PROTEIN METABOLISM OF HEMOLYTIC STREPTOCOCCI

by

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Approved: Noble P. Sherwood.

Department of Bacteriology

1921.

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THE PROTEIN METABOLISM OF HEMOLYTIC STREPTOCOCCI

INTRODUCTION

The quantitative study of bacterial metabolism is a relatively new field of investigation which has been greatly stimulated in the last few years by the evidence accumulated that bacterial metabolism and the metabolism of human cells have certain fundamental properties in common. The metabolic products of bacteria are many and variable and a complete study of such a variety of end products would require so much time that the amount of work necessary would be out of proportion to the amount of benefit derived. Thus as Sears[46] points out, a great deal of the knowledge on the subject has been derived through indirect routes, through research undertaken with some other end in view. Attempts to identify different organisms through their dissimilarities, as the reducing and fermenting powers, the coagulation of milk, the common indol test, and the search for more suitable media have all added to the list of known end products.

While the food utilized by bacteria may be as completely studied as that of higher animals, the study of metabolic products represents a much more difficult

problem than in the case of higher forms. The beginning and end products may be analysed, but the complex metabolism going on within the cell can only be judged from the enzymes at work and the end-products produced by these enzymes.

The anabolic and the catabolic phases of metabolism are found in bacteria as well as in man. And as in man the bacteria must obtain their structural material from nitrogenous sources, while their catabolic needs may be satisfied by either nitrogenous or carbohydrate material. Conceding then that a source of nitrogen is necessary for bacterial growth, we find the forms in which it is available very variable, from atmospheric nitrogen to complex protein compounds in which there is a mechanism as complicated in its involvement of enzymes and end products as the metabolism of similar protein in the human body. The metabolic products vary according to the organism studied, the substance used and the condition of growth.

For the catabolic stage most organisms are found to be capable of utilizing both carbohydrates and protein but showing a preference in their selection of food requirements. Kendall[20-28] and his coworkers have shown that the energy requirements of bacteria are best satisfied by carbohydrates, the carbohydrates exert-

ing a sparing action on the protein. This fundamental principle of metabolism they expressed as "Fermentation takes precedence over putrefaction", the first being the result of the action of the bacteria on the carbohydrates with the production of acid, and the latter the action of bacteria on the protein with the production of alkali.

The formation of ammonia has been used by Kendall[20-28] and his followers as the most definite index of proteolysis, as nitrogen is the most important element entering into the structure of the bacterial cell, and from their experience ammonia seems to be the chief end product of the breaking down of the protein material. This ammonia they considered as the result of the intracellular deamidization of the protein derivatives indicental to their transformation into energy.

From a recent article by Foster[14] on the biochemistry of hemolytic streptococci, his results on protein metabolism correlates those of Kendall. From growth curves he shows that the amino acids may also be used as an index of protein metabolism, a diminished rate of growth coinciding with an increased output of amino acid in the media, due to a decrease in the amino acid intake by the cells. He believes with Waksman[47-48] that the animo acids are intermediate products in the

breaking down of the protein as in the animal body. The low concentration of the amino acids in the media during the first part of the incubation he accounts for by explaining that the bacteria in their rapid stage of growth utilize the amino acids for structural purposes as soon as they are formed. Then, as more amino acids are assimulated, more ammonia is split off, thus correlation the findings of Kendall and Walker that ammonia formation is the result of intracellular deamidization of protein material.

