THE PREPARATION AND THE CHEMICAL AND BACTERIOLOGICAL ANALYSIS OF ANIMAL AND VEGETABLE PEPTONES

by

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INTRODUCTION*

During the early days of bacteriology the nitrogen requirements of bacteria grown by artificial means were met by the addition to media of such naturally occurring substances as blood, urine and other body fluids. Naegeli (33) in 1882 was probably the first to use egg albumin, which he called "peptone", as a source of nitrogen. Later it was found that "peptones", derived from the partial digestion of proteins, would furnish organic nitrogen in a more available form. Since then, peptones and related products have been utilized on an increasingly greater scale.

In recent times new and better peptones have been made available to the bacteriologist. As a general rule the methods of preparation and the source materials of these peptones have been kept secret. Except for the several Difco products, their chemical composition is not available. The desire and need for media prepared from chemically pure constituents has created an interest in the chemical composition of peptones. While it is doubtful that a chemically pure peptone will ever be produced by present methods, it is desirable to know as much as possible of their preparation and chemical make-up. Therefore peptones were prepared from various animal tissues and from samples of hydrolyzed and unhydrolyzed corn gluten. These peptones, as well as samples of twenty-four commercial brands, were analyzed for various nitrogen fractions, and were tested bacteriologically for their ability to support the growth of bacteria.

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PREPARATION OF ANIMAL AND VEGETABLE PEPTONES

The methods of preparing peptones, as found in the literature, fall into three general groups as follows: (1) hydrolysis of proteins by enzymes; (2) hydrolysis of proteins by acids and alkalies and (3) combinations of these two methods. Examples of the preparation of peptones by any one of these methods are numerous.

Berthelot (4) mixed equal weights of finely minced pig pancreas, pig intestinal mucosa and lean beef and allowed these to stand in a 3:100 Na_2CO_3 solution at 40° C. until the digestion was completed. The filtered broth was then evaporated to dryness *in vacuo* below 45° C. The peptone obtained was found to be especially suitable for the growth of intestinal organisms.

Soparkar (51) prepared a satisfactory substitute for Witte's peptone by digesting casein with enzymes obtained from the pancreatic glands of goats. After a few days digestion at 40° C. the enzymes were inactivated by boiling and the extract, consisting of a mixture of proteoses, peptones, polypeptides and amino acids, was evaporated to dryness and pulverized.

Boez (9) prepared a peptone especially suitable for the growth of *Mycobacterium tuberculosis* by mixing 500 grams each of lean beef, fresh pig pancreas and pig intestines with six grams of Na_2CO_3 and 40 grams of chloroform in two and one-half liters of water, and allowing the mixture to digest for 48 hours at 37° C. The digestion was arrested by making the mixture slightly acid with HCl, after which the solution was filtered and concentrated. The high amino acid content was responsible for the superiority of this medium over others for the growth of the tubercle bacillus.

Douglas (14) heated ground beef heart to 70 to 80° C., cooled and added trypsin. The mixture was then digested for two to three hours after which

it was made slightly acid with HCl and brought to 100° C. to precipitate out unaltered albumins and then filtered. Douglas obtained very good growth of the typhoid bacillus by use of this solution.

Utkin (57) found that the by-products obtained from the preparation of insulin and spermin would yield from 13 to 25 per cent of a high grade peptone when these by-products were hydrolyzed with pepsin and/or trypsin.

Itzioka (26) prepared a "tryptone" from casein by the action of rabbit pancreatic juice. Martin's peptone, prepared by the peptic digestion of whole hog stomachs, was found by Mustafa (32) to be superior to Witte's peptone for use in detecting indol production by E. coli.

Hucker and Carpenter (25) subjected finely ground beef heart to pepsin-HCl digestion for a period of eleven hours, after which the pH was adjusted to 7.4, trypsin added and the digestion allowed to continue for 36 hours. Samples removed at various time intervals to test their ability to support bacterial growth were heated to 90° C., dried in vacuo and powdered. Raw lean beef was also digested by trypsin alone and after digestion treated as was the heart muscle digest.

Grabar (19) obtained a phosphorus rich (2.2 per cent) peptone by the incomplete tryptic digestion of casein. The yield obtained was about five per cent of a peptone containing 13.1 per cent nitrogen.

Wallis (60) prepared a substitute for "Nutrose" by digesting casein and peanut flour with trypsin in the presence of Na_2CO_3. The resulting peptone was used for making Conradi-Drigalski medium for growth of the colon-typhoid group. The growth promoting properties of this peptone appeared to be due to the presence of a vitamin associated with the globulin of the peanut flour.

An excellent substitute for Witte's peptone was prepared by von Gutfeld (23) by digesting coagulated and washed cakes of horse serum and horse and human blood with pancreatin. Strauss (52) also prepared a substitute for Witte's peptone by digesting fibrin with trypsin.

Leifson and Diamond (29) prepared 115 peptones from beef, beef heart, beef spleen, beef lung, pork, hog stomach, fish, casein, wheat gluten and soybean flour. These materials were digested by pepsin, pancreatin and papain and by combinations of these enzymes. Some of the peptones thus prepared were found to be superior to the commercial brands with which they were compared.

Sadikov (47) prepared peptones by heating various proteins in aqueous solutions of $(NH_4)_2CO_3$ or NH_4OH in an autoclave for two to twelve hours at 150 to 180°C. The dried hydrolysates obtained by this procedure consisted chiefly of peptones. If the mixture was heated 24 hours, amino acids were formed. The peptones obtained from fibrin and casein supported good growth of various types of bacteria.

Grand and Lewis (20) obtained two widely different peptones when silk was hydrolyzed at 30°C. with 70 per cent H_2SO_4 for 65 to 70 minutes. One of the peptones had a low amino nitrogen content and contained more tyrosine than the original silk (12.3 per cent) while the other had a tyrosine content similar to that of many common proteins (2.5 to 5.7 per cent) and had a high amino nitrogen content.

Piccioni (38) peptonized commercial gelatin in the presence of H_2SO_4 for 80 to 100 hours, after which the solution was heated to 100°C., filtered and concentrated. Bacterial and chemical tests indicated that this method produced a satisfactory peptone.

Bramigk (10) added three liters of water and 15 ml. of H_2SO_4 to the fibrin obtained from one "pailful" of coagulated blood, and allowed the mixture to stand overnight. It was then poured into three liters of water containing 18 ml. of H_2SO_4 and heated to 50° C. The extract of the mucosa of two hogs, prepared at 35° C., in a liter of sterile water, was added and the whole digested at 37° C. for 48 hours. The resulting liquid was neutralized with either NH_4OH or $Ba(OH)_2$, clarified by heating slightly, filtered and evaporated *in vacuo*. Bramigk states that the peptone thus obtained was identical with Witte's peptone.

The utilization of plant proteins for the production of bacteriological peptones has received some attention. Snyder (49) placed convenient amount of air dried plant material in the bottom of a test tube, covered these with selenium oxychloride and heated gently over a low flame. When the material was completely peptonized it was cooled and poured into a liter of sterile distilled water and allowed to settle over night. The clear supernatant was then decanted and the precipitated material centrifuged, washed and taken up in five ml. of sterile water. Small quantities of this material, when placed in petri plates as they were poured, increased the growth of certain plant pathogens.

Berthelot and Amoureux (5) prepared a peptone by the action of pepsin and HCl on peanut press cake. This peptone was rich in arginine and supported excellent growth of several organisms. These authors, with van Deinse (7), prepared a peptone from soybean press cake by a similar method. The high content of soluble carbohydrates present in the peptone made it especially suitable for the growth of certain organisms.

Sadikov and Sinitzuin (48) prepared peptones from yeast by autolysis and by heating in the autoclave at 150° C. in the presence of 0.1 to 0.5

per cent aqueous H_3PO_4. These peptones gave highly virulent cultures of B. danich and B. mereshkovski.

Preparation of Animal Peptones

The peptones were prepared from various bovine tissues including lean muscle, heart, liver, spleen and brain and from lean pork. The finely ground tissues were suspended in distilled water in a six liter Pyrex flask and the mixture brought to a pH of 1.0 to 1.2 by the addition of concentrated HCl. One-half gram of granular pepsin (Difco, 1:10,000) was stirred in and the mixture allowed to digest in a 52° C. incubator for approximately 48 hours, shaking at intervals. The pH was adjusted to 1.0 to 1.2 every twelve hours by the addition of concentrated HCl. At the end of the digestion period the supernatant liquid was siphoned off, and the remaining liquid separated from the undigested residue by filtering on a Büchner funnel containing a layer of filter paper pulp. The residue was washed twice with 500 ml. of distilled water and the washings added to the peptone solution. The solution was then heated in the autoclave at 15 pounds pressure for 20 minutes to destroy the enzyme and to bring about coagulation of soluble proteins which are heat coagulable, after which it was cooled and filtered. The clear solution was concentrated in vacuo at a temperature of 55° C. to remove part of the HCl and to bring the volume approximately to one liter. This solution was then neutralized with 40 per cent NaOH to pH 7.00 at a temperature below 30° C., after which it was placed in a tall battery jar, covered with a layer of toluene and dialyzed in running tap water for 48 to 72 hours to remove the NaCl. The dialyzing membrane used was Du Pont Cellophane seamless tubing. After dialysis the solution was again brought to pH 7.00, autoclaved as before, cooled, filtered and

poured into shallow Pyrex dishes which were placed in a forced air drier. After 48 hours in the forced air drier the dishes containing the partially dried peptone were placed in a hot air oven at 80°C. for complete drying. When thoroughly dried, the peptone was scraped from the dishes, ground to a fine powder and placed in bottles. Table 1 shows the amount of tissue, pepsin, HCl and water used in preparing each peptone, together with the grams and per cent yield of peptone obtained. Four batches of beef peptone, two batches each of spleen, liver, brain and heart peptone, and one batch of pork peptone were prepared. The batches of beef peptone were mixed; batches of other peptones were kept separate. These peptones will be designated by the source material and batch number, for example, spleen peptone (1); heart peptone (2), etc.

Preparation of Vegetable Peptones

The material used in the preparation of the vegetable peptones was obtained from the Corn Products Refining Company of Argo, Illinois. It consisted of corn gluten which had been hydrolyzed by boiling with 20 per cent HCl for varying lengths of time. The various treatments were as follows: sample 1 was hydrolyzed for two hours; sample 2 for ten hours; sample 3 for 16 hours after which the glutamic acid was removed; and sample 4 for 16 hours after which the glutamic acid, leucine and tyrosine were removed.

The hydrolysates were first concentrated *in vacuo* to a thick syrup at a temperature of 55°C. The solution was then diluted to two liters and brought to pH 7.00 by the addition of 40 per cent NaOH, keeping the temperature below 30°C. It was then dialyzed to remove the NaCl, adjusted

to pH 7.00, autoclaved, cooled and filtered as were the animal peptones. The resulting solution was black due to the presence of humin nitrogen formed during hydrolysis. Therefore, to prepare a satisfactory peptone it was necessary to decolorize the solution. This was done by adding 100 grams of Norite "A" per liter of solution, boiling for 10 minutes and filtering on a Büchner funnel containing a layer of filter paper pulp. The decolorizing process was repeated if necessary, and the decolorized solution dried in the forced air drier and hot air oven as described above for animal peptones. Table 2 shows the yields of vegetable peptones obtained from the four hydrolysates. Several batches of peptone were prepared from each sample of hydrolysate, the batches being mixed after preparation. For purposes of identification these peptones were labelled according to the hydrolysate sample from which they were prepared; for example: vegetable peptone No. 1 was prepared from hydrolysate sample No. 1, etc.

In addition to the above peptones prepared from hydrolysates of corn gluten, two additional peptones were prepared from corn gluten before it was hydrolyzed. A vegetable peptone (labelled vegetable peptone No. 5) was prepared according to the technique employed for animal peptones and a vegetable tryptone (labelled vegetable tryptone) made by digesting corn gluten with trypsin in the presence of Na_2CO_3. In both cases it was necessary to allow the digestion to proceed for three weeks before it was complete. The procedure for preparing the vegetable tryptone was as follows: Nine hundred grams of the dry corn gluten was suspended in three liters of distilled water by mechanical stirring and sufficient saturated aqueous Na_2CO_3 added to bring the pH between 8.0 and 8.5. Three grams of trypsin (Difco, 1:360) was stirred in, the mixture covered with an inch layer of

Table 1. Amount of various ingredients used in preparing animal peptones showing the yield in grams and per cent obtained.

Kind of Tissue	Grams tissue*	Grams pepsin	Ml. conc. HCl	Liters water	Grams peptone obtained	Per cent yield**
Lean beef	450	0.1	20	1	15.2	16.9
Lean beef	896	0.1	32	2	36.7	20.4
Lean beef	5,453	0.5	38	5	169.0	15.5
Lean beef	5,489	0.5	36	5	219.5	19.9
Beef spleen (1)	4,894	0.5	42	5	87.1	8.9
Beef spleen (2)	4,672	0.5	39	5	207.1	21.9
Beef brain (1)	1,242	0.5	25	4	33.4	12.9
Beef brain (2)	3,780	0.5	43	4	107.2	13.9
Beef liver (1)	413	0.1	20	1	10.7	13.4
Beef liver (2)	4.180	0.5	31	5	403.0	47.7
Beef heart (1)	5,175	0.5	50	5	182.0	17.4
Beef heart (2)	3,445	0.5	46	5	257.0	36.8
Lean pork	1,400	0.5	39	5	90.0	31.8

* Wet weight of tissue

** Per cent yields figured on dry weight of tissue taken as 20 per cent of wet weight.

Table 2. Yield of vegetable peptones obtained from corn gluten hydrolysates.

Hydrolysate number	Hours hydrolyzed	Amount of hydrolysate used	Treatment of hydrolysate	Grams peptone obtained	Per cent yield obtained
1	2	1 liter 4 liters	undecolorized decolorized	77.0 505.0	7.7 12.6
2	10	1 liter 2 liters 4 liters	undecolorized decolorized decolorized	11.0 41.0 556.0	1.1 2.0 13.3
3*	16	1 liter 2 liters 4 liters	undecolorized decolorized decolorized	24.5 46.0 497.0	2.4 2.3 12.4
4**	16	1 liter 2 liters 4 liters	undecolorized decolorized decolorized	6.0 12.0 34.0	0.6 0.6 0.8

* Glutamic acid removed
** Glutamic acid, leucine and tyrosine removed.

Table 3. Amount of ingredients used in preparing vegetable peptone No. 5 and vegetable tryptone from unhydrolyzed corn gluten.

Grams dry corn gluten	Liters water	Grams enzyme	Yield in grams	Per cent yield
900	3	0.1 pepsin	335	37.2
900	3	3.0 trypsin	452	50.2

toluene, corked and placed in the 37°C. incubator for three weeks. The mixture was shaken daily and the pH adjusted to between 8.0 and 8.5 every two days. At the end of the digestion period the undigested residue was filtered off, and the solution brought to pH 7.00, autoclaved, cooled and filtered. It was then dialyzed and dried as described for animal peptones. Table 3 shows the amounts of material used in preparing these vegetable peptones, together with the grams and per cent yield obtained.

Discussion

The various tissues were selected for preparing the animal peptones with the idea in mind that certain of the tissues would yield a peptone especially suitable for the growth of certain organisms. The bacteriological results, especially the growth of pathogenic organisms, demonstrated that this theory is incorrect. The data obtained would indicate that the value of a peptone depends not so much on the original tissue used in its preparation as on the treatment accorded the tissue during the preparation of the peptone.

An examination of Table 1 shows that the per cent yield varies from 8.9 for one batch of spleen peptone to 47.7 for one batch of liver peptone. Table 1 also shows that the per cent yield for two different batches of the same peptone varies considerably. Variation in the per cent yield between tissues might be expected; variation in the per cent yield when using the same tissue is not so easily explained. The batches of peptone were prepared under as nearly identical conditions as possible. These variations may be partially explained on the basis of differences in the composition of the tissues themselves, and some variations may be expected to enter in the preparation of the peptones, especially during

dialysis. Undoubtedly some amino acids and lower peptides as well as other forms of nitrogen were lost during dialysis. However, it is unlikely that this loss would account for as large a variation in per cent yield as is found in the beef liver peptone, whereas, it could account for the difference in per cent yield obtained for the beef brain peptone. Certainly the dialysis is the one step in the preparation of the peptones that is most unpredictable as to its influence on the yield of the peptone. No attempt was made to determine the amount of the various forms of nitrogen lost during dialysis. It may be great or small, depending upon the tissues used and upon the extent of hydrolysis. A one hundred per cent yield could not be obtained because not all of the tissue was digested by the pepsin.

While the procedure used for the commercial production of peptone is secret, it is felt that if the yield of peptones under reasonably controlled laboratory conditions varies with different batches, so too could different batches of commercial peptones vary in yield and composition. Since it is practically impossible to obtain any two samples of raw materials that are identical in every respect, especially when the samples do not come from the same source, it will be equally impossible, even under ideal conditions, to prepare two batches of peptone that will be identical in composition.

An examination of Table 2 shows that the yield of vegetable peptones from corn gluten hydrolysates No. 1,2 and 3 was larger when larger amounts of the hydrolysate was used. The reason for this increased yield if not clear. Hydrolysate No. 4 gave a lower yield because the treatment to remove the amino acids diluted the protein content, resulting in less

dry matter per liter of solution than was found in the other three hydrolysates.

As in the case of the animal peptones, some nutrient materials were undoubtedly lost during dialysis, especially from those hydrolysates that had been hydrolyzed for more than ten hours, because here most of the material was in the form of amino acids which were dialyzable to more or less extent.

The decolorization of the vegetable peptones undoubtedly causes the loss of a small amount of certain essential growth factors, as is shown by the growth curves given in Table 4 and in Figures 1,2 and 3. (The method of determining the rate of growth of E. coli is given under Bacteriological Analysis.) The organisms grown in decolorized media show a much longer lag phase than do those grown in the undecolorized media, indicating that the materials removed by decolorization are essential in the initial stages of growth for E. coli.

