	tr.	(-)	tr.	½+	+
Et-R	-	++	*	tr.	++	½+	½+	+	*	(-)	-	+	***	½+	++	++	(-)	-	-	+
Et-H	-	+	**	tr.	+	+	½+	½+	**	(-)	tr.	½+	**	½+	+	tr.	(-)	(?)	tr.	++
Ao	*	*	*	-	-	-	-	½*	++	(-)	½+	(?)	-	½*	(?)	½+	(-)	(?)	½+	½+
Aa-1	-	½+	*	-	-	½+	-	+	*	-	½+	+	-	½*	(?)	-	(-)	-	tr.	(-)
Aa-2	-	½*	½*	tr.	-	½+	tr.	½+	½+	tr.	tr.	(?)	-	½*	½+	-	(-)	-	tr.	½+
Aa-3	**	++	***	+++	tr.	++	½+	½+	**	-	+++	***	***	**	++	-	+++	***	+++	++
Saal	-	½+	+	tr.	+	-	-	½+	++	(-)	-	*	(?)	-	(?)	-	(-)	-	(?)	tr.
Ss	*	½+	**	+	-	*	*	(?)	**	(-)	+	**	**	*	++	-	(-)	+	++	*
Ss-1	*	+++	***	++	+	+	+	+	**	(-)	++	***	***	**	++	-	(-)	****	++	++
Sg-1	-	-	+	-	tr.	-	-	-	*	-	-	-	-	-	(-)	-	(-)	-	tr.	-

Table 12 (continued)

Org.	Peptones																		
	P	W	PD	S	AS	AP	AB	AC	PL	CHP	CH	CB	DT	Dl	DP	DY	DN	DPP	M-4	B-l
Sg-2	*	+++	*	+++	+	+	+	½+	**	(-)	++	++	**	**	++	-	(-)	***	½+	+
Shs	-	+	½+	tr.	½+	-	-	+	++	(-)	tr.	-	(?)	(?)	(?)	-	(-)	-	(?)	(?)
Sd-Y	-	tr.	tr.	-	tr.	-	-	tr.	+	(-)	-	½+	-	-	-	-	(-)	-	-	½+
Sd-4	-	tr.	tr.	-	-	-	-	-	+	(-)	-	-	-	-	-	-	(-)	-	-	tr
Sl	+	+	+	tr.	++	-	tr.	++	++	(-)	½+	+	½+	+	½+	-	(-)	-	½+	+
Cs	*	***	***	+	½+	+	½+	½*	***	(-)	++	**	***	***	*	-	(-)	***	+	+
Pl	*	½+	(-)	-	½+	+	+	+	**	(-)	-	+	**	**	++	-	(-)	***	+	+
P292	½*	++	**	++	½+	+	+	*	**	(-)	++	++	**	**	++	-	(-)	**	+	+

+ = blackening along line of inoculation

* = blackening diffuses into media

(-) = no growth

To Medium IV, 20.0 grams of peptone were added. The pH was adjusted to 7.1 and the medium was tubed, plugged and sterilized at 15 pounds for 15 minutes.

The addition of skimmed milk was supposed to prevent precipitation but in all media there was a precipitate in the bottom of the tube. All the peptones gave clear, light colored media except Armour's infusion peptone, proteose-peptone #3 (DPP), Witte's, and protone. These were dark colored and cloudy making readings very difficult.

After inoculation with organisms of Group III the tubes were observed at 2, 4, and 7 days. A summary of the results after 7 days is given in Table 13. It was impossible to make any observations on the medium containing Witte's peptone because of its dark color. Wilson, Armour peptic digest of casein, Bacto-tryptose and Bacto-tryptone peptones gave good results in bismuth sulfite media.

Ferric Citrate Method:

The basic medium, made up according to Levine (1937), is as follows:

Medium V

K_2HPO_4, Anal. Reagent ..... 1.0 g.
Ferric citrate..... 0.5 g.
Dextrose, C. P. ..... 1.0 g.
Agar, Bacto..... 10.0 g.
Distilled Water.....1000.0 cc.

Twenty grams of peptone were added to Medium V. After tubing and plugging it was sterilized at 15 pounds pressure for 15 minutes.