Kleigler[41] in reviewing the work of Kendall and his associates called attention to the conclusiong of these workers as to the sparing action of carbohydrates on protein. He thought that other important factors were involved, chiefly the concentration of sugar and peptone and the presence of inorganic salts as buffers. He also used ammonia production as an index of protein metabolism. By using varying concentrations of peptone in 0.5% glucose he found that increasing the amount of peptone led to an increase in the ammonia evolved, when the concentration of glucose was relatively low. More ammonia was produced when an American make of peptone was used than with Witte's peptone, which he explains be referring to Retter's[45] work showing that certain bacteria do not digest proteoses and peptones, leading to the conclusion that American

products are richer in amino acids and give a higher yield of ammonia. Kleigler also used hydrogen ion as an index of metabolism, the increase in hydrogen ion denoting utilization of protein. This method also gave data as to the amount of sugar a particular organism could use without inhibiting growth and the rate at which the sugar could be utilized. By adding primary sodium phosphate to the broth culture the hydrogen ion concentration was kept down, so that some of the organisms were able to use up all the sugar before producing sufficient acid to inhibit their growth. When this happened the P_H jumped from the acid range of about 4.6 to the alkaline range above 7 showing that as soon as the carbohydrates were utilized, the protein was attacked, neutralizing the acid and giving an alkaline reaction. The varying amounts of glucose utilized depend sometimes upon the process of fermentation, some organisms carrying the process far enough to do away with the acid. For the gas producing organisms Kleigler suggests two explanations for this difference, either that inhibiting factors of a specific character are produced, or that specific differences exist in the way the glucose molecule is split by these different organisms, giving rise to different amounts of toxic substances.

Sears[46] while granting that the production of

ammonia was sometimes a correct index of protein metabolism, calls attention to the fact that the metabolic stage might go only as far as the amino acids; or that at certain stages there might be an unusual amount of amino acids broken down to ammonia indicating a vigorous metabolism when but one stage of the process was being performed. He, therefore, not only showed a sparing action of carbohydrates on protein from ammonia production but also from the concentration of amino acids produced. Most micro-organisms also showed evidence of the existence of large amounts of nitrogen products intermediary between amino acids and ammonia. Urea and uric acid were not found in his cultures of bacteria because of the ease with which these substances are decomposed by most species. Varying concentrations of amino acids showed that these bodies were formed and broken down by the organisms. Most species showed an inclination to utilize the simple compounds of nitrogen before the complex. He found a few specimens of bacteria capable of producing creatine and creatinine in sugar-free peptone cultures.

Berman and Rettger[5] used cultures of B. subtilis, B. coli, Proteus vulgaris and B. cloacae for studying nitrogen metabolism. In media containing 0.25 per cent Witte's peptone and 1 per cent glucose, B. coli and

Proteus vulgaris fermented the sugar so rapidly that an inhibiting hydrogen ion concentration was produced before the sugar was used up. But B. subtilis had a slow carbohydrate metabolism and remained in balance with the protein metabolism. B. cloacae decomposed sugar rapidly but broke it down beyond the acid stage and so did not produce an inhibiting hydrogen ion concentration. These last two organisms he showed to be exceptions to the general rule that carbohydrates spare the protein.

Rettger[45] studied the metabolic products of bacteria growing anaerobically. His purpose was to investigate the process of putrefaction and his conclusion reached was that putrefaction was caused by anaerobic organisms alone. By the use of meat and egg media and by employing various methods of testing for the end products he determined, qualitatively or quantitatively, mercaptan hydrogen sulfide, indol, skatol, phenol, aromatic-oxy acids, skatol-carbonic acid, tyrosine, leucine, tryptophane, albumose, and peptone.

Different organisms are able to utilize different forms of nitrogen, from simple atmospheric nitrogen to complex nitrogenous compounds. Althouth the range of nitrogenous food stuffs is usually large, preference for the simpler forms of protein is usually manifest.[46]

Waynick and Woodhouse[49] in studying the nitrogen fixation of Azobacter found amino acids in large quantities in the first few days of incubation indicating that nitrogen goes through the simple organic forms before being made into protein nitrogen. According to Waksman[47-48] protein and amino acids are the best sources of nitrogen for Actinomycetes, while the amides are not. The utilization of both amides and ammonium salts seems to depend on the source of carbon available and the reaction of the media. Coagulated egg albumen was found by Breman and Retter[5] to be too complex for even the most powerful proteolytic bacteria unless first split by some enzyme.