Table 4. The rate of growth of E. coli in decolorized and undecolorized vegetable peptone broth, with Bacto-peptone as control. (Average of five trials)

Peptone	Number of bacteria per ml.					
	0 hours	2 hours	6 hours	12 hours	24 hours	48 hours
No.2 ud.	11	37	27,000	2,000,000	138,000,000	342,000,000
No.2 d.	10	12	2,800	80,000	11,000,000	30,000,000
No.3 ud.	10	47	31,000	10,000,000	188,000,000	424,000, 000
No.3 d.	10	12	3,100	150,000	4,600,000	55,000,000
No.4 ud.	10	23	16,000	940,000	38,500,000	185,000,000
No.4 d.	10	11	1,400	175,000	450,000	4,600,000
Bacto-peptone	12	35	14,500	32,000,000	279,000,000	513,000,000

d. = Decolorized peptone

ud. = Undecolorized peptone

Figure 1

Growth curves for *E. coli* in broth prepared from decolorised and undecolorised vegetable peptone No. 2

Control: Bacto-peptone

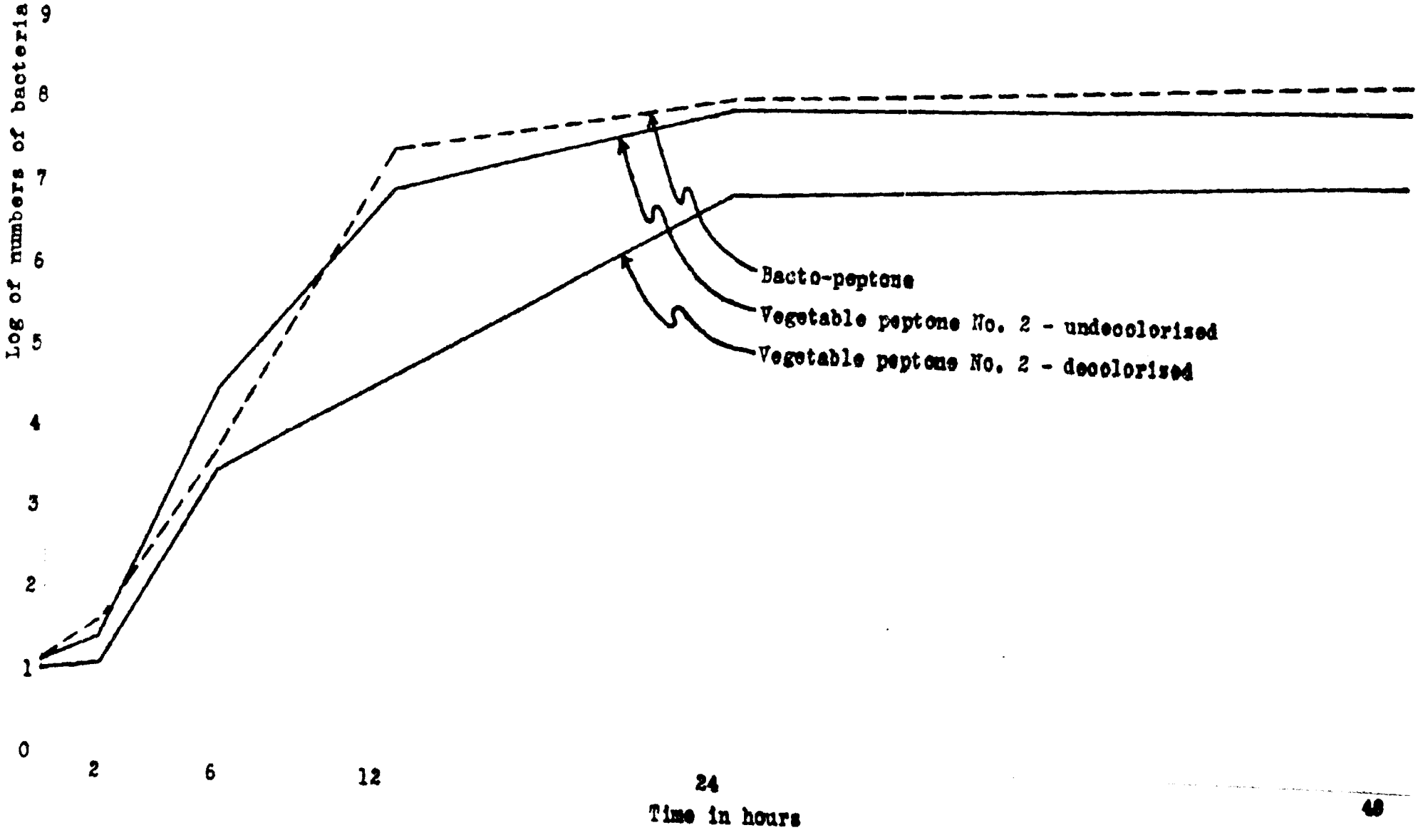


Figure 2

Growth curves for *E. coli* in broth prepared from decolorized and undecolorized vegetable peptone No. 3

Control: Bacto-peptone

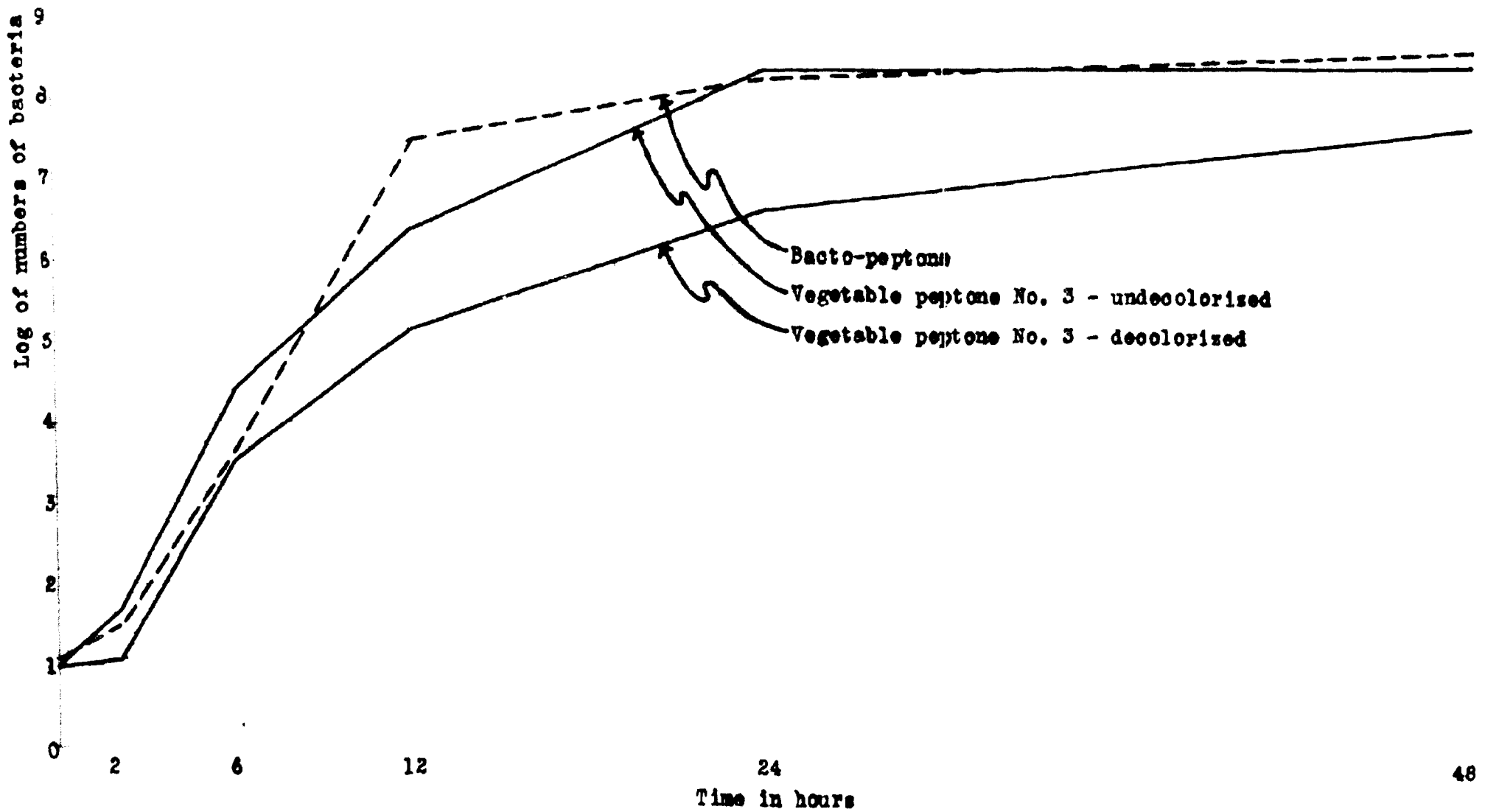
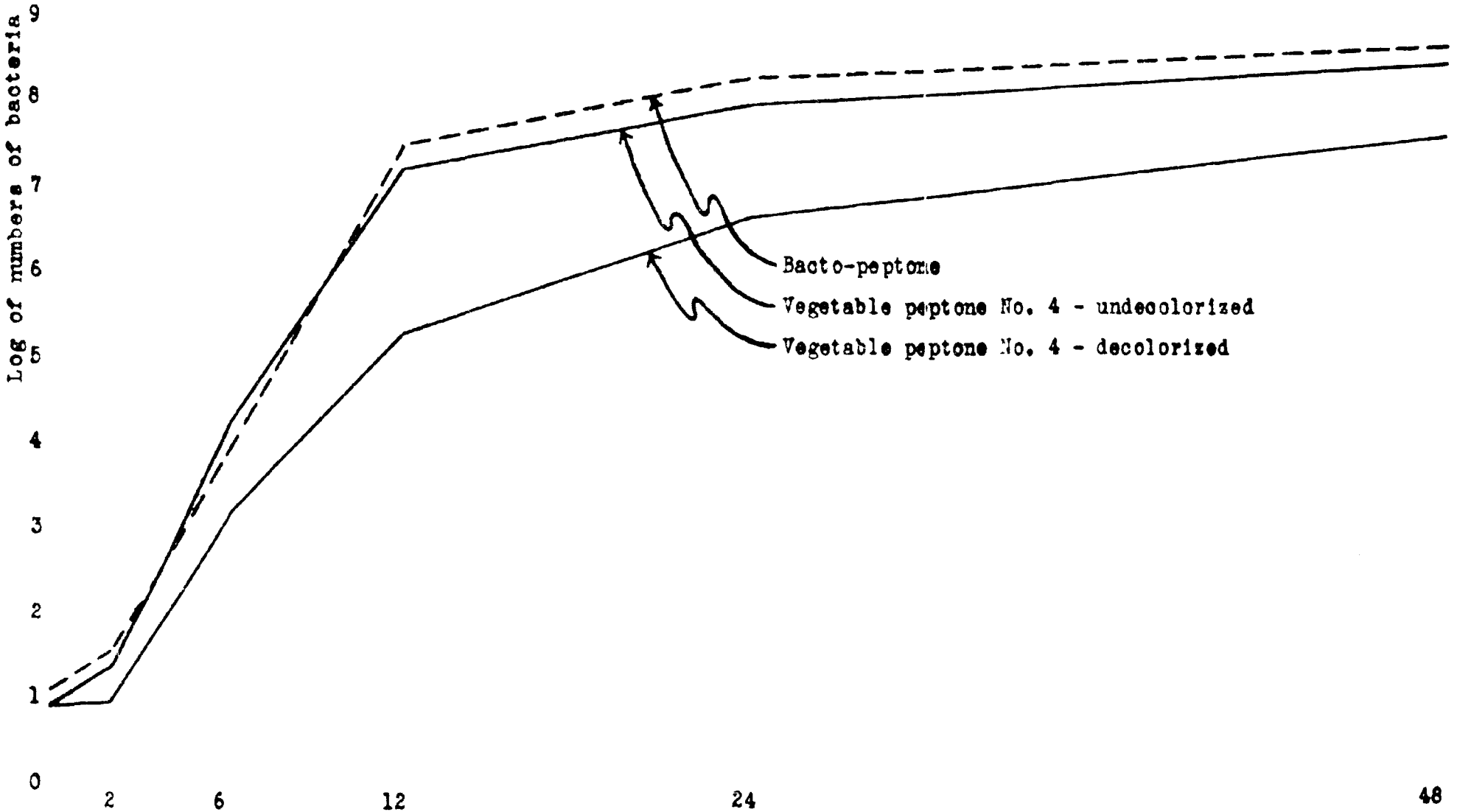


Figure 3

Growth curves for *E. coli* in broth prepared from decolorized and undecolorized vegetable peptone No. 4

Control: Bacto-peptone



SUMMARY

A method is given whereby peptones may be made from various animal tissues by digestion with pepsin in a medium made acid by HCl. Methods for the preparation of vegetable peptones from hydrolyzed and unhydrolyzed corn gluten are also given. The results show that: (1) The per cent yield of peptone obtained varies considerably even when two batches of peptone are made by the same method from the same source material. (2) The per cent yield of vegetable peptones prepared from samples of hydrolyzed corn gluten was larger when larger amounts of the hydrolysates were used. (3) Decolorization of the vegetable peptones prepared from hydrolyzed corn gluten resulted in the loss of nutrient materials as shown by the inferior growth and increased lag phase of E. coli in the decolorized as compared with the undecolorized peptones.

THE CHEMICAL ANALYSIS OF PEPTONES

Considerable work has been done on the chemical composition of peptones and related substances. However, with a few exceptions, the work has been limited to one constituent of the peptones and to a very few different brands. The most complete analysis of a large group of peptones may be found in the Difco Manual, published by the Digestive Ferments Company of Detroit, Michigan. McAlpine and Brigham (31) have given a fairly complete nitrogen distribution of four peptones, including Fairchild's, Witte's, Difco Bacto- and proteose-peptones. These authors analyzed the peptones mentioned for total nitrogen, non-protein nitrogen, ammonia nitrogen and amino nitrogen. The protein and polypeptide nitrogen fractions were mathematically determined from these analyses. The total nitrogen content of the peptones was found to be approximately the same, but the content of the nitrogen fractions differed greatly in the various brands.

Hucker and Carpenter (25) determined the total nitrogen and amino nitrogen in samples of digested beef heart muscle. Their results showed that as long as the digestion mixture remained acid the total nitrogen content remained fairly constant. However, as soon as the digestion mixture was made alkaline, the total nitrogen content decreased rapidly, due to loss of ammonia, and, as would be expected from a tryptic digestion, the amino nitrogen content increased rapidly.

Blanchetière (8) determined the diketopiperazine nitrogen in eight peptones by Kjeldahl analysis of the solution which remained after the amino acids and peptides were precipitated out by $Ba(OH)_2$. He found from 1.82 to 4.35 per cent of this type of nitrogen to be present in the peptones.

Tilley (53) stated that commercial peptones contain unoxidized, partly oxidized and oxidized sulphur compounds. No hydrogen sulfide was liberated by bacteria from compounds containing oxidized sulphur, but it was given off freely from those compounds containing partly oxidized or unoxidized sulphur. The peptones used were not listed as to brands.

O'Meara and Macsween (34) (35) found that copper and iron present in commercial peptones hindered the growth of all Gram positive organisms tested. Again the peptones were not listed as to brands.

Yaoi (62) showed that commercial peptones differed greatly in their cystine content. Witte's contained the greatest amount, followed by Gehe's, Riedel's and Shiono's peptones. Basle peptone was third and Champoteant's, Tamba's and Billault's were in fourth place with approximately equal amounts. Less cystine was found in Terunchi's, Difco and May-Baker's peptones than in any of the peptones analyzed.

Fürth and Deutschberger (18) found Witte's peptone and fibrin contained seven per cent arginine.

Wherry (61) found Witte's and Grübler's peptones contained nitrites and nitrates in sufficient quantities to interfere with tests for the production of these substances by bacteria.

Abel and Geiling (1) prepared primary and secondary albuminoses from Witte's peptone by repeated salting out in acid and alkaline mediums with $(NH_4)_2SO_4$ and precipitation with ethyl alcohol. A more toxic fraction, which was not precipitated by $(NH_4)_2SO_4$ in acid-alcohol and which contained histamine, was also prepared. The authors state that the albuminoses thus prepared cannot be regarded as chemical entities.

Underhill and Gross (56) separated a solution of Witte's peptone into various fractions by electrolysis. The solution obtained from the

anode chamber was acid to litmus; low in ash content; low in tryptophane as compared with the original solution; and low in basic nitrogen, especially arginine and lysine. The solution obtained from the cathode chamber was alkaline to litmus; high in ash content; higher in tryptophane than that of the anode material, but lower than the original solution; and high in basic nitrogen, especially arginine and histidine. The solution obtained from the center chamber was neutral to litmus.

Kitamura (28) found from 0.6 to 1.0 per cent of free sugar, but no combined sugar in Terunchi's and Witte's peptones. Treece (55) found that gaswas produced from Difco and Parke, Davis and Company peptones, but not from Armour's and Witte's peptones. Evidence was presented which indicated that this gas was due to free sugar in the peptone and that the carbohydrate radicals of the peptones were not the source of this gas. Further evidence of the presence of free carbohydrates in peptones was presented by Anderson (2) who found that E. coli would form gas from peptones. Van Slyke and Hart (59) and Eldridge and Rogers (15) found that CO_2 was produced from peptones by the action of certain cheese organisms; Evans (16), and Ayers, Rupp and Mudge (3) found that CO_2 was produced by certain streptococci from peptones.

Gorini (22) found that E. coli produced indol from Witte's peptone but not from an Italian peptone. Porcher and Panisset (39) showed that the ability of four brands of peptones to produce indol when tested with the same strain of E. coli, differed greatly. These results show that different peptones vary in their tryptophane content, from which indol is derived.

That peptones contain growth promoting substances is indicated by the fact that Ottensooser (37) found Witte's peptone contained group "A" specific substance, while Orla-Jensen, Otte and Snog-Kjaer (36) found that

bacteriologic peptones contained sufficient lactoflavin and enough bios to permit the growth of streptococci. On the other hand, Roberts and Baldwin (41) showed that some commercial peptones such as Bacto-peptone may contain a principle, removable by certain colloids, which is inhibiting to sporulation. Hydrophilic colloids such as agar removed more of the principle from peptones than did the hydrophobic ones as examplified by charcoal.

Methods of Chemical Analysis

The peptones, both commercial and prepared, were analyzed for total nitrogen, total and primary proteose nitrogen, peptone nitrogen, free amino acid nitrogen, free ammonia nitrogen, amino nitrogen and ash content. They were also tested to determine their reactions to various color tests.

A stock solution of each peptone was prepared by accurately weighing twenty grams, dissolving in approximately 300 ml. of distilled water and making up to 500 ml. in a volumetric flask. After mixing thoroughly the solution was poured into a sterile bottle, covered with a layer of toluene and stored in the ice box at 4° C. All chemical analyses except the ash determinations and certain color tests were performed on aliquots of this stock solution. All determinations were made in triplicate.

Total Nitrogen. To determine the total nitrogen content of the peptones, ten ml. aliquots of the stock solution were analyzed according to the Kjeldahl-Gunning method. The indicator used in this and in other nitrogen determinations was a mixture of methylene blue and methyl red as suggested by Johnson and Green (27).

Total Proteose Nitrogen. Small test tubes (88 x 13 mm.) were filled approximately one-half full of C.P. $ZnSO_4$ crystals and five ml. of the stock

peptone solution added to each tube. The tubes were heated in a water bath to dissolve the $ZnSO_4$ and allowed to cool slowly to room temperature. If necessary, more $ZnSO_4$ was added until the peptone solution was saturated, as shown by the presence of crystals of $ZnSO_4$ in the bottom of the tubes. The pH of the precipitation mixture was not adjusted, but the precipitation was carried out at the pH of saturated $ZnSO_4$, which is approximately 3.50. The salt error was not considered in determining this pH. The precipitate of proteose nitrogen was filtered off on a mat of shredded asbestos in a Gooch crucible, and washed several times with saturated $ZnSO_4$ solution to remove the non-proteose nitrogen. The crucible containing the precipitate was then placed in a 50 ml. beaker containing approximately fifteen ml. of dilute (1:1) H_2SO_4 and the acid heated to dissolve the precipitate. When the precipitate was dissolved, the crucible was removed and rinsed with distilled water, the washings being added to the acid-proteose solution. The shredded asbestos was removed by filtering through a second Gooch crucible containing a layer of shredded asbestos and the filter washed with distilled water. The solution was then placed in a Micro-Kjeldahl flask and the amount of proteose nitrogen determined by performing a Micro-Kjeldahl analysis.

Primary Proteose Nitrogen. Five ml. aliquots of the stock peptone solution were placed in small test tubes and five ml. of saturated aqueous $ZnSO_4$ added, giving a final solution one-half saturated with $ZnSO_4$. The tubes were inverted several times to mix the contents and allowed to stand at room temperature over night to complete precipitation. As in the case of the total proteose nitrogen, the pH of the precipitation mixture was not adjusted, but the precipitation was carried out at the pH of one-half saturated $ZnSO_4$ which is approximately 3.90. The tubes were then centri-

fugalized for one-half hour at 3000 r.p.m., and the precipitate washed twice with one-half saturated $ZnSO_4$ solution, and dissolved in dilute (1:1) H_2SO_4. The amount of primary proteose nitrogen was determined by Micro-Kjeldahl analysis.