Protone, tryptone, and Cudahy hog protein peptone gave good growth, but the organisms produced no hydrogen sulfide in these media so

Table 13

Hydrogen Sulfide Production from the Various Peptones Using Bismuth Sulfite as the Indicator

Org.	Peptones																				
	P	W	PD	S	AS	AP	AB	AC	AI	PL	CH	CB	DT	Dl	DP	DY	DH	DPr	DPP	M-1	M-2	B-1
Pv-1	++	***	**	+	+	***	***	+	++++	***	++	++++	*	++	+	+++	**	-	***	++	+	**
Pv-2	(-)	++	½+	+	tr.	(-)	½+	½+	++	(?)	½+	+	½+	+	½+	++	++	++	**	+	+	+
Et-R	-	***	-	**	tr.	**	***	***	***	++	½+	***	**	**	-	****	***	-	+	*	*	***
Et-H	-	**	*	½+	(?)	**	***	****	***	(?)	-	***	**	***	-	****	***	-	-	*	*	½+
Ao	++	+++	+++	+++	+	++	+++	+++	++++	+	++++	++++	**	+++	+	+++	+++	+	+	+++	+++	++++
Aa-1	+++	+++	+++	+++	+++	++++	+++	+++	+++	++	+++	++++	**	++++	++	+++	+++	++	+	+++	+++	+++
Aa-2	+	+	½+	+	½+	++	+++	+	+++	½+	½+	½+	++	+++	½+	+++	+++	½+	-	+++	+++	**
Sa-a	½+	****	****	**	tr.	****	***	****	***	***	***	*****	**	***	**	***	***		-	***	****	***
Saal	-	½+	(-)	tr.	(-)	++	tr.	½+	++	(-)	-	+	+	½+	-	++	++	tr.	+	½+	½+	+
Ss	-	*	½+	**	(?)	****	***	+	***	+	+	*****	**	***	(?)	***	***		+	***	**	***
Ss-1	(-)	****	½+	tr.	tr.	**	***	**	**	****	½+	(-)	**	***	*	++	***		***	***	***	***
Sg-1	(-)	**	(-)	(-)	(-)	(-)	**	+	+	½+	½+	(?)	(?)	+	+	++++	+++	½+	+	+++	(-)	+++

Table 13 (continued)

Org.	Peptones																				
	P	W	PD	S	AS	AP	AB	AC	AI	PL	CH	CB	DT	Dl	DP	DY	DN	DPr	DPP	M-1	M-2	B-1
Sg-2	(-)	+++	½+	++	++	+++	+++	+++	++	½+	++	+++	+++	+++	+	+	+++		-	++	+++	++
Shs	(-)	½+	(-)	½+	(-)	++	(?)	½+	++	+	(-)	(-)	+	++	½+	++	++		tr.	½+	++	+
Sd-Y	(-)	tr.	(-)	(-)	(-)	(-)	-	(?)	tr.	(-)	(-)	(-)	½+	(-)	(-)	½+	-		+	-	tr.	tr.
Sd-B	(-)	½+	(-)	(-)	(-)	(-)	(-)	½+	tr.	(-)	(-)	(-)	½+	(-)	(-)	½+	½+		½+	tr.	(?)	tr.
Sl	+++	+++	++++	(-)	(-)	++++	+++	++	+++	+++	+++	++++	++++	+++	(-)	+++	+++		+++	½+	(-)	+
Cs	(-)	+	(-)	-	-	++++	++	++	++	-	½+	++	*	½+	(-)	½+	+		-	½+	(?)	½+
Pl	(?)	+++	½+	++	++	+++	+++	++++	++	++	++	++	+++	+	++	++	++		-	++	++	++
P292	+	+++	++	++	++	+++	+++	+++	++	++	+++	+++	++	++	++	++	++		-	++	++	++

+ = blackening along line of inoculation

* = blackening diffuses into media

(-) = no growth

they were omitted from Table 14. Wilson, Armour infusion, and Witte's peptones were dark colored and cloudy which made observations very difficult. Parke Davis and Cudahy beef peptones gave the best hydrogen sulfide production when ferric citrate was used as an indicator. Proteose-peptone gave a number of traces of hydrogen sulfide, but the results were not easily interpreted. Stearns, Armour, Armour pancreatic digest of casein, Cudahy protein, Bacto-tryptone and Bacto-protone peptones gave no positive tests, and were omitted from Table 14.