For simplifying the study of metabolism simple proteins and synthetic media containing compounds of simple and known composition have been used. Thus Kendall[32] succeeded in growing the tubercle bacillus, an organism usually considered to require very complex food, in media having as a source of nitrogen di-ammonium hydrogen phosphate and various sugars as sources of carbon. Grassner[13] and others have used media having asparagin as the only nitrogen source.

The growth and activity of an organism is known to depend to a great extent on the reaction of the media. The metabolic products of many organisms will soon retard their growth unless removed or neutralized.

Foster[14] found a direct correlation between the maximum acidity and maximum growth and glucose utilization in his strains of streptococci. The end of the period of activity in growth occurred when a constant P_H had been reached. Lactic acid was the chief acid produced in his cultures. Smaller portions of volatile acids, mostly formic and acetic, were found. Forty per cent of the acids produced were non-volatile. The presence of horse serum in the media not only stimulated the growth rate and acid production, but also permitted a wider range in the hyrdogen ion concentration. The presence of a little carbohydrate showed a decrease in the ammonia production over that found in 1% glucose. The ammonia production was higher when extra protein in the form of horse serum was added, but a 1% glucose protected a 5% serum.

Dragstedt[10] showed that streptococci could grow in serum-enriched media up to P_H 4.63. According to Grace and Highberg[14] Streptococcus veridans grew best in a broth having a reaction of P_H 6.8, while a reaction more alkaline than P_H 7.6 distinctly retards growth. Jones[18] found that the initial hydrogen ion concentration influences the final hydrogen ion. He suggests that the limiting hydrogen ion concentration be defined in terms of initial reaction, composition of media and conditions

of growth.

The proteolytic enzymes concerned in the breaking down of the protein are of interest in this connection because it is by means of these agents that the bacteria are able to prepare their food for assimulation. The simularity of the action of bacterial enzymes to those of the human body has already been mentioned. Kendall[15], by using a culture of B. proteus obtained the enzyme in free state by filtering through a Berkfeld filter. The enzyme was not found in active state in media containing utilizable carbohydrates, appearing only when protein or protein derivatives were being used for energy of B. proteus.

Jones[17] grew cultures of B. proteus in 4% dextrose-peptone broth, adding sodium hydroxide from time to time in order to keep the hydrogen ion concentration about P_H 7.2. The cultures were under observation 32 days in an attempt to find proteolytic enzymes but without success, thus correlating the findings of Kendall that the enzymes were found only when protein was being utilized.

Wolfe[51-52] found a powerful urea-splitting enzyme in cultures of B. proteus, capable of transforming 45% of the total nitrogen of the urine into ammonia.

The study of the protein metabolism of these seven strains of hemolytic streptococci was undertaken with three objects in view.

1. To investigate the general sparing action of carbohydrates for protein as shown by the papers reviewed in the introduction.

2. To determine whether or not there is a variation in the sparing action within a group similar to that found by Kendall and others between different groups of organisms.

3. To determine whether or not the ammonia lost by volatilization from the surface of the culture media during incubation should be regarded as negligible, as has been customary in all previous work in metabolism.

History of the Organisms

Six of the seven strains used were obtained from Dr. B. J. Clawson of the University of North Dakota, the histories of which were taken from his paper on the Constancy of Hemolytic Streptococci,[23] and are given by number as follows:

,Organism 292, carcinoma of larynx and lobar pneumonia.

Organism 297, pericardial fluid after death following nephrectomy.

Organism 293, pus from frontal sinus.

Organism 294 and 295, from removed tonsils.