Secondary Proteose Nitrogen. The figure for secondary proteose nitrogen was obtained by subtracting the value obtained for primary proteose nitrogen from that obtained for total proteose nitrogen.

Peptone Nitrogen. Five ml. aliquots of the stock solution were placed in small test tubes and five ml. of cold 20 per cent aqueous tannic acid solution added. The tubes were inverted several times to mix the contents and placed in the ice box for one-half hour. Preliminary experiments indicated that this amount of tannic acid was sufficient to precipitate all the peptone nitrogen in the peptones tested. A large excess was avoided since it would exert a solvent action on the tannic acid-peptone complex, as shown by Lundin and Schröderheim (30). The pH of the precipitation mixture was not adjusted, but performed at the pH of 20 per cent tannic acid, which is approximately 2.80. After completion of precipitation the tubes were centrifugalized for five minutes at 3000 r.p.m. and the precipitate washed twice with cold five per cent tannic acid solution. The precipitate was then dissolved in dilute (1:1) H_2SO_4 by heating in an Arnold steamer. The amount of peptone nitrogen was determined by Micro-Kjeldahl analysis.

Free Amino Acid Nitrogen. Phospho-tungstic acid was chosen as the protein precipitant in determining the free amino acid nitrogen. The procedure used was as follows: Ten ml. of the stock solution was pipetted into a 50 ml. beaker and 20 ml. of a five per cent aqueous phospho-tungstic acid solution was slowly added by means of a pipette. The mixture was

stirred continuously during the addition of the acid. After standing for 30 minutes at room temperature the precipitate was filtered off on a Büchner funnel containing a layer of filter paper pulp which was covered with a layer of Dicalite filter-aid, and washed three times with 25 ml. portions of one per cent phospho-tungstic acid. The filtrate was tested with a few drops of five per cent phospho-tungstic acid to test for the complete precipitation of the protein intermediate products, placed in a 200 ml. volumetric flask and diluted to the mark with distilled water. Fifty ml. aliquots were concentrated and the amount of free amino acid nitrogen by the Micro-Kjeldahl method.

Ammonia Nitrogen: Free ammonia present in the peptones was determined by the aeration method of Folin (17) as modified by Van Slyke and Cullen (58).

Amino Nitrogen: Amino nitrogen was determined by the Van Slyke and Sørenson methods.

Ash Content: To determine the amount of inorganic matter present in the peptones, approximately two grams was accurately weighed into porcelain crucibles of a known constant weight. These were then heated by a Meeker burner until the peptone ceased to smoke, and then placed in an electric furnace at a temperature of approximately 500° C. for one hour. At the end of this time the crucibles were placed in a desiccator over calcium chloride for one-half hour to cool and then weighed. This procedure was followed until a constant weight of ash was obtained.

Materials

In addition to the 16 prepared peptones, the following commercial brands were included in the chemical analysis of peptones. Numbers in

parentheses are the manufacturers batch numbers. Bacto-peptone (297614); Bacto-neopeptone (294983); Bacto-proteose-peptone (296837); Bacto proteose-peptone No. 2 (316258); Bacto proteose-peptone No. 3 (312275); Bacto-tryptone (306619); Bacto-tryptose (308035) and Bacto-protone (315785). Peptonum Siccum (100817) and Armour's Special Peptone (111025); Baker's Bacteriological Peptone (3239). Cenco Peptone, dry - from meat (9-2739). Chaissiang Peptone, a French product. Albumin peptone (dry) and meat peptone (dry) (900427) from Eimer and Amend. Fairchild'd peptone (360427). Merck's peptone from meat, dried (33119). Parke, Davis and Company Bacteriologic Peptone (3229678). Pfanstiehl Bacteriological Peptone (1033). Stearn's Bacteriological Peptone, N.P., (3474-K). Witte peptone made prior to 1912 and Witte peptone obtained in 1940 (3147) from F. Witte, Rostock, Germany. Wilson Peptone "CB" (31080) and Wilson Peptone "C" (30732).

Discussion

An examination of Tables 5 and 6 shows that the peptones differ widely in chemical composition. These results are in accord with those of McAlpine and Brigham (31) who found that Witte's, Fairchild's, Bacto-peptone and proteose-peptone varies considerably in the amounts of various nitrogen fractions for which they were tested. With the exception of Bacto-protone, all commercial peptones were higher in peptone nitrogen than in any other fraction for which they were tested. Protone was highest in proteose nitrogen. The amino nitrogen as determined by Van Slyke's method was, without exception, slightly higher than the figure obtained by Sørenson's formol titration.

Table 5. The Chemical Analysis of Commercial Peptones

Expressed in Per Cent.

Name of peptone	Total nitrogen	Total proteose nitrogen	Primary proteose nitrogen	Secondary proteose nitrogen	Peptone nitrogen	Free Ammonia nitrogen	Free amino acid nitrogen	Free nitrogen (Van Slyke)	Amino nitrogen (Srenson)	Ash
Bacto-peptone	15.72	0.52	0.07	0.45	9.69	0.07	1.16	3.33	3.27	2.55
Neopeptone	13.73	2.10	0.19	1.91	8.33	0.11	0.99	2.67	2.54	2.76
Proteose-peptone	13.55	3.82	0.19	3.63	7.99	0.11	0.85	2.72	2.69	3.88
Proteose-peptone No. 2	12.45	6.40	0.90	5.50	3.64	0.17	1.50	3.29	3.11	3.64
Proteose-peptone No. 3	12.87	8.25	3.30	4.95	6.45	0.13	1.06	2.55	2.46	3.24
Tryptone	12.77	2.69	0.16	2.53	3.16	0.17	2.06	4.56	4.53	6.48
Tryptose	12.99	2.53	0.25	2.28	5.76	0.05	1.41	3.57	3.48	5.02
Protone	15.24	13.27	5.27	8.00	1.04	0.05	0.37	1.83	1.75	2.43
Armour	14.27	4.37	0.64	3.73	6.05	0.33	1.69	3.94	3.84	2.07
Armour Special*	13.89	4.32	0.74	3.58	3.75	0.12	0.57	1.08	1.05	2.23
Baker	15.43	3.71	0.03	3.68	13.92	0.09	0.25	1.39	1.36	1.07
Cenco	15.38	5.45	0.41	5.04	11.91	0.09	0.48	0.85	0.81	1.26
Chaissiang	13.15	0.50	0.00	0.50	5.02	0.07	1.21	3.25	3.24	1.58
E & A Albumin	11.21	1.47	0.36	1.16	6.58	0.08	0.45	1.41	1.36	3.98
E & A Meat	16.14	1.27	0.01	1.25	13.86	0.06	0.27	1.13	1.05	0.70
Fairchild	14.44	0.50	0.16	0.34	4.80	0.23	1.92	5.21	5.09	1.62
Merck	15.83	0.37	0.00	0.37	12.53	0.06	0.36	1.83	1.74	1.18
Pfansteihl	13.56	1.48	0.08	1.40	6.69	0.17	1.31	3.94	2.89	3.21
Parke-Davis	14.42	0.63	0.02	0.61	7.81	0.09	1.12	2.53	2.48	1.86
Stearn	15.45	0.52	0.03	0.50	10.68	0.12	0.76	1.76	1.66	1.11
Witte (1912)	14.48	8.50	1.71	6.79	10.55	0.02	0.32	1.85	1.79	1.57
Witte (1940)	13.45	6.59	1.07	5.52	10.59	0.00	0.52	2.54	2.48	1.31
Wilson "CB"	11.86	1.09	0.11	0.98	4.46	0.13	1.58	3.71	3.67	4.69
Wilson "C"	11.48	2.32	0.19	2.13	2.65	0.13	1.58	3.90	3.81	6.36
Average	13.90	3.44	0.66	2.78	7.41	0.11	0.99	2.90	2.59	2.74

* Not a bacteriological peptone

Table 6. The Chemical Analysis of Prepared Peptones

Expressed in Per cent.

Name of peptone	Total nitrogen	Total proteose nitrogen	Primary proteose nitrogen	Secondary proteose nitrogen	Peptone nitrogen	Free ammonia nitrogen	Free amino acid nitrogen	Amino nitrogen (Van Slyke)	Amino nitrogen (Sorenson)	Ash
Beef peptone	14.19	6.47	0.22	6.23	10.54	0.21	0.47	1.63	1.57	1.19
Spleen peptone (1)	14.84	4.16	1.22	2.94	10.94	0.24	0.71	2.11	2.07	1.75
Spleen peptone (2)	14.64	2.88	0.57	2.31	9.92	0.02	0.67	2.39	2.29	2.16
Liver peptone (1)	16.44	2.30	0.75	1.56	2.85	0.19	1.17	6.17	5.97	6.04
Liver peptone (2)	13.44	5.64	1.14	4.51	9.23	0.13	0.57	1.61	1.52	3.11
Heart peptone (1)	15.08	4.36	0.46	3.90	10.63	0.05	0.50	2.06	2.04	1.99
Heart peptone (2)	14.20	6.08	1.40	4.68	10.34	0.14	0.45	1.82	1.80	2.86
Brain peptone (1)	15.00	0.77	0.08	0.69	3.12	0.47	1.54	4.28	4.26	2.88
Brain peptone (2)	11.99	1.44	0.33	1.11	6.44	0.02	1.00	2.94	2.92	6.75
Pork peptone	12.58	3.65	0.90	2.75	8.64	0.14	0.82	1.64	1.57	4.48
Average	14.24	3.77	0.70	3.07	8.26	0.16	0.79	2.66	2.60	3.52
Veg. peptone No. 1*	9.11	0.23	0.09	0.14	0.27	0.00	2.87	5.54	5.28	2.43
Veg. peptone No. 2*	7.66	0.13	0.09	0.04	0.15	0.00	2.95	7.06	7.00	1.47
Veg. peptone No. 3*	8.50	0.19	0.13	0.06	0.08	0.00	3.26	7.16	7.01	1.32
Veg. peptone No. 4*	12.24	0.19	0.13	0.06	0.08	0.00	3.75	7.16	7.10	2.79
Veg. peptone No. 5**	11.45	2.38	0.41	1.98	2.52	0.37	1.76	2.36	2.30	3.94
Veg. tryptone**	11.37	0.12	0.05	0.07	0.41	0.51	0.00	5.24	5.19	3.04
Average	10.05	0.54	0.15	0.39	0.73	0.14	2.48	5.76	5.64	2.49

* Prepared from unhydrolyzed corn gluten.

** Prepared from hydrolysates of corn gluten.

In the case of the prepared animal peptones, the results (Table 7) show that two batches of peptone prepared from the same source material vary considerably in chemical composition. This again emphasizes the difficulty of preparing standardized peptones under either laboratory or commercial conditions. As in the case of per cent yield, the variation in chemical composition of two batches of peptone from the same source is probably due either to variations in the original material, or to the treatment of the peptone during preparation. As a general rule, the forms of nitrogen were present in larger amounts in the prepared animal peptones than in the commercial peptones, with the exception of free amino acid and amino nitrogen.

The vegetable peptones prepared from hydrolysates of corn gluten were lower than either the commercial or animal peptones in all forms of nitrogen, with the exception of the free amino acid and amino nitrogen. The low amount of peptone and proteose nitrogen is due to the acid hydrolsis, liberating amino acids. This same hydrolysis would also account for the higher amounts of amino nitrogen found. In the case of the peptone prepared from dry corn gluten, the combination of a fairly high acid content and a temperature of 55°C. for three weeks was evidently enough to hydrolyze the peptone nitrogen formed. This resulted in a lowering of the amount of this form of nitrogen over that found in other peptones prepared by pepsin-HCl digestion, and in the production of a larger percentage of free amino acid and amino nitrogen. The vegetable tryptone was digested for a sufficient length of time to allow the trypsin to form a comparatively large amount of free amino acids and amino nitrogen. The Tables also show that the free ammonia nitrogen is very low in all cases.

Tables 5 and 6 show that the sum of the various nitrogen fractions does not equal the figure obtained for total nitrogen. This indicates that in some cases one or more forms of nitrogen are being determined by more than one method, and in other cases that not all the nitrogen is being determined. The dividing line between proteose nitrogen and peptone nitrogen is undoubtedly very indistinct. Thus some proteose nitrogen is probably being determined both by saturating with $ZnSO_4$ and by precipitation with tannic acid, the same being true of peptone nitrogen. Also it is true that some amino acids, especially the basic ones such as arginine, lysine and histidine, are precipitated by the addition of phospho-tungstic acid and therefore are not included in the figures for free amino acid nitrogen. The only truly reliable determinations are those for total nitrogen and free ammonia nitrogen. Lysine is not determined by Van Slyke's method unless the deaminization is carried on for one-half hour, so the figures given for amino nitrogen are slightly lower than they would be had this procedure been followed. Thus the figures given in Tables 5 and 6 are most useful for comparative purposes in that they indicate which peptone is higher than another in any one nitrogen fraction. As stated by Abel and Geiling (1), nitrogen fractions such as peptone nitrogen and the several proteose nitrogens cannot be regarded as chemical entities. They are defined from their reactions under definite conditions and not by their chemical make-up. The fact that the vegetable peptones prepared from 16 hour hydrolysates of corn gluten show the presence of small amounts of peptone and proteose nitrogen indicates that free amino acids are precipitated to a slight extent by saturating with $ZnSO_4$ and by the addition of tannic acid.

While the amount of heat coagulable nitrogen is not included in

Tables 5 and 6, it may be mentioned here that none of the peptones contained a significant amount of this type of nitrogen. However, if the pH of any peptone is adjusted by the addition of either an acid or a base and the peptone solution is then autoclaved, a precipitate is usually formed.

Mention should be made at this time of the work of Rimington and Kay (46) and of Rimington (42) (43) (44) (45) on the structure of a phospho-peptone isolated from tryptic digests of casein. This peptone had an empirical formula of $C_{37}H_{62}O_{33}N_9P_3$, a molecular weight of 1245, was strongly levo-rotatory, and acted as a nine-basic acid. One ninth of the nitrogen was in the form of amino nitrogen. However, after hydrolysis with HCl, the amino nitrogen was equal to the total nitrogen. This indicated that the substance was a peptone consisting of nine amino acids, joined by peptid linkages, all of which were acyclic mono-amino acids. After acid hydrolysis hydroxyglutamic acid, hydroxyaminobutyric acid and serine were isolated. Further analysis showed that the peptones was probably made up of three molecules of hydroxyglutamic acid, four molecules of hydroxyaminobutyric acid, two molecules of serine and three molecules of phosphoric acid. Of the phosphorus present, two thirds was removed by the action of bone phosphatase, the remaining one-third being removed by phosphoric esterase of kidney extracts, indicating the presence of an ester linkage. A structural formula was suggested which satisfied all of the experimental findings, although the sequence of the amino acids and the attachment of the phosphorus atoms were not determined.

COLOR REACTIONS OF PEPTONES

Various color reactions have been devised to determine the presence or absence of certain components of proteins. The peptones were tested for their reaction to the following tests: The biuret test for peptide linkages; the Millon test for tyrosine; the Rosenheim test for tryptophane, the Sakaguchi test for arginine; the ninhydrin reaction for the alpha amino and free carboxyl groups; the xanthoproteic test for the benzene nucleus; the Fleitmann test for the presence of loosely bound sulphur as found in cystine and cysteine; and the Molish test for the presence of carbohydrates. The results obtained for these color reactions are shown in Table 7.

Discussion

Table 7 shows that the commercial peptones are positive to all except the Molish reaction. This indicates that all the commercial peptones are free from carbohydrates. The positive biuret test indicates that hydrolysis has not been completed. The positive Rosenheim test indicates that none of the peptones tested are prepared by acid hydrolysis, or if they are, it is only a very mild treatment, since a more drastic hydrolysis would destroy the identity of the tryptophane present.

Table 7. The Color Reactions of Peptones

Peptone	Biuret Test	Millon test	Rosen-heim test	Sakguchi test	Ninhydrin test	Xantho-proteic test	Fleit-mann test	Molisch test
A. Commercial								
Bacto-peptone	+	+	+	+	+	+	+	-
Neopeptone	+	+	+	+	+	+	+	-
Proteose-peptone	+	+	+	+	+	+	+	-
Proteose-peptone No. 2	+	+	+	+	+	+	+	-
Proteose-peptone No. 3	+	+	+	+	+	+	+	-
Tryptone	+	+	+	+	+	+	+	-
Tryptose	+	+	+	+	+	+	+	-
Protone	+	+	+	+	+	+	+	-
Wilson "CB"	+	+	+	+	+	+	+	-
Wilson "C"	+	+	+	+	+	+	+	-
Witte (1912)	+	+	+	+	+	+	+	-
Witte (1940)	+	+	+	+	+	+	+	-
Armour	+	+	+	+	+	+	+	-
Armour Special	+	+	+	+	+	+	+	-
Pfansteihl	+	+	+	+	+	+	+	-
Baker's	+	+	+	+	+	+	+	-
Cenco	+	+	+	+	+	+	+	-
Albumin	+	+	+	+	+	+	+	-
E & A Meat	+	+	+	+	+	+	+	-
Fairchild	+	+	+	+	+	+	+	-
Chaissiang	+	+	+	+	+	+	+	-
Merck	+	+	+	+	+	+	+	-
Stearn	+	+	+	+	+	+	+	-
Parke-Davis & Co.	+	+	+	+	+	+	+	-
B. Animal								
Beef	+	+	+	+	+	+	+	-
Spleen (1)	+	+	+	+	+	+	+	+
Spleen (2)	+	+	+	+	+	+	+	+
Liver (1)	+	+	+	+	+	+	+	+
Liver (2)	+	+	+	+	+	+	+	+
Heart (1)	+	+	+	+	+	+	+	-
Heart (2)	+	+	+	+	+	+	+	-
Brain (1)	+	+	+	+	+	+	+	-
Brain (2)	+	+	+	+	+	+	+	-
Pork								
C. Vegetable								
Number 1	-	+	-	+	+	+	+	+
Number 2	-	+	-	+	+	+	+	+
Number 3	-	+	-	+	+	+	+	+
Number 4	-	-	-	+	+	+	+	+
Number 5	+	+	+	+	+	+	+	+
Tryptone	+	+	+	+	+	+	+	+

The prepared animal peptones were positive to all tests except the Molish reaction, with the exception of the two liver and two spleen peptones, which were positive to this test. In the case of the liver peptones this may be explained by the presence of glycogen in the liver. The presence of carbohydrates in the spleen peptone is not so easily explained.

The four vegetable peptones prepared from hydrolyzed corn gluten gave positive Sakaguchi, ninhydrin, xanthoproteic, Fleitmann and Molish reactions and negative biuret and Rosenheim tests. The first three were positive to Millon's test, while the fourth, from which the tyrosine had been removed, was negative. The positive Molish test indicates that not all the corn starch had been removed from the protein. The positive xanthoproteic test was probably due to the presence of phenylalanine and/or tyrosine, since the tryptophane was destroyed by the acid hydrolysis, as indicated by the negative Rosenheim test. The peptone prepared from the sample of corn gluten that had been hydrolyzed only two hours (Vegetable peptone No. 1) gave a faintly positive biuret test, indicating that not all the peptide linkages were broken, as they were in the remaining vegetable peptones.

Vegetable peptone No. 5 and vegetable tryptone were positive to all tests. This again indicates the presence of carbohydrates in the original protein, and also shows that the hydrolysis was not as complete as in the case of the other vegetable peptones.