Comparison of Methods:

In Table 15 the peptones are compared as to the number of hydrogen sulfide positive tests which were obtained by each of the three methods. From the results it can be seen that the indicator used for the detection of hydrogen sulfide must be taken into account when a suitable peptone is being chosen. Some peptones such as Cudahy hog protein peptone, protone, and Witte's peptone are not suited to demonstrate the presence of hydrogen sulfide with any of the three indicators.

Table 14

Hydrogen Sulfide Production from the Various Peptones Using Ferric Citrate as the Indicator

Org.	Peptones																
	P	W	PD	AP	AB	AI	PL	CH	CB	DT	Dl	DP	DN	WT	DPP	B-1	M-1
Pv-1	(?)	-	½+	½+	-	-	-	-	**	½*	tr.	-	-	-	*	-	-
Pv-2	-	-	tr.	-	-	-	tr.	-	tr.	-	tr.	-	-	-	-	-	-
Et-R	tr.	-	½+	-	-	-	½+	-	tr.	**	tr.	tr.	-	-	*	-	-
Et-H	-	-	½+	-	-	-	tr.	-	½*	**	tr.	(?)	-	-	½*	-	-
Ao	*	½+	*	½*	-	½+	½*	tr.	tr.	-	tr.	-	-	-	-	tr.	-
Aa-1	-	-	*	½*	-	-	-	-	½*	-	tr.	-	-	-	-	-	-
Aa-2	-	-	*	½*	-	-	-	-	½*	-	tr.	-	-	-	-	-	-
Sa-a	***	-	****	**	tr.	-	**	**	***	****	***	-	tr.	*	***	½*	½*
Saal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ss	-	-	½*	½*	-	-	-	tr.	*	-	tr.	-	-	-	-	-	-
Ss-1	***	½*	****	***	tr.	-	**	***	***	***	**	tr.	tr.	*	***	½*	*
Sg-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 14 (continued)

Org.	Peptones																
	P	W	PD	AP	AB	AI	PL	CH	CB	DT	Dl	DP	DN	WT	DPP	B-l	M-l
Sg-2	½+	-	*	tr.	-	-	*	tr.	½*	tr.	tr.	-	-	tr.	*	-	-
Shs	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sd-Y	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sd-B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sl	(-)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cs	**	tr.	***	**	tr.	-	**	*	***	**	*	tr.	tr.	***	***	½*	½*
Pl	*	-	*	tr.	-	-	*	½*	½*	½*	tr.	-	-	-	*	(?)	tr.
P292	*	-	*	tr.	-	-	*	½*	½*	½*	tr.	-	-	-	*	(?)	tr.

+ = blackening along line of inoculation

*. = blackening diffuses into media

(-) = no growth

Table 15

Comparison of the Number of Positive Hydrogen Sulfide Tests Using Lead, Bismuth, and Iron as Indicators

Peptones	Indicators		
	Lead Acetate	Bismuth Sulfide	Ferric Citrate
P	10	8	8
W	19	20	3
PD	18	13	14
S	14	15	0
AS	15	11	0
AP	12	16	11
AB	12	17	3
AC	3	19	0
AI	0	20	1
PL	20	14	10
CHP	3	3	0
CH	14	15	8
CB	14	5	14
DT	12	19	9
Dl	15	18	14
DP	13	12	3
DY	5	20	0
DN	1	19	3
DPr	0	6	0
WT	0	0	4
DPP	9	13	9
M-4	14	19	5
B-1	17	20	6

DISCUSSION

The various peptones differed considerably in their ability to support growth of bacteria at low concentrations. Likewise, the organisms also vary among themselves in their ability to grow in the presence of low concentrations of peptones. The minimum concentration of most of the peptones for the majority of the organisms was somewhere below 0.001 per cent. The minimum concentration of most of the peptones tested, necessary for the growth of Shigella dysenteriae, was somewhere between 0.1 and 0.01 per cent. The minimum concentration of most of the peptones for Proteus vulgaris, Streptococcus lactis, and Salmonella pullorum was somewhere between 0.01 and 0.001 per cent. Of the ten organisms which showed good growth in 0.001 per cent peptone media, most were still exhibiting some growth when the peptone content was lowered to 0.00005 per cent.