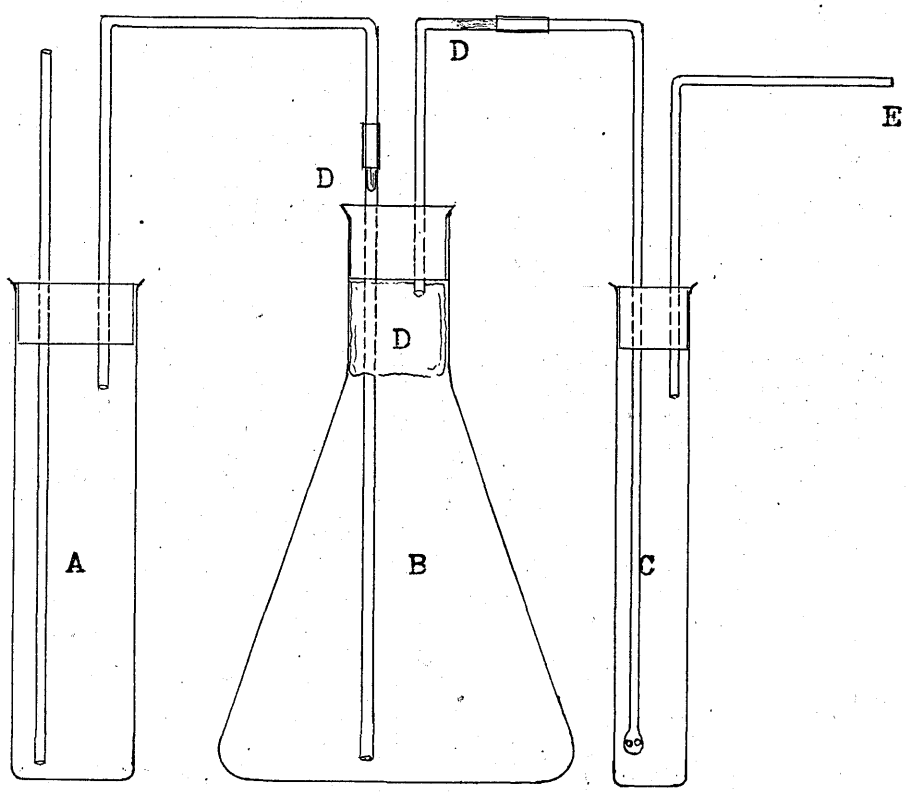
Organism 296, pus from mastoid infection.

The history of the seventh strain, 291, is unknown.

Technique.

When it was found that a better growth over a longer length of time was given by most of the organisms in calcium carbonate broth, the idea of continual aeration was conceived, since this method would do away with any loss of ammonia from the surface of the alkaline broth during incubation. Apparatus was then constructed as shown in the drawing consisting of a 250 Erlenmeyer flask fitted with a rubber stopper through which passed two glass tubes, a straight one to extend below the surface of the broth, and the other U shaped, extending only through the stopper. The tubes were both plugged with cotton and the flask also contained a cotton plug beneath the rubber stopper as a precaution against contamination, through which only the long tube extended. The other end of the U tube extended to the bottom of a test tube also fitted with a rubber stopper. An outlet tube for attaching a suction pump passed through this second stopper. The flask and test tube was then essentially a Folin ammonia aeration apparatus, in which the

Culture-Aeration Apparatus.



A. Wash bottle.

B. Culture flask.

D. Cotton plugs.

C. Acid tube.

E. To suction pump.

substance being aerated was the entire volume of the broth and the test tube the acid container for catching the ammonia as it was drawn over with the air current. One suction pump leading to the incubator gave sufficient air current when divided by two Y tubes, for eight culture flasks, a plain and a dextrose broth being connected in series, A wash bottle containing sulphuric acid was connected to the first flask of each series in order to take out any ammonia that might be in the air.