BUFFERING ACTION OF PEPTONES

To determine the buffering action a one per cent solution of each peptone was made up by dissolving exactly two grams in 150 ml. of distilled water. When dissolved, the solution was placed in a 200 ml. volu-

metric flask, diluted to the mark with distilled water and thoroughly mixed. Twenty ml. of this solution was pipetted into a 50 ml. beaker and the pH value taken before and after the addition of various amounts of 0.01N HCl and 0.01N NaOH. A duplicate set of determinations was run on each peptone. The pH readings were taken using a Beckman pH meter.

A second set of buffer determinations was made on one per cent peptone solutions whose initial pH had been adjusted to exactly 7.00. To do this two grams of the peptone were weighed and dissolved in approximately 190 ml. of distilled water. The pH was adjusted to 7.00 by the addition of either 0.01N HCl or 0.01N NaOH, and the solution diluted to 200 ml. in a volumetric flask. The pH value was then determined after various amounts of 0.01N HCl and 0.01N NaOH were added to 20 ml. of the neutral solution.

Table 8 gives the pH values obtained after adding various amounts of 0.01N HCl to the unadjusted peptone solutions and Table 9 the pH values after adding 0.01N NaOH. Tables 10 and 11 give the pH values obtained after adding HCl and NaOH, respectively, to the adjusted peptone solutions.

Discussion

The peptones are listed in Tables 8 and 10 in the order of their increasing initial acidity. They may be roughly divided into four groups as follows: (1) those that are alkalin in reaction, pH 7.00 to 7.60; (2) those that are neutral in reaction, pH 7.00; (3) those that are slightly acid, pH 6.00 to 7.00; and (4) those that are strongly acid, pH 4.89 to 6.00. This method of grouping the peptones shows that twelve are alkaline, four are neutral, twelve are slightly acid and twelve are strongly acid.

Table 8. Change in pH of a one per cent peptone solution(unadjusted) after adding various amounts of 0.01 N HCl.

Ml. HCl added.	Witte peptone (1912)	Bacto-tryptose	Proteose-peptone No. 3	Bacto-protone	Vegetable peptone No. 1	Bacto-tryptone	Proteose-peptone	Liver peptone (1)	Brain peptone (2)	Vegetable peptone No. 4	Pork peptone	Wilson peptone "O"	Vegetable peptone No. 5	Proteose-peptone No. 2	Vegetable peptone No. 3	Witte peptone (1940)	Bacto-peptone	Heart peptone (2)	Vegetable peptone No. 2	Spleen peptone (2)
0.00	7.60	7.51	7.28	7.18	7.14	7.14	7.13	7.12	7.12	7.10	7.09	7.08	7.00	7.00	7.00	7.00	6.97	6.92	6.92	6.82
1.00	7.41	7.43	7.05	7.00	6.89	6.85	6.91	6.96	7.03	6.80	6.94	6.97	6.94	6.75	6.62	6.89	6.40	6.82	6.55	6.59
2.00	7.24	7.29	6.79	6.83	6.61	6.67	6.68	6.74	6.94	6.59	6.79	6.81	6.82	6.48	6.32	6.71	5.97	6.69	6.21	6.37
3.00	7.02	7.12	6.55	6.69	6.32	6.50	6.44	6.53	6.83	6.40	6.61	6.68	6.63	6.19	6.06	6.51	5.61	6.51	5.96	6.16
4.00	6.83	6.95	6.29	6.50	6.06	6.33	6.12	6.33	6.73	6.27	6.42	6.53	6.42	5.90	5.81	6.31	5.38	6.53	5.75	5.95
5.00	6.53	6.76	6.05	6.27	5.89	6.17	5.81	6.15	6.61	6.11	6.24	6.37	6.20	5.60	5.61	6.09	5.21	6.15	5.60	5.71
6.00	6.29	6.51	5.80	6.01	5.72	6.01	5.57	5.97	6.49	5.97	6.06	6.22	5.98	5.31	5.44	5.87	5.07	5.97	5.48	5.48
7.00	6.00	6.25	5.58	5.81	5.58	5.85	5.39	5.82	6.39	5.85	5.91	6.04	5.78	5.10	5.31	5.63	4.97	5.77	5.37	5.31
8.00	5.71	6.01	5.34	5.57	5.46	5.69	5.22	5.69	6.24	5.72	5.74	5.87	5.61	4.95	5.19	5.45	4.85	5.60	5.27	5.14
9.00	5.51	5.71	5.11	5.31	5.36	5.52	5.01	5.56	6.09	5.61	5.60	5.70	5.45	4.79	5.06	5.29	4.74	5.46	5.17	5.00
10.0	5.31	5.49	4.92	5.10	5.28	5.41	4.82	5.48	5.93	5.51	5.42	5.55	5.35	4.66	4.99	5.15	4.63	5.31	5.10	4.87
12.0	5.00	5.24	4.63	4.76	5.11	5.17	4.63	5.30	5.65	5.37	5.24	5.29	5.16	4.37	4.82	4.91	4.41	5.09	4.98	4.66
14.0	4.71	4.98	4.42	4.51	4.99	4.95	4.45	5.16	5.40	5.21	5.04	5.02	4.97	4.12	4.67	4.72	4.26	4.90	4.88	4.44
16.0	4.53	4.82	4.25	4.30	4.88	4.76	4.30	5.01	5.19	5.08	4.89	4.87	4.78	4.00	4.54	4.52	4.12	4.71	4.77	4.31
18.0	4.37	4.64	4.08	4.11	4.78	4.55	4.16	4.90	5.00	4.96	4.71	4.69	4.61	3.90	4.43	4.38	4.01	4.54	4.67	4.13
20.0	4.19	4.51	3.93	3.97	4.67	4.37	4.04	4.80	4.85	4.82	4.59	5.45	4.47	3.81	4.33	4.22	3.90	4.31	4.58	3.99

Table 8. (Continued)

Ml. 0.01N HCl added	Spleen peptone (1)	Bacto-meopep-tone	Beef peptone	Heart peptone (1)	Wilson peptone "CB"	Brain peptone (1)	Armour peptone	Vegetable tryp-tone	E&A Albumin peptone	Steam peptone	Armour special peptone	Liver peptone (2)	Cenco peptone	Fairchild pep-tone	Baker peptone	Parke, Davis peptone	Pfansteihl peptone	E&A Meat peptone	Chaissiane peptone	Merck peptone
0.00	6.72	6.55	6.46	6.43	6.93	6.34	6.31	6.10	5.98	5.91	5.82	5.62	5.53	5.51	5.21	5.20	5.17	5.15	4.91	4.89
1.00	6.37	6.23	6.32	6.31	6.74	6.18	6.14	5.74	5.60	5.51	5.72	5.55	5.30	5.33	5.02	5.03	5.12	4.92	4.79	4.68
2.00	6.07	5.98	6.08	6.13	6.56	5.99	5.95	5.53	5.37	5.15	5.49	5.41	5.06	5.18	4.82	4.93	5.06	4.70	4.68	4.51
3.00	5.83	5.76	5.84	5.93	6.36	5.80	5.82	5.35	5.20	4.94	5.30	5.29	4.80	5.06	4.64	4.85	4.97	4.52	4.58	4.34
4.00	5.62	5.53	5.62	5.73	6.19	5.62	5.69	5.17	5.05	4.75	5.16	5.18	4.62	4.94	4.47	4.77	4.87	4.36	4.49	4.22
5.00	5.41	5.37	5.44	5.54	5.99	5.48	5.56	5.02	4.91	4.61	5.05	5.09	4.49	4.81	4.34	4.70	4.79	4.20	4.38	4.12
6.00	5.21	5.22	5.25	5.37	5.78	5.34	5.42	4.88	4.77	4.49	4.93	5.00	4.42	4.72	4.23	4.62	4.72	4.08	4.30	4.02
7.00	5.02	5.06	5.09	5.21	5.60	5.22	5.28	4.75	4.66	4.38	4.86	4.90	4.29	4.61	4.11	4.55	4.64	3.96	4.22	3.96
8.00	4.90	4.91	4.96	5.10	5.45	5.11	5.15	4.62	4.55	4.29	4.76	4.80	4.18	4.52	4.00	4.51	4.58	3.84	4.16	3.87
9.00	4.78	4.78	4.83	4.98	5.31	5.02	5.02	4.52	4.45	4.19	4.67	4.71	4.09	4.43	3.88	4.42	4.51	3.74	4.10	3.79
10.0	4.67	4.71	4.73	4.89	5.18	4.92	4.90	4.43	4.38	4.10	4.60	4.61	3.97	4.35	3.76	4.34	4.45	3.65	4.03	3.72
12.0	4.48	4.52	4.57	4.69	4.97	4.76	4.73	4.30	4.23	3.96	4.45	4.37	3.77	4.21	3.53	4.19	4.33	3.49	3.92	3.58
14.0	4.28	4.36	4.40	4.51	4.81	4.62	4.57	4.17	4.07	3.81	4.27	4.20	3.60	4.07	3.39	4.06	4.22	3.31	3.81	3.44
16.0	4.14	4.21	4.23	4.36	4.63	4.47	4.42	4.08	3.91	3.69	4.11	4.00	3.46	3.94	3.25	3.96	4.10	3.19	3.71	3.32
18.0	3.99	4.07	4.11	4.20	4.48	4.31	4.29	4.02	3.73	3.55	3.99	3.86	3.29	3.81	3.15	3.87	4.00	3.07	3.60	3.23
20.0	3.84	3.95	3.97	4.02	4.44	4.20	4.17	3.96	3.56	3.45	3.87	3.75	3.21	3.61	3.10	3.73	3.92	3.00	3.52	3.17

Table 9. Change in pH of a one per cent peptone solution (adjusted to pH 7.0) after adding various amounts of 0.01N HCl.

Ml. 0.01N HCl added	Vegetable peptone No. 4	Parke, Davis peptone	Liver peptone (1)	Brain peptone (2)	Vegetable tryptone	Pork peptone	Heart peptone (2)	Vegetable peptone No. 5	Steam's peptone	Wilson peptone "C"	Vegetable peptone No. 2	Vegetable peptone No. 1	Brain peptone (1)	Fairchild's peptone	Pfanstehl peptone	Vegetable peptone No. 3	Bacto-tryptone	Liver peptone (2)	Armour's special peptone	Heart peptone (1)
0.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
1.00	6.82	6.70	6.78	6.95	6.68	6.89	6.90	6.94	6.61	6.89	6.75	6.84	6.88	6.89	6.86	6.62	6.79	6.82	6.91	6.88
2.00	6.59	6.50	6.54	6.84	6.35	6.71	6.72	6.82	6.27	6.72	6.37	6.56	6.66	6.72	6.72	6.32	6.60	6.61	6.79	6.71
3.00	6.38	6.29	6.32	6.76	6.06	6.54	6.55	6.63	5.95	6.58	6.01	6.27	6.42	6.53	6.54	6.06	6.42	6.39	6.55	6.50
4.00	6.18	6.12	6.11	6.65	5.85	6.36	6.38	6.42	5.68	6.40	5.77	5.95	6.20	6.32	6.37	5.81	6.26	6.14	6.34	6.29
5.00	6.00	5.96	5.95	6.55	5.64	6.19	6.19	6.20	5.46	6.21	5.56	5.75	5.96	6.08	6.16	5.61	6.13	5.92	6.10	6.08
6.00	5.90	5.83	5.80	6.41	5.45	5.99	6.00	5.98	5.27	6.03	5.41	5.57	5.75	5.89	5.93	5.44	5.97	5.70	5.90	5.88
7.00	5.76	5.72	5.66	6.31	5.33	5.81	5.82	5.78	5.13	5.88	5.27	5.40	5.58	5.67	5.73	5.31	5.81	5.52	5.70	5.67
8.00	5.64	5.62	5.55	6.16	5.22	5.64	5.66	5.61	5.00	5.71	5.15	5.28	5.42	5.49	5.54	5.19	5.65	5.39	5.51	5.55
9.00	5.54	5.55	5.47	6.03	5.13	5.50	5.51	5.45	4.92	5.52	5.06	5.16	5.29	5.34	5.39	5.06	5.50	5.22	5.38	5.34
10.0	5.45	5.46	5.39	5.90	5.05	5.38	5.39	5.35	4.85	5.38	4.99	5.06	5.19	5.20	5.26	4.99	5.33	5.11	5.22	5.20
12.0	5.29	5.31	5.21	5.60	4.90	5.16	5.17	5.16	4.75	5.16	4.84	4.90	4.99	5.00	5.00	4.82	5.12	4.91	5.01	4.99
14.0	5.13	5.16	5.08	5.35	4.80	4.98	4.97	4.97	4.63	4.96	4.72	4.74	4.81	4.80	4.82	4.67	4.90	4.74	4.81	4.80
16.0	5.00	5.00	4.97	5.10	4.70	4.82	4.80	4.78	4.56	4.78	4.61	4.61	4.65	4.64	4.63	4.54	4.70	4.60	4.66	4.60
18.0	4.87	4.85	4.83	4.89	4.65	4.66	4.63	4.61	4.50	4.60	4.51	4.50	4.51	4.50	4.50	4.43	4.60	4.45	4.46	4.43
20.0	4.71	4.70	4.70	4.69	4.61	4.51	4.50	4.47	4.46	4.41	4.41	4.40	4.40	4.36	4.36	4.33	4.31	4.30	4.30	4.29

Table 9. Change in pH of a one per cent peptone solution (adjusted to pH 7.0) after adding various amounts of 0.01N HCl.

Ml. 0.01N HCl added.	Armour peptone	Spleen peptone (2)	Wilson peptone "CB"	Beef peptone	Witte peptone (1940)	Chaissiang peptone	Bacto-tryptose	E&A Albumin peptone	Bacto-protone	Spleen peptone (1)	Witte peptone (1912)	Bacto-neopeptone	Bacto proteose-peptone	Bacto peptone	Bacto proteose-peptone No. 3	Bacto proteose-peptone No. 2	Merck's peptone	Cenco peptone	E&A Meat peptone	Baker's peptone
0.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
1.00	6.89	6.88	6.85	6.85	6.89	6.83	6.90	6.79	6.87	6.80	6.80	6.82	6.86	6.55	6.70	6.75	6.83	6.48	6.40	6.41
2.00	6.73	6.71	6.66	6.63	6.71	6.68	6.75	6.57	6.71	6.56	6.62	6.62	6.55	6.15	6.45	6.48	6.49	6.04	5.91	5.85
3.00	6.60	6.53	6.46	6.42	6.51	6.51	6.59	6.29	6.54	6.26	6.40	6.38	6.31	5.78	6.20	6.19	6.00	5.65	5.50	5.42
4.00	6.45	6.33	6.23	6.20	6.31	6.31	6.39	6.01	6.36	6.00	6.17	6.09	5.99	5.52	5.97	5.90	5.56	5.38	5.21	5.13
5.00	6.29	6.11	6.00	5.98	5.09	6.09	6.18	5.78	6.15	5.71	5.91	5.81	5.75	5.30	5.75	5.60	5.24	5.16	5.00	4.93
6.00	6.10	5.91	5.76	5.76	5.87	5.84	5.91	5.59	5.93	5.50	5.65	5.52	5.53	5.14	5.55	5.31	5.01	5.00	4.82	4.75
7.00	5.91	5.69	5.56	5.53	5.63	5.60	5.65	5.40	5.68	5.31	5.41	5.29	5.33	4.99	5.35	5.10	4.82	4.83	4.67	4.60
8.00	5.72	5.50	5.40	5.38	5.45	5.40	5.42	5.22	5.45	5.13	5.22	5.10	5.13	4.84	5.14	4.95	4.69	4.70	4.52	4.46
9.00	5.56	5.33	5.25	5.24	5.29	5.20	5.24	5.11	5.24	5.01	5.06	4.94	4.96	4.75	4.97	4.79	4.55	4.56	4.40	4.31
10.0	5.41	5.19	5.11	5.12	5.15	5.08	5.09	5.00	5.08	4.90	4.92	4.81	4.80	4.62	4.80	4.66	4.42	4.42	4.28	4.13
12.0	5.12	4.95	4.89	4.91	4.91	4.85	4.86	4.80	4.81	4.66	4.65	4.59	4.56	4.42	4.53	4.37	4.21	4.23	4.07	3.96
14.0	4.89	4.78	4.70	4.70	4.72	4.65	4.64	4.60	4.59	4.45	4.36	4.39	4.35	4.26	4.30	4.12	4.01	4.02	3.88	3.78
16.0	4.68	4.59	4.52	4.52	4.51	4.49	4.47	4.41	4.40	4.27	4.22	4.20	4.18	4.12	4.14	4.00	3.89	3.88	3.72	3.60
18.0	4.47	4.40	4.39	4.38	4.38	4.32	4.30	4.27	4.26	4.14	4.08	4.05	4.02	4.00	3.98	3.90	3.73	3.72	3.56	3.45
20.0	4.27	4.26	4.25	4.22	4.22	4.19	4.18	4.10	4.10	3.97	3.96	3.91	3.89	3.88	3.83	3.81	3.68	3.58	3.42	3.32

Table 10. Change in pH of a one per cent peptone solution (unadjusted) after adding various amounts of 0.01N NaOH.

Ml. 0.01N NaOH added	Witte peptone (1912)	Bacto-tryptose	Proteose-peptone No. 3	Bacto-protone	Vegetable peptone No. 1	Bacto-tryptone	Proteose-peptone	Liver peptone (1)	Brain peptone (2)	Vegetable peptone No. 4	Pork peptone	Wilson peptone "C"	Vegetable peptone No. 5	Proteose-peptone No. 2	Vegetable peptone No. 3	Witte peptone (1940)	Bacto-peptone	Wilson peptone "CB"	Heart peptone (2)	Vegetable peptone No. 2
0.00	7.60	7.51	7.28	7.18	7.14	7.14	7.13	7.12	7.12	7.10	7.09	7.08	7.00	7.00	7.00	7.00	6.97	6.93	6.92	6.92
1.00	7.67	7.62	7.41	7.36	7.22	7.25	7.32	7.44	7.28	7.27	7.27	7.32	7.25	7.12	7.19	7.19	7.32	7.15	7.22	7.16
2.00	7.85	7.74	7.50	7.49	7.41	7.37	7.43	7.64	7.37	7.48	7.41	7.42	7.43	7.23	7.42	7.31	7.52	7.29	7.38	7.38
3.00	8.00	7.85	7.60	7.61	7.55	7.49	7.54	7.78	7.45	7.70	7.52	7.54	7.59	7.33	7.39	7.44	7.70	7.42	7.50	7.56
4.00	8.18	7.94	7.72	7.73	7.69	7.61	7.64	7.96	7.50	7.85	7.64	7.65	7.70	7.45	7.76	7.53	7.85	7.51	7.63	7.71
5.00	8.34	8.02	7.81	7.85	7.81	7.73	7.76	8.11	7.55	7.99	7.73	7.74	7.83	7.57	7.89	7.59	7.99	7.61	7.78	7.86
6.00	8.48	8.10	7.91	7.97	7.92	7.85	7.87	8.22	7.59	8.08	7.83	7.85	7.92	7.67	7.99	7.66	8.10	7.71	7.90	7.98
7.00	8.62	8.21	8.01	8.09	8.01	7.97	7.97	8.32	7.68	8.17	7.93	7.93	8.00	7.77	8.08	7.77	8.20	7.81	8.02	8.03
8.00	8.78	8.28	8.10	8.21	8.10	8.11	8.08	8.40	7.76	8.23	8.03	8.02	8.10	7.87	8.15	7.89	8.31	7.91	8.17	8.16
9.00	8.91	8.36	8.20	8.37	8.19	8.25	8.20	8.50	7.88	8.31	8.12	8.10	8.19	7.94	8.21	8.01	8.40	8.00	8.31	8.24
10.0	9.05	8.46	8.29	8.53	8.25	8.34	8.30	8.60	7.96	8.38	8.22	8.18	8.27	8.02	8.29	8.12	8.45	8.08	8.46	8.31
12.0	9.35	8.61	8.48	8.90	8.39	8.54	8.51	8.73	8.13	8.49	8.49	8.36	8.41	8.20	8.41	8.41	8.62	8.26	8.79	8.46
14.0	9.57	7.85	8.66	9.26	8.49	8.72	8.74	8.85	8.28	8.59	8.65	8.52	8.58	8.39	8.50	8.61	8.78	8.42	9.17	8.56
16.0	9.85	8.89	8.86	9.57	8.59	8.93	8.95	8.97	8.41	8.68	8.89	8.67	8.72	8.53	8.59	8.84	8.91	8.59	9.41	8.68
18.0	9.95	9.00	9.00	9.79	8.69	9.07	9.13	9.10	8.56	8.75	9.04	8.80	8.85	8.70	8.68	9.12	9.04	8.71	9.70	8.75
20.0	10.13	9.12	9.11	10.00	8.79	9.16	9.28	9.20	8.77	8.80	9.29	8.90	8.95	8.85	8.73	9.37	9.17	8.86	9.89	8.81

Table 10. Change in pH of a one per cent peptone solution (unadjusted) after adding various amounts of 0.01N NaOH.