It is difficult to account for the growth of the organisms in concentrations of peptones as low as 0.00005 per cent. Possibly there were enough nutrients carried over with the inoculum to initate growth, but this hardly seems possible since all tubes were inoculated from suspensions in physiological salt solutions. Most of the organisms which grew in these low concentrations are proteolytic and possibly are able to utilize more of the molecule and thus require less peptone. Another possibility is the presence of a growth promoting factor which would permit the growth of these organisms in low concentrations of peptone.

It was found that of the nine strains of Salmonella pullorum tested, all failed to grow in 0.01 per cent concentration with the six

peptones. Koser et al (1938) listed either nicotinic acid or nicotinamide as growth promoting factors of the dysentery organisms. It was thought that perhaps these peptones were low in, or lacking in this factor, but the addition of nicotinic acid did not improve growth.

Bacto-peptone was extracted with various solvents in the hope that a toxic substance, a growth promoting factor, or an essential amino acid might be removed. As far as growth supporting properties were concerned all extracts and extracted residues gave the same amount of growth as the unextracted Bacto-peptone. The methyl alcohol extracted residue gave less hydrogen sulfide production by the lead acetate method. This would correlate well with the report of Redfield (1915) who stated that much less hydrogen sulfide was produced from the alcohol insoluble peptone than from the unextracted peptone. However, in media containing bismuth sulfite or ferric citrate, both the methyl alcohol extract and extracted residue and the benzene extracted residue gave better hydrogen sulfide production than media containing the unextracted peptone.

Acetyl methyl carbinol is produced by some bacteria as a result of the fermentation of glucose. It is probably formed by the condensation of two molecules of acetaldehyde. In the Voges-Proskauer test, the acetyl methyl carbinol is oxidized to diacetyl by the alkali. This condenses with constituents of the peptone containing the guanidine nucleus to give a red color which indicates a positive test. The peptone is important in this test only as it promotes growth of the organism and furnishes compounds containing the guanidine nucleus. The tests for acetyl methyl carbinol were good in all the peptones.

Herzfeldt and Klinger proved there was a stoichiometric relationship between the indol formed by bacteria and the tryptophane disappearing from the media. Woods (1935) using thick washed suspension of Escherichia coli, showed that l(-) tryptophane is converted quantitatively to indol, and that the rates of disappearance of tryptophane is identical with the rate of production of indol.

A peptone, then, must contain enough of the amino acid for the organism to produce indol. Armour pancreatic digest of casein gave the clearest positive tests. Eight other peptones gave very good results.

Hydrogen sulfide production by bacteria is influenced by several factors. Media containing the same peptone may produce entirely different results when different indicators are used. For example, of the twenty organisms tested none gave positive tests for hydrogen sulfide when iron was added as an indicator with Stearns peptone, but fourteen organisms gave positive tests when bismuth and lead were incorporated as indicators. An explaination for this might be that the enzyme system which causes the production of hydrogen sulfide was inactivated by the indicator in some manner, or the indicator combined with the sulfur compounds to make them unavailable. Very few organisms produced hydrogen sulfide when Cudahy hog protein, Bacto-protone or Witte's peptones were used, no matter which indicator was employed. They obviously do not contain sulfur compounds available for the production of hydrogen sulfide.

From these studies it would appear that on the whole all peptones employed in these studies gave better hydrogen sulfide production when bismuth sulfite was used as an indicator than when lead acetate or

ferric citrate were used. The production of hydrogen sulfide depends upon the peptone used as well as the detector ion.

SUMMARY

1. The growth supporting properties of the peptones under investigation varied a great deal.
2. The minimum concentration of peptone necessary for the growth of Shigella dysenteriae, Proteus vulgaris, Streptococcus lactis, and Salmonella pullorum was determined.
3. Peptones appear to contain a substance which is toxic to Salmonella pullorum or else they lack a growth stimulating substance necessary for the propagation of this organism. The absence of nicotinic acid from the peptones does not seem to be the factor involved.
4. Extraction of Bacto-peptone with several organic solvents did not effect its growth supporting properties.
5. All the peptones tested contained enough compounds possessing the guanidine nucleus to give good tests for acetyl methyl carbinol when the latter was produced by bacteria.
6. Several peptones were found which yielded superior tests for the demonstration of indol production.
7. The production of hydrogen sulfide in the various peptones was compared employing three different media. All the peptones under investigation produced better results when bismuth sulfite was the indicator than when ferric citrate was employed.

BIBLIOGRAPHY

Able, J. J. and Geiling, E. M. K. 1924
Some hitherto undescribed properties of the constituents of Witte's peptone.
Jour. Pharmacol. 23. 1-27.