The media used was fresh meat infusion broth. One pound of fresh ground lean meat was used for each 1000 c.c. of infusion. To this base 1% peptone and 0.5 percent sodium chloride were added. The broth was rendered sugar free by inoculating with B. coli and incubating 24 hours. After filtering and re-sterilizing the reaction was adjusted to neutrality. A micro-kjeldahl was then run on 1 c.c. portions of the broth to determine the total nitrogen in the media. To one half of the broth 1% of dextrose was added. One hundred c.c. of the media was then placed in each of the 250 c.c. flasks fitted as described above, about 1.5 grams of calcium carbonate added and the whole autoclaved at 15 pounds pressure for 15 minutes. After cooling the flasks were inoculated by means of a capillary pipette through the long glass tube,

from a fresh 24 hour suspension of the organisms in saline solution. Ten c.c. of N/50 sulphuric acid was measured into the acid tube, placed in the incubator and connected to the suction pump. A slow current of air was drawn continously through the apparatus.

Every 24 hours or as indicated in the data, the apparatus was removed from the incubator, the flasks shaken in order to bring the calcium carbonate into suspension, and the solution aerated rapidly for a few seconds to remove any ammonia that might be lost from the flasks during titration. The tube was disconnected and the excess of acid titrated for, using N/50 sodium hydroxide and phenol red as an indicator. Towards the close of the incubation period it was found safer to use N/20 acid and the corresponding alkali.

At the close of the incubation period the flasks were opened, tested out for pure cultures, and the volume made up to 100 c.c., the original volume, in order to avoid any error due to evaporation. A total nitrogen determination was made on 1 c.c. portions of the broth in order to check up with the first determination for the loss of nitrogen, and an ammonia determination made on 5 c.c. of the broth culture in order to account for the ammonia held in solution.

For the digestion process in the total nitrogen determination the hard glass bulbs used for combination experiments in chemistry were found to be very convenient. When the ends were bent upward on either side of the bulb in a U shape, loss of material due to bumping was impossible. A fume absorber consisting of a wash bottle having an inverted U tube, one end drawn out to a point and the other extending below the surface of the water and another tube leading from the wash bottle to the suction pump completed the apparatus. When the pointed end of the U tube was inserted into an open end of the digestion bulb, the air current drew the fumes over into the wash bottle. 1 c.c. of the broth and 3 c.c. of concentrated C.P. sulphuric acid were used for each digestion. A small crystal of copper sulphate was added as a catalytic agent. Digestion was carried on for a few minutes after the solution became clear. The acid was then washed out into an ammonia aeration apparatus tube and the process completed with the usual Folin aeration method. 40% NaOH was used to neutralize the acid and the heat from the reaction, together with very strong aeration for one hour gave results which checked with the distillation by heat method and the former method was used throughout the determinations because of its greater convenience in working with such small quantities of material.

The results together with curves showing the sparing action of the carbohydrates for the protein are shown in the tabulated data and are self explanatory.