Ml. 0.01N NaOH added	Spleen peptone (2)	Spleen peptone (1)	Bacto-neopeptone	Beef peptone	Heart peptone (1)	Brain peptone (1)	Armour peptone	Vegetable tryptone	E&A Albumin peptone	Stearn peptone	Armour special peptone	Liver peptone (2)	Cenco peptone	Fairchild peptone	Baker peptone	Parke, Davis peptone	Pfanstethl peptone	E&A Meat peptone	Chaissiang peptone	Merck peptone
0.00	6.82	6.72	6.55	6.46	6.43	6.34	6.31	6.10	5.98	5.91	5.82	5.62	5.53	5.51	5.21	5.20	5.17	5.15	4.91	4.89
1.00	6.94	6.93	6.97	6.82	6.81	6.76	6.51	6.53	6.37	6.30	6.01	6.15	6.18	5.73	5.81	5.59	5.21	5.55	5.08	5.09
2.00	7.06	7.09	7.14	6.99	6.98	6.99	6.71	6.90	6.65	6.71	6.33	6.40	6.78	5.93	6.48	5.92	5.75	5.95	5.27	5.33
3.00	7.18	7.25	7.32	7.14	7.13	7.15	6.84	7.17	6.89	6.98	6.57	6.63	7.21	6.22	6.97	6.18	6.10	6.56	5.47	5.72
4.00	7.30	7.40	7.47	7.28	7.27	7.31	7.00	7.38	7.10	7.18	6.79	6.85	7.62	6.49	7.41	6.45	6.38	7.08	5.70	6.24
5.00	7.42	7.53	7.58	7.41	7.41	7.47	7.13	7.57	7.27	7.39	7.00	7.01	7.99	6.74	7.76	6.65	6.64	7.47	6.00	6.66
6.00	7.52	7.68	7.71	7.53	7.54	7.60	7.26	7.71	7.47	7.51	7.20	7.19	8.29	6.96	8.01	6.84	6.84	7.76	6.26	6.99
7.00	7.64	7.84	7.85	7.69	7.67	7.74	7.39	7.84	7.62	7.67	7.38	7.37	8.46	7.16	8.28	7.00	7.03	8.03	6.48	7.21
8.00	7.77	8.00	8.00	7.85	7.78	7.85	7.50	7.96	7.79	7.81	7.59	7.55	8.79	7.32	8.49	7.15	7.20	8.26	6.68	7.39
9.00	7.89	8.14	8.10	7.99	7.91	7.97	7.61	8.04	7.97	7.95	7.80	7.67	8.95	7.47	8.68	7.30	7.35	8.41	6.83	7.53
10.0	8.01	8.30	8.21	8.10	8.02	8.09	7.73	8.13	8.16	8.05	7.98	7.82	9.12	7.61	8.86	7.42	7.52	8.59	6.99	7.67
12.0	8.26	8.61	8.42	8.37	8.33	8.29	7.96	8.30	8.58	8.31	8.37	8.12	9.43	7.84	9.20	7.60	7.72	8.88	7.31	8.01
14.0	8.52	8.90	8.63	8.61	8.62	8.48	8.12	8.42	8.95	8.59	8.68	8.40	9.71	8.10	9.48	7.81	7.89	9.20	7.60	8.29
16.0	8.80	9.14	8.82	8.80	8.98	8.62	8.30	8.55	9.18	8.86	8.95	8.68	9.91	8.28	9.70	8.00	8.09	9.44	7.83	8.50
18.0	9.07	9.39	8.98	9.17	9.29	8.79	8.48	8.65	9.43	9.11	9.17	8.89	10.13	8.41	9.91	8.19	8.23	9.62	8.08	8.69
20.0	9.30	9.51	9.12	9.38	9.51	8.96	8.66	8.73	9.65	9.36	9.35	9.12	10.32	8.60	10.10	8.32	8.41	9.71	8.31	8.93

Table 11. Change in pH of a one per cent peptone solution (adjusted) after adding various amounts of 0.01 N NaOH

ML. 0.01N NaOh added.	Bacto-tryptose	Armour peptone	Vegetable peptone No. 3	Bacto-tryptone	Fairchild peptone	Proteose-peptone No. 2	Pfansteihl peptone	Wilson peptone "C"	Brain peptone (2)	Vegetable peptone No. 1	Chaissiang peptone	Proteose-peptone No. 3	Vegetable peptone No. 5	Vegetable peptone No. 4	Wilson peptone "CB"	Vegetable peptone No. 2	Bacto-peptone	Brain peptone (1)	Liver peptone (1)	Bacto-neopeptone
0.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
1.00	7.12	7.08	7.19	7.12	7.20	7.12	7.14	7.24	7.12	7.27	7.18	7.12	7.25	7.36	7.30	7.37	7.21	7.19	7.23	7.15
2.00	7.24	7.19	7.42	7.22	7.39	7.23	7.28	7.38	7.21	7.48	7.32	7.23	7.43	7.60	7.42	7.60	7.40	7.35	7.46	7.30
3.00	7.34	7.31	7.59	7.36	7.51	7.33	7.40	7.49	7.31	7.63	7.48	7.34	7.59	7.81	7.52	7.79	7.57	7.50	7.65	7.44
4.00	7.44	7.39	7.76	7.46	7.65	7.45	7.51	7.58	7.41	7.80	7.60	7.45	7.70	8.00	7.63	7.95	7.71	7.65	7.81	7.56
5.00	7.52	7.50	7.89	7.56	7.78	7.57	7.63	7.70	7.50	7.91	7.71	7.55	7.83	8.11	7.78	8.09	7.83	7.78	7.98	7.69
6.00	7.62	7.60	7.99	7.68	7.90	7.67	7.72	7.81	7.59	8.01	7.85	7.65	7.92	8.22	7.88	8.21	7.94	7.91	8.10	7.82
7.00	7.71	7.70	8.08	7.79	8.00	7.77	7.84	7.91	7.68	8.12	7.98	7.75	8.00	8.31	7.98	8.30	8.05	8.01	8.20	7.94
8.00	7.80	7.80	8.15	7.90	8.08	7.87	7.95	8.01	7.75	8.21	8.10	7.85	8.10	8.40	8.07	8.38	8.15	8.12	8.30	8.04
9.00	7.89	7.90	8.21	7.98	8.18	7.94	8.02	8.09	7.86	8.30	8.18	7.94	8.19	8.47	8.13	8.47	8.23	8.22	8.40	8.16
10.0	7.97	7.98	8.29	8.05	8.25	8.02	8.13	8.19	7.94	8.39	8.28	8.05	8.27	8.51	8.24	8.52	8.31	8.33	8.50	8.27
12.0	8.11	8.15	8.41	8.21	8.40	8.20	8.31	8.37	8.12	8.52	8.46	8.25	8.41	8.62	8.43	8.65	8.49	8.51	8.64	8.48
14.0	8.28	8.30	8.50	8.40	8.50	8.39	8.50	8.52	8.30	8.64	8.61	8.44	8.58	8.73	8.58	8.78	8.64	8.68	8.78	8.70
16.0	8.42	8.47	8.59	8.51	8.63	8.53	8.64	8.67	8.50	8.76	8.77	8.63	8.72	8.81	8.73	8.86	8.76	8.81	8.89	8.85
18.0	8.56	8.61	8.68	8.67	8.73	8.70	8.80	8.80	8.70	8.86	8.89	8.80	8.85	8.90	8.89	8.94	8.89	8.96	9.00	9.01
20.0	8.70	8.72	8.73	8.81	8.82	8.85	8.88	8.90	8.90	8.94	8.94	8.95	8.95	8.96	9.00	9.00	9.01	9.09	9.10	9.10

Table 11. Change in pH of a one per cent peptone solution (adjusted) after adding various amount of 0.01N NaOH.

Ml. 0.01N NaOH added	Vegetable tryptone	Proteose-peptone	Steam peptone	Witte peptone (1940)	Parke, Davis peptone	Beef peptone	Bacto-peptone	Merck peptone	Spleen peptone (2)	Pork peptone	Spleen peptone (1)	Liver peptone (2)	Witte peptone (1912)	Armour special peptone	Heart peptone (1)	Heart peptone (2)	E&A Albumin peptone	Cenco peptone	E&A Meat peptone	Baker peptone
0.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00	7.00
1.00	7.41	7.18	7.30	7.19	7.27	7.29	7.18	7.30	7.18	7.21	7.25	7.12	7.15	7.21	7.12	7.15	7.12	7.45	7.46	7.44
2.00	7.65	7.31	7.57	7.31	7.53	7.43	7.30	7.50	7.29	7.32	7.45	7.31	7.30	7.43	7.29	7.30	7.31	7.82	7.82	7.82
3.00	7.80	7.44	7.78	7.44	7.75	7.57	7.43	7.70	7.42	7.47	7.61	7.49	7.44	7.65	7.42	7.45	7.49	8.19	8.11	8.13
4.00	7.95	7.55	7.97	7.53	7.95	7.71	7.56	7.88	7.52	7.60	7.78	7.69	7.60	7.88	7.54	7.59	7.68	8.48	8.36	8.39
5.00	8.09	7.68	8.15	7.59	8.13	7.88	7.69	8.02	7.64	7.74	7.95	7.81	7.73	8.10	7.69	7.72	7.85	8.78	8.60	8.61
6.00	8.22	7.79	8.31	7.66	8.31	8.01	7.81	8.19	7.77	7.86	8.10	7.97	7.89	8.30	7.83	7.89	7.98	8.98	8.83	8.82
7.00	8.33	7.92	8.45	7.77	8.48	8.15	7.97	8.31	7.89	7.99	8.25	8.17	8.06	8.50	7.98	8.01	8.13	9.13	8.98	8.99
8.00	8.44	8.01	8.54	7.89	8.61	8.30	8.09	8.43	8.00	8.10	8.40	8.32	8.22	8.68	8.11	8.17	8.32	9.30	9.11	9.12
9.00	8.56	8.13	8.63	8.01	8.74	8.40	8.26	8.57	8.11	8.25	8.58	8.45	8.38	8.88	8.25	8.31	8.51	9.40	9.26	9.26
10.0	8.65	8.23	8.73	8.12	8.86	8.52	8.40	8.69	8.23	8.38	8.71	8.66	8.56	9.00	8.42	8.48	8.70	9.53	9.36	9.35
12.0	8.80	8.47	8.88	8.51	9.11	8.79	8.72	8.90	8.50	8.65	9.00	8.97	8.90	9.18	8.80	8.85	9.08	9.78	9.58	9.50
14.0	8.90	8.68	9.03	8.61	9.29	9.03	9.01	9.10	8.80	8.91	9.22	9.18	9.20	9.38	9.13	9.18	9.37	9.98	9;79	9.65
16.0	9.00	8.87	9.15	8.84	9.42	9.24	9.28	9.30	9.08	9.15	9.41	9.42	9.49	9.55	9.42	9.45	9.61	10.13	9.95	9.80
18.0	9.08	9.05	9.26	9.12	9.50	9.40	9.44	9.47	9.30	9.40	9.58	9.55	9.70	9.70	9.67	9.67	9.82		10.13	9.95
20.0	9.14	9.19	9.37	9.37	9.55	9.60	9.60	9.60	9.62	9.63	9.71	9.76	9.80	9.83	9.84	9.85	10.00			10.14

These results agree to some extent with those of Chamot and Georgia (12), who divided the peptones which they tested into three groups as follows: slightly alkaline, slightly acid and highly acid. These authors found Witte's (1912), Bacto-peptone and Proteose-peptone to be slightly alkaline. The present work shows Witte's (1912) and Proteose-peptone to be slightly alkaline, but the sample of Bacto-peptone tested was very slightly acid, pH 6.97. Chamot and Georgia found Armour's and Parke, Davis and Company's peptones to be only slightly acid (between pH 7.00 and 5.80) while the present results show Armour's in this group at a pH of 6.31 and Parke, Davis and Company's in the strongly acid group with a pH of 5.20. Chamot and Georgia's third group (highly acid - pH 5.00 and above) includes Fairchild's (pH 4.78) and Stearn's (pH 4.75). The present work places these two peptones in the same general grouping, but the pH values obtained were slightly higher, pH 5.51 and 5.91, respectively.

The relative buffering ability of the peptones is best shown in Tables 9 and 11, where the peptones were adjusted to pH 7.00 before the addition of acid or alkali. The peptones are listed in both tables in the order of their decreasing buffer ability as shown by the pH reached after 20 ml. of 0.01N HCl or 0.01N NaOH was added to 20 ml. of the adjusted peptone solution, or by the Van Slyke buffer index computed from the results. This criterion of buffering value places Vegetable peptone No. 4 first, Parke, Davis and Company's second, Liver peptone (1) third, etc., when acid was added. Table 10 also shows that the prepared peptones are, as a group, better buffers against acid than are the commercial peptones. The leading ten peptones include seven prepared peptones and three commercial brands. It is to be noted that a few of the highly acid peptones such as Parke, David and Company's, Stearn's, Fairchild's and Pfanstiehl appear to be good buffers against acid, while other highly acid peptones such as Baker's, Merck's and Cenco are

relatively poor buffers against acid.

When alkali is added to the adjusted peptone solutions the results (Table 11) show that Bacto-tryptose is the best buffer, followed by Armour's and Vegetable peptone No. 3. The commercial peptones appear to be much better buffers against alkali than are the prepared peptones as shown by the fact that seven of the leading ten peptones are commercial products. Here again some of the more acid peptones such as Fairchild's and Chaissiang appear to have a much better buffer action against alkali than do others in the same group, such as Baker's and Cenco. The fact that such highly acid peptones as Baker's, Cenco, and Merck's show no buffering action against either acid or alkali after they have been adjusted to pH 7.00 indicates that neutralization does not affect their buffering capacity. This in turn would seem to indicate that the neutralization of peptones, as is practiced in preparing media for bacteriological use, has little effect on the buffering ability of a peptone. Thus it may be concluded that, as would be expected, the buffering action of a peptone is due to inherent properties of the peptone, rather than to the acid or alkali used in neutralizing the peptone. As a general rule, when unadjusted, the peptones having a high initial pH are better buffers against acid than those having a low initial pH, and vice versa. However, when the peptones are placed on an equal basis by adjusting the solution to pH 7.00, this relationship is lost. These results (Tables 8,9,10 and 11) show that, in general, the initial pH of a peptone has no relation to its ability as a buffer, either against acid or base, if the peptone has been neutralized.

Figure 4 shows the buffer curves obtained when the pH is plotted against the ml. of acid or alkali added to the peptone solution. Since

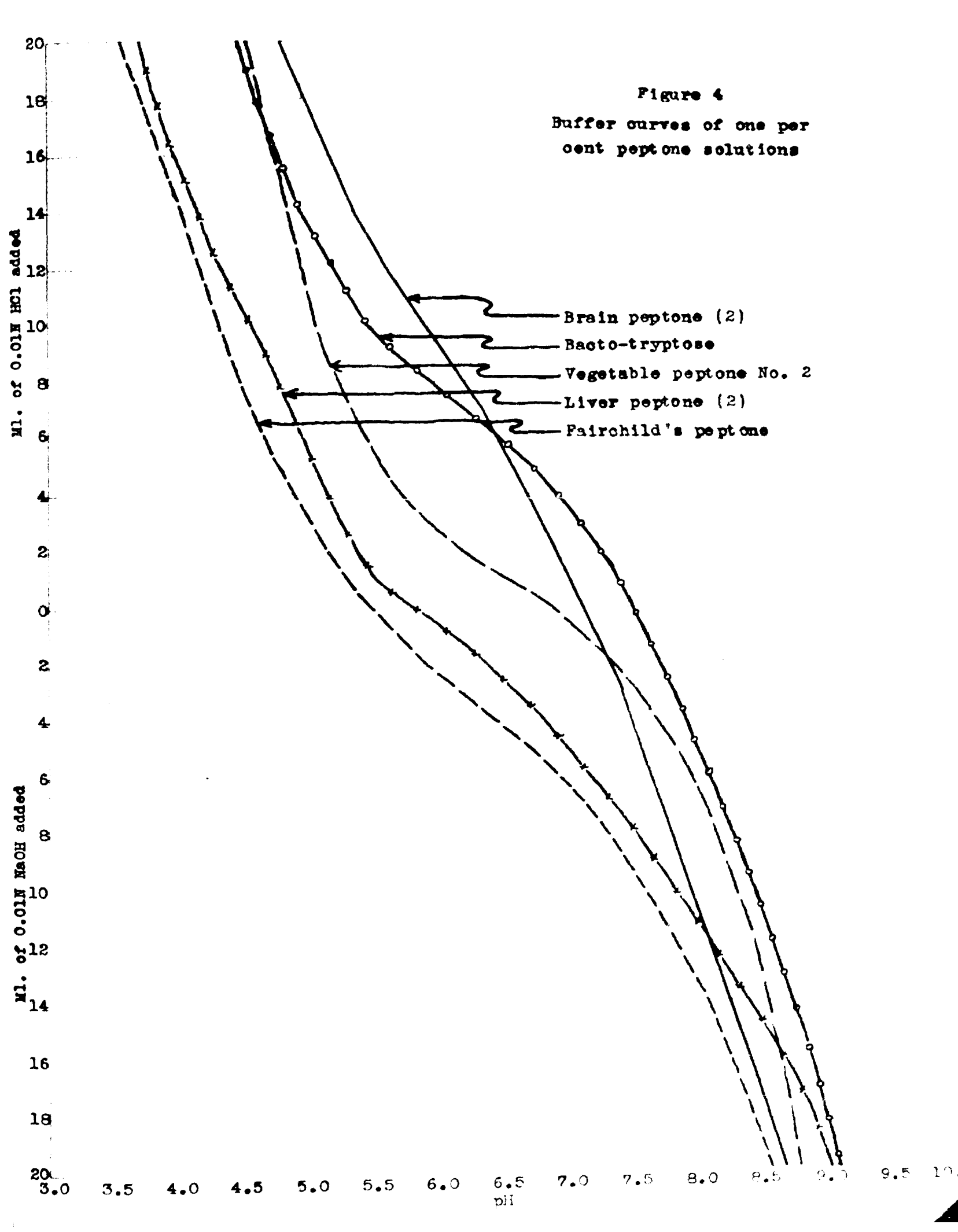


Figure 4

Buffer curves of one per cent peptone solutions

the curves for all peptones, with one exception, show the same general trend, only a few typical examples are presented. The exception is brain peptone (2), which is included in Figure 4. These curves indicate that the peptones have the least buffering action between pH 5.5 and 7.5. This is unfortunate, since it is in this range that buffering action in bacteriological media is most desirable. Bronfenbrenner, DeBord and Orr (11) studied the buffer capacity of Difco, Proteose, Witte, Aminoid, Fairchild, Roche and Armour peptones. These authors found the greatest buffer action between pH 8 and 9 and the least between pH 4 and 5, which is somewhat lower than the results obtained in the present work.