Almy, L. H. and James, L. H. 1928
A method for the study of the formation of volitile sulfur compounds by bacteria.
Jour. Bact. 12. 319-331.

Andrews, J. C. 1937-38
Reduction of certain sulfur compounds to hydrogen sulfide by the intestional microorganisms of the dog.
Jour. Bio. Chem. 122. 687-692.

Barritt, M. M. 1936
The intensification of the Voges-Proskauer reaction by the addition of alpha naphthol.
Jour. Path. and Bact. 42. 441-454.

Bedford, R. H. 1929
A rapid method for obtaining the Voges-Proskauer reaction.
Jour. Bact. 18. 93-94.

Blanchetiere, A. 1927
Composition of peptones.
Compt. Rend. Soc. Biol. 96. 381-383.

Chamot, E. M. and Georgia, F. R. 1925
Hydrogen-ion concentration and peptones used in Bacteriology.
Jour. Amer. Water Works Ass. 13. 661-674.

Chamot, E. M. and Redfield, H. W. 1915
Studies on the culture media employed for the bacteriological examination of water. I The Schardinger-Dunham medium for testing the presence of hydrogen sulfide forming bacteria.

Ciaccio and Trimarchi 1927
The hyperglucemia action of certain protein degradation products.
Bull. soc. ital. biol. sper. 2. 780-783.

Clock, F. M. and Tanner, F. W. 1938
Studies on the bacterial metabolism of sulfur. III Formation of hydrogen sulfide by thermophilic bacteria.
Centralbl. f. Bakt II 98. 298-311.

Colwell, R. C. 1915
Comparative analysis of several peptones.
Science 41. 662.

Day, A. A. and Walker, A. W. 1913
The selective action of certain bacteria on peptone.
Jour. Med. Res. 28. 465-470.

Difco Manual of Dehydrated Culture Media and Reagents 1939
Difco Laboratories
Detroit, Mich.

Dubos, Rene 1930
The bacteriostatic action of certain components of commercial peptones as affected by conditions of oxidation and reduction.
Jour. of Exp. Med. 52. 331-345.

Fellers, C. R., Shostrom, C. E. and Clark, E. D. 1924
Hydrogen sulfide determination in bacterial cultures and in certain canned foods.
Jour. Bact. 9. 235-249.

Gerini, Costantino 1916
Influence of the quality of peptone on bacterial function.
Arch. farm. sper. 21. 209-214 (Chem. Abs. 10. 1875).

Hunter, C. A. and Creecelius, H. G. 1938
Hydrogen sulfide studies I Detection of hydrogen sulfide in cultures.
Jour. Bact. 35. 185-196.

Kahn, M. C. 1925
Hydrogen sulfide production by anaerobic spore-bearing bacteria.
Jour. Bact. 10. 439-447.

Koser, S. A., Dorfman, A. and Saunders, F. 1938
Nicotinic acid as an essential growth substance for dysentery bacilli.
Proc. Soc. Exp. Bio. and Med. 38. 311-313.

Kulp, W. L. 1925
Indol studies.
Jour. Bact. 10. 459-471.

Levine, Max 1921
Notes on Bact. coli and Bact. aerogenes
Amer. Jour. Pub. Health 11. 21-23.

Levine, Max 1937
Laboratory Techniques in Bacteriology p.231.
MacMillan Company.

Levine, M., Vaughn, R., Epstein, S. S. and Anderson, D. O. 1932
Some differential reactions in the colon-aerogenes group of bacteria.
Proc. Soc. Exp. Biol. and Med. 29. 1022-1024.

McAlpine, J. G. and Brigham, G. D. 1923
Some chemical studies of commercial bacteriological peptones.
Jour. Bact. 16. 251-256.

Myers, J. T. 1920
The production of hydrogen sulfide by bacteria.
Jour. Bact. 5. 231-252.

Norton, J. F. and Sawyer, M. V. 1921
Indol production by bacteria.
Jour. Bact. 6. 471-478.

O'Meara, R. A. Q. and Macsween, J. C. 1937
The influences of copper in peptones on the growth of certain pathogens in peptone broth.
Jour. Path. and Bact. 44. 225-234.

Paine, F. S. 1927
The destruction of acetyl methyl carbinol by members of the colon-aerogenes group.
Jour. Bact. 13. 269-274.