When used in bacteriological media, the ability of a peptone to buffer a solution against the production of acid is more important than its ability to buffer against the production of alkali, since the presence of carbohydrates in many media usually results in the formation of acids. Also the buffering ability of a peptone between pH 7.00 and 6.00 is more important than its buffering ability between pH 5.00 and 4.00, because it is desirable to maintain neutrality for as long a period as possible. With these facts in mind, the approximate amount of 0.01N HCl needed to change a one per cent peptone solution from pH 7.00 to pH 6.00 was determined from Table 9. The results are given in Table 12. This table shows that under the conditions of the experiment, Brain peptone (2) is by far the best buffer against acid, followed by Armour's peptone. It also shows that, while a prepared peptone is best, the buffer values of the prepared peptones as a group is slightly less than that of commercial peptones, since six of the leading ten peptones are commercial products.

When the amount of acid needed to change a one per cent peptone solution from pH 7.00 to 6.00 is compared with the amount needed to change

Table 12. Approximate amount of 0.01N HCl needed to bring a one per cent peptone solution (adjusted to pH 7.0) to pH 6.00 and 5.00.

Peptone	Ml. to bring to pH 6.00	Peptone	Ml. to bring to pH 5.00
1. Brain peptone (2)	9.50	1. Brain peptone (2)	17.00
2. Armour peptone	6.50	2. Vegetable peptone No. 4	16.00
3. Bacto-tryptone	6.00	3. Liver peptone (1)	16.00
4. Wilson peptone "C"	6.00	4. Parke, Davis peptone	16.00
5. Vegetable peptone No. 5	6.00	5. Pork peptone	14.00
6. Heart peptone (2)	6.00	6. Heart peptone (2)	14.00
7. Pork peptone	6.00	7. Vegetable peptone No. 5	14.00
8. Bacto-tryptose	5.90	8. Wilson peptone "C"	13.80
9. Pfansteihl peptone	5.60	9. Bacto-tryptone	13.00
10. Bacto-protone	5.60	10. Armour peptone	13.00
11. Spleen peptone (2)	5.50	11. Brain peptone (1)	12.00
12. Armour's special peptone	5.50	12. Fairchild's peptone	12.00
13. Heart peptone (1)	5.40	13. Pfansteihl peptone	12.00
14. Vegetable peptone No. 4	5.00	14. Armour's special peptone	12.00
15. Wilson peptone "CB"	5.00	15. Heart peptone (1)	12.00
16. Beef peptone	5.00	16. Spleen peptone (2)	11.60
17. Fairchild's peptone	4.95	17. Vegetable tryptone	11.30
18. Witte peptone (1940)	4.90	18. Witte peptone (1940)	11.20
19. Chaissiang peptone	4.90	19. Liver peptone (2)	11.00
20. Witte peptone (1912)	4.90	20. Wilson peptone "CB"	11.00
21. Brain peptone (1)	4.80	21. Beef peptone	11.00
22. Parke, Davis peptone	4.75	22. Vegetable peptone No. 1	10.60
23. Liver peptone (1)	4.60	23. Chaissiang peptone	10.60
24. Liver peptone (2)	4.60	24. Bacto-protone	10.60
25. Bacto-neopeptone	4.20	25. Bacto-tryptose	10.40
26. E & A Albumin peptone	4.00	26. Vegetable peptone No. 2	10.00
27. Spleen peptone (1)	4.00	27. Vegetable peptone No. 3	10.00
28. Proteose-peptone	4.00	28. E & A Albumin peptone	10.00
29. Proteose-peptone No. 3	3.95	29. Witte peptone (1912)	9.50
30. Vegetable peptone No. 1	3.90	30. Spleen peptone (1)	9.00
31. Proteose-peptone No. 2	3.75	31. Proteose peptone No. 3	9.00
32. Vegetable tryptone	3.00	32. Proteose peptone	8.75
33. Vegetable peptone No. 2	3.00	33. Bacto-neopeptone	8.60
34. Vegetable peptone No. 3	3.00	34. Stearn's peptone	8.00
35. Merck peptone	3.00	35. Proteose-peptone No. 2	7.60
36. Stearn's peptone	2.90	36. Bacto-peptone	7.00
37. E & A Meat peptone	2.80	37. Merck's peptone	6.00
38. Baker's peptone	2.75	38. Cenco peptone	6.00
39. Bacto-peptone	2.50	39. E & A Meat peptone	5.00
40. Cenco peptone	2.00	40. Baker's peptone	4.60

the pH from 6.00 to 5.00, the results (Table 13) show that Brain peptone (2) maintains its position as the best buffer against acid. This table also shows that a peptone which is a good buffer between pH 6.00 and 5.00 is not necessarily a good buffer between pH 7.00 and 6.00, and vice versa. Thus the results show that peptones vary considerably in their buffer action between any two pH values, and that there is no correlation between their buffering ability at two different sets of pH units. These results support the findings of Bronfenbrenner, DeBord and Orr (11).

Attempts to correlate the buffering ability of the peptones with their chemical composition failed to show any relationship between the buffer index and the amount of any of the various nitrogen fractions. This indicates that the buffering capacity of a peptone is not due to any one nitrogenous component, but rather it is due to some component not analyzed or, more likely, to the way the various components are combined in the peptone molecule. The ash content of a peptone should have some influence on the buffering ability, especially if it contains phosphates of calcium or sodium. The presence in a peptone of a small amount of such salts as these would result in a better buffer than if a large amount of ash consisting of neutral salts, such as NaCl, was present.

Summary

Chemical analysis was made of large numbers of peptones for total nitrogen, total, primary and secondary proteose nitrogen, peptone nitrogen, free ammonia nitrogen, free amino acid nitrogen, amino nitrogen and ash content. The results obtained show that: (1) The commercial and prepared peptones are fairly uniform as to total nitrogen content. (2) The vegetable peptones are, as a group, lower than the commercial peptones in

total nitrogen. (3) The peptones, both commercial and prepared, vary widely in their content of the various nitrogen fractions. (4) The commercial and prepared animal peptones, are, with the exception of Bacto-protone, higher in peptone nitrogen than any other nitrogen fraction. (5) The amino nitrogen as determined by Sørenson's formol titration consistently gave slightly lower results than the same fraction as determined by Van Slyke's method. (6) The vegetable peptones prepared from hydrolyzed corn gluten are higher in free amino acid and amino nitrogen than are the commercial and prepared peptones. (7) The content of free ammonia nitrogen is low in all peptones. (8) The sum of the nitrogen fractions does not equal the figure for total nitrogen, indicating that more than one nitrogen fraction was determined by the same analysis, or that not all of certain forms of nitrogen is being determined. (9) The commercial peptones gave positive biuret, Millon, Rosenheim, Sakaguchi, ninhydrin, xanthoproteic and Fleitmann tests. They were all negative to the Molish reaction. (10) The prepared animal peptones, with the exception of the two spleen and two liver peptones, were positive to all except the Molish reaction. The four exceptions were positive to the Molish reactions. (11) The four vegetable peptones prepared from hydrolyzed corn gluten gave positive Sakaguchi, ninhydrin, xanthoproteic, Fleitmann and Molish reactions, and negative Rosenheim and biuret tests. Vegetable peptones number 1,2 and 3 were positive to Millon's test, while number 4 was negative. Vegetable peptone No. 5 and vegetable tryptone were positive to all tests. (12) The initial pH of a one per cent peptone solution may be used as the basis for roughly dividing the peptones into four groups as follows: alkaline, pH 7.00 to 7.60; neutral, pH 7.00; slightly acid,

pH 6.00 to 7.00 and strongly acid, pH 4.89 to 6.00. (13) The initial pH of all peptones varies widely. (14) When the buffer index, obtained after adding 20 ml. of 0.01N HCl or NaOH to a neutralized peptone solution is used as the criterion of buffering ability, the prepared peptones, as a group, are shown to be better buffers against acids and the commercial peptones to be better buffers against bases. When the amount of 0.01N HCl needed to change a peptone solution from pH 6.00 to 7.00 is used as the criterion, the commercial peptones are shown to be slightly superior to the prepared peptones as buffers against acids. (13) Brain peptone (2) is the best buffer against acid. (14) The peptones, as a general rule, show the least buffering action between pH 5.5 and 7.5.

BACTERIOLOGICAL ANALYSIS OF PREPARED PEPTONES

The methods of determining the efficacy of bacteriological peptones to support the growth of bacteria are legion. A new test may be devised to solve each problem as it arises. The tests used to determine the ability of the prepared peptones to support the growth of bacteria include the determination of the rate of growth of E. coli; plating of samples of raw milk; growth and gas production by coliform organisms found in naturally contaminated water and the testing for the growth of certain pathogenic organisms. Whenever possible standard or recommended media were used as controls in these experiments.

Determination of Rate of Growth of E. coli.

The medium used for the determination of the rate of growth of E. coli consisted of five-tenths per cent peptone and five-tenths per cent NaCl at a pH of 7.00. Fifty ml. of this medium was inoculated with

one ml. of a 1:1,000,000 dilution of a twelve hour broth culture of E. coli and incubated at 37°C. After inoculation the medium was shaken to distribute the organisms and plated out immediately and at two hour intervals for the first twelve hours, and again at the end of 24 and 48 hours. Duplicate plates were poured for each peptone tested. The plating medium used was as follows:

Bacto-peptone	0.5%
Bacto Beef-extract	0.3%
NaCl	0.5%
Agar	1.5%
Water	1000.0 ml.
pH	7.00

The plates were incubated for 48 hours at 37°C. and the colonies counted with the aid of a Quebec Colony Counter. The agerage of the results of five experiments using four vegetable peptones prepared from hydrolysates of corn gluten in comparison with Bacto-tryptone, tryptose and peptone is given in Table 13. Figure 14 shows the curves obtained when the log of the numbers of bacteria was plotted against the time in hours. The vegetable peptones used in these experiments were decolorized with Norite "A".

An examination of the results given in Table 13 shows that vegetable peptones number 1,2 and 3 give better growth of E. coli during the first six hours than do the Difco products. After six hours Bacto-tryptose is better than any of the vegetable peptones, and at the end of 12 hours the Difco peptones gave better growth than did the vegetable peptones. Reference to Tables 5 and 6 shows that the vegetable peptones are higher in free amino acid nitrogen than are any of the Difco products. This seems to indicate that a peptone containing a large amount of this type of nitrogen will initiate a faster rate of growth, resulting in a shorter lag phase, than will a peptone which contains a smaller amount of free amino acids. On the other hand, the presence of proteose and/or peptone nitrogen in a peptone makes it capable of carrying out a more extended growth, as is shown by

Time in hours	Bacteria per ml.						
	Bacto-peptone	Bacto-tryptone	Bacto-tryptose	Vegetable peptone No. 1	Vegetable peptone No. 2	Vegetable peptone No. 3	Vegetable peptone No. 4
0	19	23	19	20	19	19	18
2	51	58	64	69	74	79	45
4	590	810	1355	1549	1636	1960	393
6	14,600	19,300	20,400	20,800	21,500	29,200	8,800
8	289,000	503,000	1,550,000	534,000	536,000	1,487,000	63,000
10	2,280,000	3,770,000	4,470,000	2,540,000	3,540,000	4,000,000	584,000
12	54,000,000	66,000,000	77,000,000	39,000,000	43,000,000	47,000,000	7,000,000
24	283,000,000	394,000,000	675,000,000	189,000,000	240,000,000	254,000,000	42,000,000
48	229,000,000	333,000,000	351,000,000	102,000,000	185.000,000	205,000,000	76,000,000

Table 13. Comparison of the rate of growth of E. coli in broth prepared from four vegetable peptones and three Difco peptones.. Average of five trials.

the Difco products.

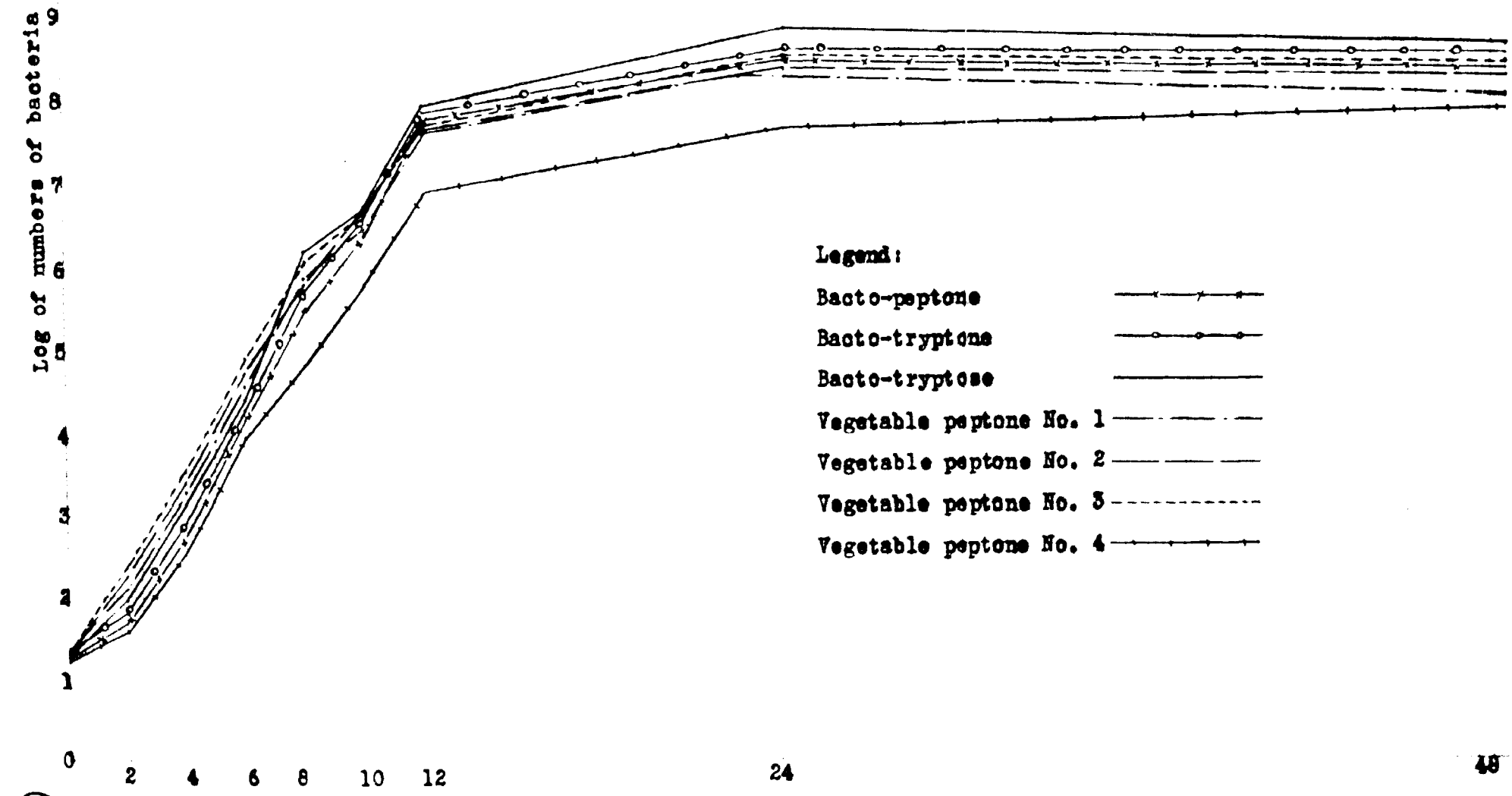
These results support the findings of Rettger, Berman and Sturges (40), who found that the presence in a medium of free amino acids is desirable or even necessary before many organisms can ititiate growth. Some organisms cannot utilize the more complex nitrogen fractions until growth has been initiated by utilization of free amino acids. However, once growth has started the bacteria will produce enzymes to break down these more complex fractions. Hartley (24) has pointed out that, as a general rule, the more complete the digestion of the protein, the better the growth of certain organisms. Gordon and M'Leod (21) found that certain amino acids, tryptophane in particular, are toxic to bacteria. Since the tryptophane in the samples of hydrolyzed corn gluten has been destroyed by the hydrolyzed corn gluten has been destroyed by the hydrolysis treatment, the peptones prepared from these samples may be less toxic to E. coli than the commercial peptones tested, in which tryptophane is present as shown by the positive Rosenheim test.

The positive Molish test shows the presence of carbohydrates, probably glucose, in the vegetable peptones, which would also aid in the growth of the organisms during the early stages.

Figure 14 shows that the growth rates of the three vegetable peptones and the three Difco peptones are very similar, and that Vegetable peptone No. 4 is much inferior to the others. Both Table 13 and Figure 14 show that the vegetable peptones No. 1,2 and 3 and the Difco peptones gave maximum numbers of E. coli in 24 hours, and then started to recede, while vegetable peptone No. 4 did not show maximum growth until 48 hours or later. This was doubtless due to the removal of leucine and tyrosine as previously stated. This would also indicate

Figure 5

Growth curves for E. coli in broth prepared from vegetable and Difco peptones



that one or both of these amino acids were necessary for growth during the initial period.

Plating of Samples of Raw Milk.

The medium used in testing the ability of the various prepared peptones to grow the organisms found in raw milk was Standard Milk Agar in which the tryptone was replaced with one of the prepared peptones. Standard Milk Agar (51) was used as the control.

The method was as follows: A 1:1,000 dilution of the raw milk sample was prepared and shaken for at least five minutes in a shaking machine to break up clumps of bacteria and to thoroughly distribute the organisms in the dilution water. Duplicate plates of this dilution were poured for each peptone to be tested. The plates were incubated at 37° C. for 48 hours and the colonies were then counted with the aid of a Quebec Colony Counter. Samples of milk showing more than 300 or less than 30 colonies per plate were discarded.

Table 14 gives the results obtained from 100 samples of raw milk tested with vegetable peptones No. 1,2 and 3, as compared with Bacto-tryptone. Table 15 gives the results obtained with undecolorized vegetable peptones No. 1, 3 and 5 and vegetable tryptone as compared with Bacto-tryptone and tryptose. Table 16 gives the results obtained with 50 samples of raw milk tested with vegetable peptone No. 3 and 5 and vegetable tryptone as compared with Bacto-tryptose. Tables 17 and 18 give the results obtained with 100 samples of raw milk tested with eight animal peptones as compared with Bacto-tryptone.

Discussion

The average results obtained when comparing the vegetable peptones with Bacto-tryptone and tryptose (Tables 14, and 15 and 16) show

Table 14. Comparison of three vegetable peptones and Bacto-tryptone for growth or organisms in samples of raw milk.

Sample number	Bacteria per ml.			
	Vegetable peptone No.1	Vegetable peptone No.2	Vegetable peptone No.3	Bacto-tryptone
1	34,000	34,000	41,000	47,000
2	40,000	43,000	41,000	44,000
3	245,000	145,000	112,000	126,000
4	105,000	112,000	98,000	109,000
5	44,000	63,000	84,000	65,000
6	78,000	80,000	70,000	50,000
7	98,000	69,000	59,000	54,000
8	44,000	41,000	49,000	42,000
9	44,000	41,000	41,000	41,000
10	50,000	36,000	32,000	26,000
11	102,000	61,000	78,000	119,000
12	57,000	102,000	93,000	76,000
13	33,000	34,000	40,000	33,000
14	77,000	87,000	65,000	144,000
15	79,000	61,000	59,000	63,000
16	37,000	94,000	45,000	43,000
17	37,000	59,000	25,000	23,000
18	184,000	144,000	112,000	91,000
19	58,000	62,000	58,000	43,000
20	33,000	21,000	44,000	66,000
21	53,000	52,000	81,000	55,000
22	31,000	41,000	61,000	43,000
23	53,000	55,000	88,000	53,000
24	21,000	30,000	39,000	33,000
25	120,000	124,000	128,000	128,000
26	42,000	46,000	46,000	43,000
27	44,000	42,000	60,000	40,000
28	19,000	22,000	30,000	28,000
29	102,000	54,000	108,000	70,000
30.	113,000	114,000	120,000	116,000
31	107,000	128,000	130,000	119,000
32	148,000	178,000	211,000	221,000
33	186,000	182,000	197,000	164,000
34	41,000	67,000	82,000	81,000
35	86,000	90,000	97,000	91,000
36	40,000	34,000	43,000	83,000
37	81,000	78,000	87,000	84,000
38	68,000	94,000	95,000	94,000
39	72,000	78,000	83,000	89,000
40	20,000	21,000	22,000	36,000
41	30,000	31,000	34,000	33,000
42	52,000	64,000	68,000	68,000
43	31,000	30,000	36,000	38,000
44	37,000	40,000	60,000	48,000
45	41,000	51,000	64,000	57,000
46	50,000	49,000	53,000	32,000
47	30,000	37,000	39,000	46,000
48	254,000	258,000	282,000	278,000
49	272,000	301,000	377,000	327,000
50	31,000	36,000	39,000	35,000
Average	75,000	77,800	82,100	78,400

Table 14. (Continued)

Sample number	Bacteria per ml.			
	Vegetable peptone No.1	Vegetable peptone No.2	Vegetable peptone No.3	Bacto-tryptone
51	155,000	124,000	136,000	71,000
52	108,000	152,000	132,000	144,000
53	116,000	118,000	112,000	112,000
54	48,000	47,000	48,000	52,000
55	31,000	39,000	44,000	62,000
56	121,000	122,000	184,000	150,000
57	49,000	56,000	60,000	54,000
58	109,000	122,000	126,000	109,000
59	177,000	196,000	215,000	194,000
60	39,000	38,000	48,000	33,000
61	49,000	40,000	39,000	41,000
62	54,000	39,000	32,000	38,000
63	48,000	49,000	47,000	48,000
64	53,000	37,000	35,000	46,000
65	126,000	126,000	103,000	152,000
66	354,000	282,000	340,000	248,000
67	263,000	249,000	189,000	224,000
68	104,000	88,000	61,000	96,000
69	65,000	87,000	144,000	77,000
70	81,000	52,000	55,000	53,000
71	122,000	123,000	128,000	120,000
72	279,000	211,000	273,000	235,000
73	18,000	26,000	18,000	34,000
74	43,000	46,000	51,000	58,000
75	44,000	47,000	48,000	56,000
76	198,000	220,000	178,000	184,000
77	165,000	134,000	177,000	142,000
78	25,000	28,000	41,000	31,000
79	59,000	63,000	63,000	76,000
80	239,000	260,000	290,000	239,000
81	47,000	43,000	52,000	48,000
82	23,000	40,000	67,000	49,000
83	32,000	31,000	33,000	33,000
84	40,000	33,000	38,000	43,000
85	18,000	13,000	21,000	32,000
86	169,000	179,000	234,000	182,000
87	105,000	97,000	138,000	114,000
88	43,000	44,000	58,000	49,000
89	145,000	192,000	209,000	203,000
90	29,000	30,000	28,000	20,000
91	33,000	95,000	112,000	81,000
92	12,000	10,000	15,000	30,000
93	23,000	33,000	39,000	38,000
94	24,000	27,000	26,000	30,000
95	15,000	19,000	36,000	25,000
96	44,000	46,000	47,000	39,000
97	40,000	48,000	45,000	46,000
98	26,000	44,000	22,000	29,000
99	45,000	47,000	45,000	48,000
100	59,000	64,000	79,000	63,000
Average	86,200	87,100	95,000	93,700
Average of 100 samples	80,600	82,900	88,500	86,000

Table 15. Comparison of four undecolorized vegetable peptones with Bacto-tryptone and tryptose for growth of organisms in samples of raw milk.

Sample Number	Bacteria per ml.					
	Vegetable peptone No. 1	Vegetable peptone No.3	Vegetable peptone No.	Vegetable tryptone	Bacto-tryptone	Bacto-tryptose
1	23,000	19,000	24,000	23,000	30,000	29,000
2	44,000	37,000	38,000	42,000	45,000	46,000
3	67,000	88,000	74,000	87,000	96,000	86,000
4	258,000	258,000	367,000	209,000	369,000	321,000
5	29,000	47,000	59,000	36,000	63,000	46,000
6	73,000	178,000	304,000	155,000	315,000	278,000
7	30,000	80,000	52,000	27,000	43,000	74,000
8	60,000	66,000	66,000	56,000	60,000	67,000
9	27,000	162,000	51,000	135,000	113,000	156,000
10	16,000	27,000	7,000	21,000	28,000	37,000
11	38,000	52,000	73,000	102,000	110,000	121,000
12	42,000	24,000	31,000	38,000	45,000	46,000
13	51,000	46,000	54,000	51,000	51,000	48,000
14	45,000	35,000	39,000	39,000	48,000	47,000
15	14,000	14,000	35,000	21,000	23,000	27,000
16	29,000	31,000	36,000	32,000	39,000	43,000
17	17,000	20,000	51,000	23,000	51,000	62,000
18	43,000	43,000	52,000	57,000	46,000	52,000
19	46,000	50,000	48,000	57,000	47,000	58,000
20	21,000	24,000	31,000	41,000	35,000	46,000
21	69,000	73,000	88,000	71,000	44,000	74,000
22	41,000	43,000	42,000	43,000	42,000	44,000
23	4,000	1,000	66,000	52,000	28,000	53,000
24	1,000	5,000	32,000	30,000	26,000	25,000
25	6,000	18,000	24,000	60,000	30,000	82,000
26	24,000	4,000	8,000	38,000	36,000	35,000
27	4,000	6,000	17,000	14,000	25,000	47,000
28	5,000	12,000	31,000	91,000	75,000	91,000
29	36,000	43,000	52,000	51,000	46,000	51,000
30	107,000	170,000	221,000	198,000	136,000	182,000
31	3,000	44,000	46,000	58,000	24,000	60,000
32	15,000	34,000	97,000	99,000	98,000	97,000
33	1,000	1,000	28,000	21,000	17,000	37,000
34	3,000	9,000	30,000	44,000	23,000	33,000
35	4,000	11,000	42,000	38,000	35,000	44,000
36	45,000	41,000	41,000	55,000	51,000	55,000
37	33,000	73,000	87,000	87,000	75,000	92,000
38	37,000	65,000	102,000	108,000	64,000	113,000
39	4,000	8,000	25,000	33,000	28,000	34,000
40	7,000	17,000	24,000	29,000	20,000	37,000
41	19,000	21,000	36,000	34,000	28,000	37,000
42	10,000	21,000	65,000	82,000	30,000	86,000
43	5,000	2,000	26,000	25,000	25,000	39,000
44	6,000	41,000	154,000	41,000	42,000	154,000
45	3,000	5,000	29,000	12,000	8,000	33,000
46	6,000	23,000	47,000	34,000	29,000	30,000
47	4,000	9,000	26,000	27,000	21,000	36,000
48	6,000	7,000	20,000	27,000	20,000	34,000
49	8,000	13,000	53,000	12,000	17,000	43,000
50	32,000	57,000	51,000	70,000	64,000	76,000
Average	30,400	47,340	62,000	58,100	57,200	70,000

Table 16. Comparison of three vegetable peptones and Bacto-tryptone for growth of organisms in samples of raw milk.

Sample number	Bacteria per ml.			
	Vegetable peptone No. 3	Vegetable peptone No. 5	Vegetable tryptone	Bacto-tryptone
1	66,000	21,000	44,000	34,000
2	33,000	66,000	19,000	29,000
3	37,000	41,000	32,000	35,000
4	129,000	139,000	82,000	114,000
5	127,000	218,000	108,000	123,000
6	119,000	169,000	97,000	119,000
7	118,000	124,000	123,000	107,000
8	124,000	118,000	116,000	118,000
9	38,000	25,000	31,000	13,000
10	144,000	190,000	195,000	125,000
11	32,000	95,000	38,000	20,000
12	42,000	48,000	41,000	37,000
13	257,000	306,000	353,000	261,000
14	157,000	144,000	122,000	138,000
15	35,000	83,000	70,000	37,000
16	87,000	95,000	80,000	66,000
17	27,000	30,000	32,000	21,000
18	17,000	36,000	16,000	14,000
19	196,000	274,000	220,000	128,000
20	119,000	122,000	10[illegible],000	111,000
21	125,000	125,000	121,000	120,000
22	106,000	122,000	74 000	92,000
23	42,000	82,000	33,000	35,000
24	114,000	112,000	109,000	118,000
25	15,000	10,000	5,000	38,000
26	247,000	289,000	233,000	295,000
27	191,000	190,000	184,000	172,000
28	93,000	94,000	89,000	85,000
29	149,000	172,000	180,000	191,000
30	240,000	210,000	295,000	232,000
31	42,000	45,000	41,000	44,000
32	42,000	56,000	38,000	38,000
33	46,000	44,000	31,000	35,000
34	43,000	43,000	40,000	44,000
35	47,000	53,000	44,000	42,000
36	32,000	47,000	32,000	35,000
37	61,000	92,000	93,000	90,000
38.	248,000	268,000	248,000	215,000
39	42,000	46,000	42,000	48,000
40	18,000	30,000	27,000	16,000
41	98,000	109,000	114,000	94,000
42	124,000	131,000	122,000	117,000
43	99,000	94,000	104,000	88,000
44	245,000	246,000	215,000	274,000
45	152,000	214,000	160,000	192,000
46	32,000	45,000	44,000	20,000
47	116,000	112,000	119,000	112,000
48	119,000	59,000	82,000	117,000
49	30,000	12,000	23,000	44,000
50	82,000	65,000	94,000	75,000
Average	98,600	111,200	99,200	95,300

Table 17. Comparison of prepared animal peptones with Bacto-tryptone for growth of organisms in raw milk.

Sample number	Beef peptone	Spleen peptone (1)	Spleen peptone (2)	Liver peptone (2)	Bacto-tryptone
1	36,000	40,000	40,000	42,000	40,000
2	35,000	32,000	55,000	32,000	41,000
3	22,000	34,000	37,000	24,000	38,000
4	44,000	47,000	46,000	38,000	42,000
5	33,000	37,000	43,000	33,000	35,000
6	38,000	43,000	38,000	40,000	40,000
7	88,000	139,000	123,000	138,000	120,000
8	121,000	150,000	153,000	102,000	148,000
9	42,000	39,000	45,000	45,000	41,000
10	136,000	107,000	113,000	134,000	152,000
11	35,000	41,000	47,000	31,000	50,000
12	21,000	34,000	41,000	31,000	36,000
13	35,000	44,000	51,000	37,000	43,000
14	33,000	51,000	57,000	33,000	46,000
15	51,000	54,000	55,000	51,000	44,000
16	68,000	56,000	61,000	58,000	49,000
17	37,000	27,000	20,000	51,000	65,000
18	98,000	86,000	83,000	137,000	97,000
19	31,000	43,000	52,000	52,000	67,000
20	124,000	111,000	155,000	113,000	122,000
21	102,000	112,000	106,000	91,000	93,000
22	43,000	51,000	51,000	43,000	41,000
23	28,000	30,000	45,000	35,000	42,000
24	68,000	65,000	60,000	66,000	63,000
25	36,000	35,000	49,000	38,000	41,000
26	127,000	125,000	127,000	118,000	121,000
27	116,000	124,000	117,000	116,000	123,000
28	112,000	112,000	115,000	113,000	115,000
29	82,000	75,000	91,000	93,000	89,000
30	48,000	48,000	50,000	47,000	44,000
31	55,000	64,000	51,000	52,000	43,000
32	115,000	114,000	115,000	104,000	117,000
33	118,000	114,000	113,000	106,000	112,000
34	134,000	149,000	176,000	178,000	216,000
35	46,000	50,000	42,000	55,000	42,000
36	44,000	44,000	45,000	48,000	25,000
37	45,000	96,000	87,000	84,000	75,000
38	46,000	37,000	49,000	34,000	27,000
39	64,000	42,000	38,000	49,000	70,000
40	101,000	110,000	109,000	111,000	110,000
41	105,000	108,000	104,000	106,000	110,000
42	34,000	43,000	45,000	39,000	47,000
43	48,000	45,000	51,000	51,000	63,000
44	23,000	24,000	26,000	43,000	26,000
45	32,000	35,000	46,000	45,000	47,000
46	40,000	55,000	71,000	52,000	68,000
47	41,000	40,000	48,000	39,000	47,000
48	62,000	66,000	78,000	69,000	31,000
49	27,000	20,000	31,000	32,000	41,000
50	39,000	52,000	36,000	37,000	21,000
Average	62,100	66,000	69,700	66,500	68,500

Table 18. Comparison of prepared animal peptones with Bacto-tryptone for growth of organisms in raw milk.

Sample number	Bacteria per ml.				
	Pork peptone	Brain peptone (2)	Heart peptone (1)	Heart peptone (2)	Bacto-tryptone
1	42,000	41,000	42,000	42,000	32,000
2	38,000	38,000	36,000	37,000	42,000
3	42,000	41,000	39,000	41,000	39,000
4	32,000	33,000	34,000	35,000	31,000
5	40,000	42,000	44,000	46,000	47,000
6	38,000	37,000	40,000	41,000	39,000
7	32,000	36,000	35,000	38,000	37,000
8	231,000	193,000	236,000	241,000	199,000
9	103,000	106,000	129,000	126,000	111,000
10	41,000	43,000	42,000	44,000	42,000
11	49,000	48,000	44,000	53,000	52,000
12	32,000	35,000	34,000	37,000	37,000
13	36,000	35,000	33,000	39,000	44,000
14	37,000	46,000	39,000	45,000	37,000
15	32,000	33,000	33,000	37,000	38,000
16	31,000	32,000	34,000	38,000	35,000
17	40,000	42,000	45,000	44,000	38,000
18	257,000	273,000	215,000	268,000	231,000
19	65,000	55,000	56,000	64,000	62,000
20	96,000	72,000	64,000	85,000	67,000
21	43,000	45,000	43,000	44,000	46,000
22	59,000	26,000	48,000	56,000	33,000
23	24,000	37,000	29,000	34,000	34,000
24	117,000	101,000	114,000	121,000	116,000
25	32,000	34,000	43,000	41,000	30,000
26	55,000	42,000	48,000	49,000	39,000
27	43,000	40,000	45,000	41,000	39,000
28	39,000	40,000	41,000	40,000	37,000
29	51,000	23,000	22,000	31,000	31,000
30	64,000	43,000	44,000	54,000	54,000
31	107,000	78,000	94,000	113,000	105,000
32	52,000	51,000	52,000	53,000	42,000
33	37,000	37,000	35,000	36,000	41,000
34	130,000	135,000	124,000	136,000	134,000
35	21,000	21,000	21,000	21,000	31,000
36	48,000	49,000	43,000	53,000	46,000
37	42,000	40,000	40,000	42,000	42,000
38	90,000	98,000	92,000	113,000	109,000
39	39,000	34,000	37,000	31,000	30,000
40	40,000	37,000	43,000	45,000	51,000
41	48,000	45,000	42,000	42,000	48,000
42	58,000	51,000	52,000	55,000	51,000
43	52,000	43,000	45,000	63,000	59,000
44	42,000	41,000	42,000	43,000	42,000
45	55,000	56,000	56,000	61,000	51,000
46	42,000	44,000	45,000	47,000	45,000
47	114,000	106,000	104,000	111,000	106,000
48	90,000	91,000	95,000	92,000	99,000
49	112,000	121,000	102,000	118,000	119,000
50	296,000	212,000	234,000	222,000	245,000
Average	67,100	62,000	62,900	68,180	64,100

that vegetable peptone No. 3, vegetable peptone No. 5 and vegetable tryptone are slightly superior to Bacto-tryptone but inferior to Bacto-tryptose in their ability to grow organisms found in samples of raw milk. However, the averages obtained for vegetable peptone No. 1 and vegetable peptone No. 2 are not significantly lower than the average for Bacto-tryptone, indicating that these peptones are approximately on a par with Bacto-tryptone in their ability to grow organisms found in raw milk. Further comparative tests showed vegetable peptone No. 5 and vegetable tryptone to be superior to vegetable peptone No. 3. This indicates that peptones prepared from unhydrolyzed corn gluten are superior to those prepared from hydrolyzed corn gluten. Table 16 shows that the undecolorized peptones are much inferior to vegetable peptone No. 5 and vegetable tryptone, and are probably inferior to the decolorized peptones prepared from the same hydrolysate sample. The dark color of the media prepared from these undecolorized peptones accounts for their inferiority, since the plates poured with these media were very hard to count.

The average results given in Tables 17 and 18 show that the spleen peptone (1), heart peptone (2) and pork peptone are superior to Bacto-tryptone for growing organisms found in samples of raw milk. The averages for the other prepared animal peptones are only very slightly lower than those for Bacto-tryptone, indicating that all these peptones are approximately as efficient as Bacto-tryptone for this purpose.

Growth and Gas Production by Coliform Organisms from Naturally Contaminated Water.

The medium used in determining the usefulness of the prepared peptones for growth and gas production by coliform organisms found in naturally contaminated water was as follows:

K_2HPO_4	4.0 grams
KH_2PO_4	1.5 grams
NaCl	5.0 grams
Lactose	5.0 grams
Tryptose or a prepared peptone	20.0 grams
Distilled water	1000.0 ml.
pH	7.0

The above medium, with tryptose, as recommended by Darby and Mallmann (13) was used as the control.

The medium was tubed in 10 ml. amounts with Durham fermentation tubes, and sterilized for 20 minutes at fifteen pounds pressure. The tubes were inoculated with one ml. of contaminated river water and incubated at 37°C. for 36 hours. The number of organisms of the coliform type present in the inoculum was determined by plating out one ml. on Violet Red Bile Agar. The inoculated tubes were observed for the time of first visible growth and for first gas production and for the amount of gas at the end of 36 hours. The results are shown in Tables 19 to 26.

Discussion

When the prepared animal and vegetable peptones are compared with Bacto-tryptose for their ability to grow coliform organisms in a lactose-buffered broth the results (Table 19 to 26) show that tryptose is much superior to any of the prepared peptones, both in ability to produce the first visible growth and the first visible gas. The amount of gas pro-

Table 19. Growth and gas production by coliform organisms from naturally contaminated water in lactose buffered broth prepared with vegetable peptones and Bacto-tryptose.