Perau, H. and Simonnet, M. 1922
Simple diets deficient in fat-soluble factor A. Importance of the albuminoid material employed.
Bull. soc. chem. biol. 4. 192-205 (Chem. Abs. 16. 2883).

Penfold, W. J. and Norris, D. 1912
Relation of concentration of food supply to the generation time of bacteria.
Jour. Hyg. 12. 527-531.

Pepin, G.
Physical constants and analysis of some commercial peptones.
Bull. soc. pharmacology 17. 594-599 (Chem. Abs. 5. 2857).

Pierce, H. B. 1931
The effect of yeast on ammonia and indol production by bacteria in cultures on feces suspensions.
Jour. Bact. 21. 225-237.

Redfield, H. W. and Huckle, C. 1915
A comparative study of methods for the quantitative determination of sulfur in peptones.
Jour. Amer. Chem. Soc. 37. 607-611.

Redfield, H. W. and Huckle, C. 1915
Quantitative determinations of sulfur in the culture medium for the detection of the bacteria producing hydrogen sulfide.
Jour. Amer. Chem. Soc. 37. 612-623.

Regnier, J. and Lambin, S. 1938
Influence of the quantity of nutritive substances in the medium on the multiplication of bacteria. Colon bacillus cultures in liquid media containing variable amounts of peptone.
Compt. rend. soc. biol. 128. 883-884 (Chem. Abs. 32. 9146).

Robbins, W. J. 1941
Further observations on factor "Z".
Bot. Gazette 102. 520-535.

Segal, B. 1940
The utilization of acetyl methyl carbinol by Staphylococcus albus and aureus.
Jour. Bact. 37. 747-749.

Standard Method for Water Analysis 1936 8th Ed.
Am. Pub. Health Ass.
N. Y.

Tarr, H. L. A. 1933
The enzymic formation of hydrogen sulfide by certain heterotrophic bacteria.
Biochem. Jour. 27. 1869-1874.

Tarr, H. L. A. 1934
The enzymic formation of hydrogen sulfide by certain heterotrophic bacteria II.
Biochem. Jour. 28. 192-198.

Tilley, F. W. 1921
Influence of peptone on indol formation by Bacillus coli.
Amer. Jour. Pub. Health 11. 834-836.

Tilley, F. W. 1923
Variations in hydrogen sulfide production by bacteria.
Jour. Bact. 8. 115-120 (1923).

Tilley, F. W. 1923
The relation between chemical composition of peptones and hydrogen sulfide production by bacteria.
Jour. Bact. 8. 287-295.

Tittsler, R. P. 1931
The effect of temperature upon the production of hydrogen sulfide by Salmonella pullorum.
Jour. Bact. 21. 111-117.

Tittsler, R. P. 1938
The fermentation of acetyl methyl carbinol by the Escherichia-

Aerobacter groups and its significance in the Voges-Proskauer reaction.
Jour. Bact. 35. 157-162.

Topshtein, R. E. 1941
Preparation of bacteriological peptones and comparison of chemical compositions.
Microbiology (U.S.S.R.) 9. 197-205 (Chem. Abs. 35. 487)

Utermohlen, W. P. Jr. and Georgi, C. E. 1940
A comparison of cobolt and nickel salts with other agents for the detection of hydrogen sulfide in bacterial cultures.
Jour. Bact. 40. 449-459.

Vaughn, R. and Levine, M. 1936
Hydrogen sulfide production as a differential test in the colon group.
Jour. Bact. 32. 65-73.

Williams, O. B. and Morrow, M. B. 1928
The bacterial destruction of acetyl methyl carbinol.
Jour. Bact. 16. 43-48.

Williams, O. B. 1930
Spore formation by Bacillus subtilis as influenced by peptone concentration.
Jour. Bact. 19. 11 (abs)

Woods, D. D. 1935
Indol formation by Bacterium coli I The breakdown of tryptophane by washed suspension of Bacterium coli.
Biochem. Jour. 29. 640.

Yaoi, H. 1926
The cystine content of peptones for bacteriological use.
Japan Med. World 6. 114-116 (Biol. Abs. 3. 20758).

ZoBell, C. E. and Feltham, C. B. 1934
A comparison of lead, bismuth and iron as detectors of hydrogen sulfide production by bacteria.
Jour. Bact. 28. 169-176.