Sample No. 1 Inoculum = 6 E. coli per tube

Peptone	Tube No.	Hours 9	10	11	12	13	14	15	17	32
	1.	-	+	+	+	⊕	⊕	1%	1%	25%
Vegetable	2.	-	+	+	+	⊕	⊕	1	2	25
Peptone	3.	-	+	+	+	⊕	⊕	1	10	50
No. 1	4.	-	+	+	+	⊕	⊕	2	10	50
	5.	-	+	+	⊕	⊕	⊕	5	10	25
	1.	-	+	+	+	⊕	⊕	1%	2%	10%
Vegetable	2.	-	+	+	+	⊕	⊕	1	2	10
Peptone	3.	-	+	+	+	⊕	⊕	1	2	10
No. 2	4.	-	+	+	+	⊕	⊕	1	2	10
	5.	-	+	+	+	⊕	⊕	1	2	20
	1.	-	+	+	⊕	⊕	⊕	1%	2%	25%
Vegetable	2.	-	+	+	⊕	⊕	⊕	1	10	50
Peptone	3.	-	+	+	⊕	⊕	⊕	1	2	30
No. 3	4.	-	+	+	⊕	⊕	⊕	1	5	50
	5.	-	+	+	⊕	⊕	⊕	1	5	40
	1.	+	⊕	⊕	1%	2%	5%	10%	25%	80%
	2.	+	⊕	⊕	1	1	5	10	25	75
Tryptose	3.	+	⊕	⊕	1	3	5	25	70	80
	4.	+	⊕	⊕	1	4	10	25	75	90
	5.	+	⊕	⊕	1	2	5	50	55	80

Legend:

- = no visible growth
+ = visible growth
⊕ = visible growth and slight amount of gas
1%, etc. = one per cent gas in Dunham fermentation tubes.

Table 20. Growth and gas production by coliform organisms from naturally contaminated water in lactose buffered broth prepared with vegetable peptone and Bacto-tryptose.

Sample No. 2. Inoculum = 17 *E. coli* per tube

Peptone	Tube No.	Hours 9	10	11	12	13	14	15	17	32
Vegetable Peptone No. 1	1.	-	+	+	+	⊕	⊕	10%	20%	40%
	2.	-	+	+	+	+	⊕	1	2	30
	3.	-	+	+	+	⊕	⊕	1	1	10
	4.	-	+	+	+	⊕	⊕	1	1	20
	5.	-	+	+	+	⊕	⊕	1	1	10
Vegetable Peptone No. 2	1.	-	+	+	+	⊕	⊕	1%	10%	25%
	2.	-	+	+	+	⊕	⊕	1	2	10
	3.	-	+	+	+	⊕	⊕	1	2	10
	4.	-	+	+	+	⊕	⊕	1	5	25
	5.	-	+	+	+	⊕	⊕	1	5	10
Vegetable Peptone No. 3	1.	-	+	+	⊕	⊕	⊕	1%	2%	50%
	2.	-	+	+	⊕	⊕	⊕	1	2	50
	3.	-	+	+	⊕	⊕	⊕	1	2	25
	4.	-	+	+	⊕	⊕	⊕	1	1	30
	5.	-	+	+	⊕	⊕	⊕	1	1	10
Tryptose	1.	+	+	⊕	1%	2%	5%	50%	80%	90%
	2.	+	+	⊕	1	2	5	25	75	90
	3.	+	+	+	1	2	5	50	75	75
	4.	+	+	⊕	1	3	5	50	75	90
	5.	+	+	+	1	2	5	50	75	90

Legend:

- = no visible growth
+ = visible growth
⊕ = visible growth and slight amount of gas
1%, etc.= one per cent gas in Dunham fermentation tubes

Table 21. Growth and gas production by coliform organisms from naturally contaminated water in lactose-buffered broth prepared with vegetable peptones and Bacto-tryptose.

Sample No. 3. Inoculum = 28 *E. coli* per tube

Peptone	Tube No.	9	10	11	13	20	31
Vegetable Peptone No. 1.	1.	+	+	+	⊕	10%	20%
	2.	-	+	+	⊕	10	25
	3.	-	+	+	⊕	10	20
	4.	+	+	+	⊕	10	20
	5.	-	+	+	⊕	10	20
Vegetable Peptone No. 2	1.	-	+	+	⊕	10%	20%
	2.	-	+	+	⊕	10	20
	3.	-	+	+	⊕	10	20
	4.	-	+	+	⊕	10	25
	5.	-	+	+	⊕	20	25
Vegetable Peptone No. 3.	1.	+	+	⊕	⊕	10%	25%
	2.	-	+	⊕	⊕	5	20
	3.	-	-	⊕	⊕	15	50
	4.	+	+	⊕	⊕	5	20
	5.	-	-	⊕	⊕	5	25
Tryptose	1.	+	⊕	5%	25%	80%	85%
	2.	+	⊕	1	5	80	85
	3.	+	⊕	1	5	75	75
	4.	+	⊕	1	5	75	80
	5.	+	⊕	1	5	75	75

Legend:

- = no visible growth
+ = visible growth
⊕ = visible growth and slight amount of gas
1%, etc. = one per cent gas in Dunham fermentation tubes

Table 22. Growth and gas production by coliform organisms from naturally contaminated water in lactose-buffered broth prepared with vegetable peptones and Bacto-tryptose.

Sample No. 4. Inoculum = 169 E. coli per tube

Peptone	Tube No.	9	10	11	13	20	31
Vegetable Peptone No. 1.	1.	-	+	+	⊕	10%	20%
	2.	-	+	+	⊕	10	25
	3.	-	+	+	⊕	10	20
	4.	+	+	⊕	⊕	10	20
	5.	+	+	⊕	⊕	10	20
Vegetable Peptone No. 2.	1.	-	+	+	⊕	10%	20%
	2.	-	+	+	⊕	20	25
	3.	-	+	+	⊕	10	20
	4.	-	+	+	⊕	15	20
	5.	-	+	+	⊕	10	20
Vegetable Peptone No. 3	1.	-	+	⊕	⊕	5%	25%
	2.	-	+	⊕	⊕	5	25
	3.	+	⊕	⊕	⊕	5	40
	4.	+	⊕	⊕	⊕	25	50
	5.	+	⊕	⊕	⊕	20	45
Tryptose	1.	+	⊕	1%	5%	70%	75%
	2.	+	⊕	1	5	75	75
	3.	+	⊕	1	5	65	75
	4.	+	⊕	1	5	75	75
	5.	+	⊕	1	5	70	75

Legend:

- = no visible growth
+ = visible growth
⊕ = visible growth and slight amount of gas
1%, etc. = one per cent gas in Dunham fermentation tubes.

Table 23. Growth and gas production by coliform organisms from naturally contaminated water in lactose-buffered broth prepared with animal peptones and Bacto-tryptose.

Sample Number 1. Inoculum 14 *E. coli* per tube.

Peptone	Tube Number	Hours 8	9	10	11	12	22	36
Beef peptone	1	-	+	⊕	1%	5%	30%	70%
	2	-	+	⊕	1	5	70	80
	3	-	+	⊕	⊕	1	25	60
	4	-	+	⊕	⊕	1	40	70
	5	-	+	⊕	⊕	1	45	80
Spleen peptone (1)	1	-	+	+	⊕	1	35	75
	2	-	+	+	⊕	1	40	75
	3	-	+	+	⊕	1	50	80
	4	-	+	+	⊕	1	50	80
	5	-	+	+	⊕	1	60	80
Spleen peptone (2)	1	-	+	+	⊕	2	50	80
	2	-	+	+	⊕	2	50	80
	3	-	+	+	+	⊕	40	75
	4	-	+	+	⊕	2	50	70
	5	-	+	+	⊕	2	45	70
Liver peptone (2)	1	-	+	⊕	1	15	60	80
	2	-	+	⊕	1	5	50	75
	3	-	+	⊕	1	5	55	75
	4	-	+	⊕	1	10	60	80
	5	-	+	⊕	1	5	50	80
Bacto-tryptose	1	+	⊕	2	10	20	75	80
	2	+	⊕	2	10	15	75	85
	3	+	⊕	2	10	15	40	60
	4	+	⊕	2	10	20	50	75
	5	+	⊕	2	10	15	75	85

Legend:

- = no visible growth
+ = visible growth
⊕ = visible growth and slight amount of gas
1%, etc. = one per cent gas in Dunham fermentation tubes

Table 24. Growth and gas production by coliform organisms prepared from naturally contaminated water in lactose-buffered broth prepared with animal peptones and Bacto-tryptose.

Sample Number 2 Inoculum 39 *E. coli* per tube.

Peptone	Tube Number	Hours 9	10	11	12	20	36
Beef peptone	1	-	+	+	⊕	10%	20%
	2	-	+	+	⊕	5	75
	3	-	+	+	⊕	10	70
	4	-	+	+	⊕	15	80
	5	-	+	+	⊕	5	70
Spleen peptone (1)	1	-	+	+	⊕	20	75
	2	-	+	+	⊕	10	70
	3	-	+	+	⊕	10	65
	4	-	+	+	⊕	15	70
	5	-	+	+	⊕	15	70
Spleen peptone (2)	1	-	+	+	⊕	25	70
	2	-	+	+	⊕	20	70
	3	-	+	+	⊕	25	75
	4	-	+	+	⊕	30	80
	5	-	+	+	⊕	25	75
Liver peptone (2)	1	-	+	⊕	5	45	75
	2	-	+	⊕	1	30	70
	3	-	+	⊕	5	40	70
	4	-	+	⊕	1	35	75
	5	-	+	⊕	5	45	80
Bacto-tryptose	1	+	⊕	2	10	50	80
	2	+	⊕	5	20	50	85
	3	+	⊕	2	25	60	90
	4	+	⊕	2	15	50	80
	5	+	⊕	5	25	45	80

Legend:

- = no visible growth
+ = visible growth
⊕ = visible growth and slight amount of gas
1%, etc. = one per cent gas in Dunham fermentation tubes.

Table 25. Growth and gas production by coliform organisms from naturally contaminated water in lactose-buffered broth prepared with animal peptones and Bacto-tryptose.

Sample Number 1 Inoculum 21 *E. coli* per tube

Peptone	Tube Number	9	10	11	12	13	14	36
Heart peptone (1)	1	-	+	+	⊕	2%	10%	80%
	2	-	+	+	⊕	⊕	10	80
	3	-	+	+	⊕	10	30	80
	4	-	+	+	⊕	2	10	75
	5	-	+	+	⊕	10	40	80
Heart peptone (2)	1	-	+	+	⊕	5	15	75
	2	-	+	+	⊕	2	5	75
	3	-	+	+	⊕	5	20	75
	4	-	+	+	⊕	5	40	80
	5	-	+	+	⊕	5	45	80
Brain peptone (2)	1	-	+	+	⊕	5	25	75
	2	-	+	+	⊕	2	15	70
	3	-	+	+	⊕	5	20	75
	4	-	+	+	⊕	5	30	80
	5	-	+	+	⊕	5	25	75
Pork peptone	1	-	+	⊕	2	10	40	75
	2	-	+	⊕	5	15	40	80
	3	-	+	⊕	5	10	30	70
	4	-	+	⊕	5	15	30	75
	5	-	+	⊕	2	20	45	75
Bacto-tryptose	1	+	+	⊕	5	15	50	90
	2	+	+	⊕	5	25	45	80
	3	+	⊕	⊕	10	30	60	90
	4	+	+	⊕	5	20	55	85
	5	+	+	⊕	5	30	55	80

Legend:

- = no visible growth
+ = visible growth
⊕ = visible growth and slight amount of gas
1%, etc. = one per cent gas in Dunham fermentation tubes

Table 26. Growth and gas production by coliform organisms from naturally contaminated water in lactose-buffered broth prepared with animal peptones and Bacto-tryptose.

Sample Number 2 Inoculum 29 *E. coli* per tube

Peptone	Tube Number	9	10	11	12	13	14	36
Heart peptone (1)	1	-	+	+	⊕	2%	10%	80%
	2	-	+	+	⊕	2	10	75
	3	-	+	+	⊕	10	30	70
	4	-	+	+	⊕	2	10	75
	5	-	+	+	⊕	10	40	80
Heart peptone (2)	1	-	+	+	⊕	5	15	75
	2	-	+	+	⊕	2	15	75
	3	-	+	+	⊕	5	20	75
	4	-	+	+	⊕	4	40	75
	5	-	+	+	⊕	5	15	65
Brain peptone (2)	1	-	+	+	⊕	5	25	85
	2	-	+	+	⊕	10	30	75
	3	-	+	+	⊕	5	20	80
	4	-	+	+	⊕	10	25	80
	5	-	+	+	⊕	5	25	75
Pork peptone	1	-	+	⊕	2	25	50	80
	2	-	+	⊕	5	30	45	80
	3	-	+	⊕	5	15	40	70
	4	-	+	⊕	5	15	40	80
	5	-	+	⊕	5	30	50	75
Bacto-tryptose	1	+	⊕	2	10	40	70	85
	2	+	⊕	1	15	30	60	80
	3	+	⊕	5	15	35	60	90
	4	+	⊕	2	20	50	70	90
	5	+	⊕	5	10	30	60	80

Legend:

- = no visible growth
+ = visible growth
⊕ = visible growth and slight amount of gas
1%, etc. = one per cent gas in Dunham fermentation tubes

duced at any given time is larger in tryptose broth than in vegetable peptone broth. As in the case of the rate of growth of E. coli, (Table 13), these results show Vegetable peptone No. 3 to give the best growth of coliform organisms of any of the peptones prepared from hydrolyzed corn gluten.

Bacto-tryptose is also shown to be better than any of the prepared animal peptones, of which the pork peptone was the best. In contrast to the vegetable peptones, the animal peptones have the ability to support growth long enough for a large amount of gas to be produced, whereas the vegetable peptones do not support growth long enough to allow the maximum amount of gas which these organisms are capable of producing from the lactose present. At the end of 36 hours the coliform organisms in the animal peptone broth produced approximately the same amount of gas as they do in tryptose broth. However, they produce it more slowly in the animal peptone broth. Thus, to give a positive presumptive test for E. coli, the animal peptone broth would require a longer incubation period than would tryptose broth.

Growth of Pathogenic Organisms.

In this experiment the following organisms were employed: four unidentified hemolytic streptococci recently isolated from milk powder; an hemolytic streptococci which had been on artificial media for some time; Brucella abortus, Brucella melitensis, and Brucella suis; Eberthella typhosa, Salmonella enteritidis, Salmonella paratyphi and Salmonella schottmülleri, Pasteurella avicida; Shigella dysenteriae; Pseudomonas aeruginosa; Erysipelothrix rhusiopathiae; and Staphylococcus aureus. Prepared peptone-glucose agar slants were inoculated with the above organisms and incubated for two weeks at 37°C. before calling

them negative. Tryptose-glucose agar was used as the control. The results for this experiment are given in Table 27.

Discussion

The results show that the prepared peptones have the ability to support the growth of nearly all the pathogenic organisms tested. In this respect the animal peptones were superior to the vegetable peptones, since the hemolytic streptococci would not grow on media prepared from the latter. Growth on the animal peptones was equal to that found on tryptose, both as to amount and time of appearance. The failure of the vegetable peptones to support the growth of hemolytic streptococci probably lies in the fact that the nutrients obtained from blood are not present as they are in the animal peptones.

Berthelot, Amoureux and Petit (6) prepared peptones by digestion of peanut meal with pepsin and pancreatin. Both peptones supported good growth of Cl. tetani. It is interesting to note that this organism produced toxin from the pepsin peptone, but failed to produce toxin when grown in the pancreatic peptone. These results emphasize the importance of the effect of the method of preparation of a peptone upon its efficiency as a bacteriological medium.

While the use of vegetable proteins for the preparation of bacteriological media has received some attention, the results obtained indicate that these proteins may be more generally useful for this purpose than is generally supposed. Their ability to replace peptones in many bacteriological tests has not as yet been determined, but there is reason to believe that if further work is done on these products, more uses for them may be found. Complete amino acid analysis of corn gluten shows that it contains all of the essential amino acids in amounts necessary to support

Table 27. Comparison of the growth of pathogenic organisms on prepared peptones and Bacto-tryptose

Organism	Vegetable peptone No. 1	Vegetable peptone No. 2	Vegetable peptone No. 3	Vegetable peptone No. 4	Vegetable peptone No. 5	Vegetable tryptone	Beef peptone	Spleen peptone (1)	Spleen peptone (2)	Liver peptone (2)	Brain peptone (2)	Heart peptone (1)	Heart peptone (2)	Pork peptone	Bacto-tryptose
Hemolytic streptococci A	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Hemolytic streptococci B	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Hemolytic streptococci C	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+
Hemolytic streptococci D	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Hemolytic streptococci	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
Brucella abortus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brucella melitensis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brucella suis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sal. enteritidis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sal paratyphi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sal. schottmülleri	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Shigella dysenteriae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pasteurella avicida	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ersipelothrix rhusiopathiae	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

growth. The use of several proteins, as is the case in animal peptones, instead of a single protein, may result in the formation of peptones that will be better suited to meet the nitrogen requirements of bacteria. It is doubtful that they will ever be able to replace the animal peptones for growth of the more fastidious pathogens, since, as has been pointed out, they do not possess even the small amount of blood nutrients present in animal peptones. They are to be recommended as a cheap source of nitrogen for growing the more common saphrophytic organisms.

Summary

Methods for the comparative analysis of prepared animal and vegetable peptones with certain commercial brands as controls are given, which include the determination of the rate of growth of E. coli; the growth of organisms in raw milk; the growth and gas production of coliform organisms in naturally contaminated water; and the growth of certain pathogenic organisms. The results obtained show that: (1) Vegetable peptones number 1, 2 and 3 support growth of E. coli during the lag phase and for the first six hours of growth better than do Bacto-peptone, tryptone and tryptose. After six hours Bacto-tryptose is better than these vegetable peptones and after twelve hours the Difco products support a larger and more rapid growth than do the vegetable peptones. (2) Vegetable peptone No. 3 supports growth of E. coli better than do the other vegetable peptones tested. (3) Vegetable peptone No. 4 is inferior to any of the other peptones tested. (4) Vegetable peptone No. 4 shows increasing numbers of E. coli for 48 hours or longer, while the other

peptones tested show maximum growth at 24 hours, and then the numbers of E. coli decrease. (5) An average of the results obtained by plating out 100 samples of raw milk shows Vegetable peptone No. 3 to be very slightly superior to Bacto-tryptone in its ability to grow the organism found in raw milk. The difference is not large enough to be significant. (6) Undecolorized vegetable peptones are less efficient in growing the organisms found in raw milk than are the same peptones when decolorized. (7) Vegetable peptone No. 5 is significantly better than vegetable tryptone, vegetable peptone No. 3 or Bacto-tryptone, but inferior to Bacto-tryptose for the same purpose. (8) Of the prepared animal peptones, the two spleen peptones and the pork peptone are shown to be superior to Bacto-tryptone; the remaining animal peptones are inferior. (9) The prepared animal and vegetable peptones are inferior to Bacto-tryptose for growth and gas production by coliform organisms found in naturally contaminated water. Vegetable peptone No. 3 and pork peptone are the best of the vegetable and animal peptones, respectively. (10) The vegetable peptones did not support the growth of hemolytic streptococci, but did support the growth of all other pathogens tested. (11) Brain peptone (2), heart peptone (2), and pork peptone supported the growth of all pathogens tested. Liver peptone (2) and heart peptone (2) supported growth of all pathogens except hemolytic streptococcus C from milk powder; beef peptone, spleen peptone (1) and (2) supported growth of all pathogens except hemolytic streptococci B and C from milk powder.

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