ORGANISM NO. 291

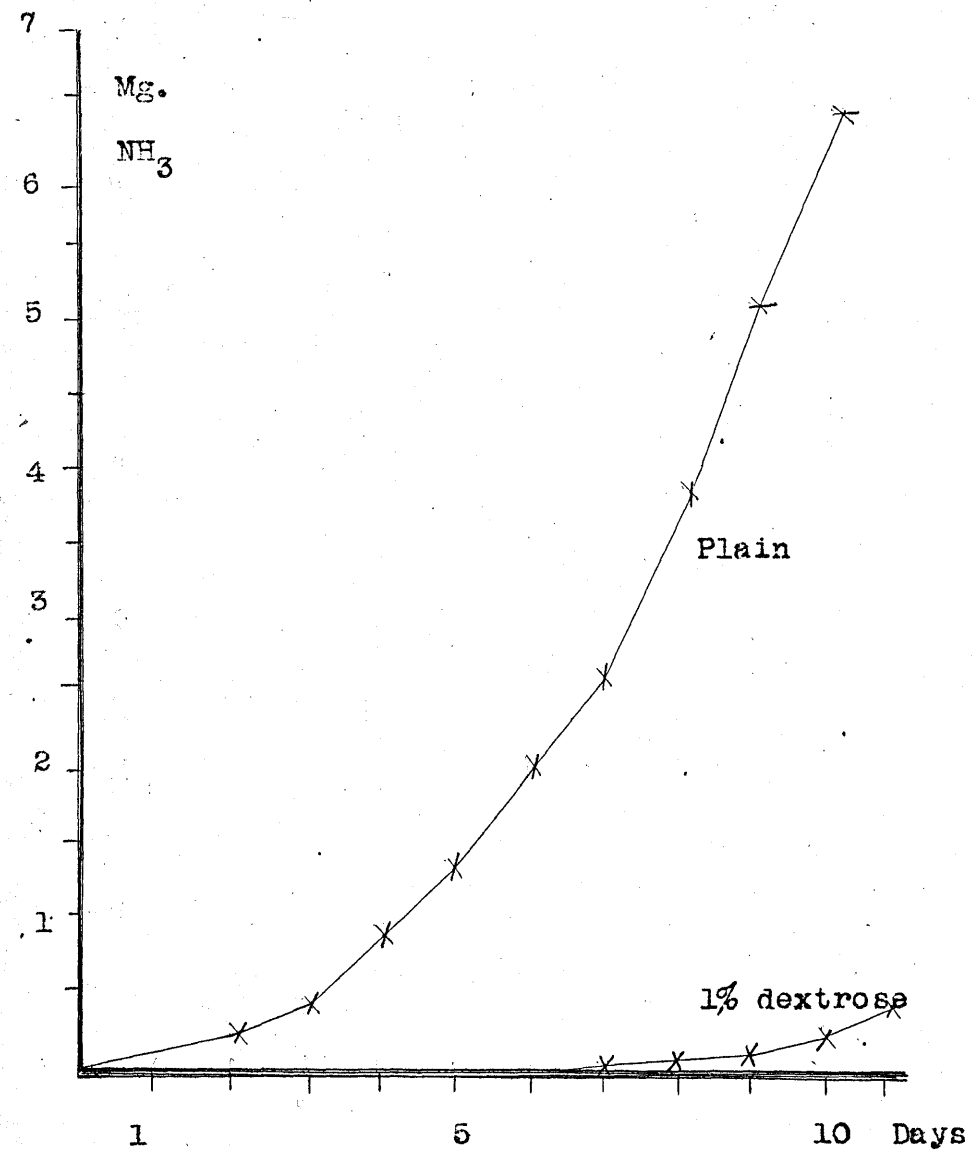
All results expressed in milligrams of ammonia nitrogen per 100 c.c. of culture media.

Uninoculated broth contained 297.8 mg. of ammonium nitrogen per 100 c.c. broth.

Days	Mg. NH per da.	Mg. total NH	NH total percent.
Dextrose broth (1%)			
1	.0	.0	.0
2	.0	.0	.0
3	.0	.0	.0
4	.0378	.0378	.013
5	.0765	.1134	.038
7	.0765	.1890	.064
8	.1134	.2646	.089
9	.1134	.3780	.130
10	.2420	.6200	.208
11	.45322	1.0730	.360
12	.4710	1.5440	.518
Plain meat infusion broth, sugar free.			
1	.3402	.3402	.114
2	.4540	.7942	.267
3	.5290	1.3232	.444
4	1.4360	2.7592	.926
5	1.5490	4.3082	1.446
7	2.1690	6.3772	2.140
8	1.6340	8.0112	2.688
9	3.7800	11.7912	3.937
10	3.7800	15.5712	5.225
11.	3.9700	19.5412	6.557
12	3.9700	23.5112	7.890

	Dextrose	Plain
Final NH determination	15.0 mg.	20.6 mg.
Final total N (as NH)	280.9	251.5

Growth Curve for Organism 291.



ORGANISM NO. 292

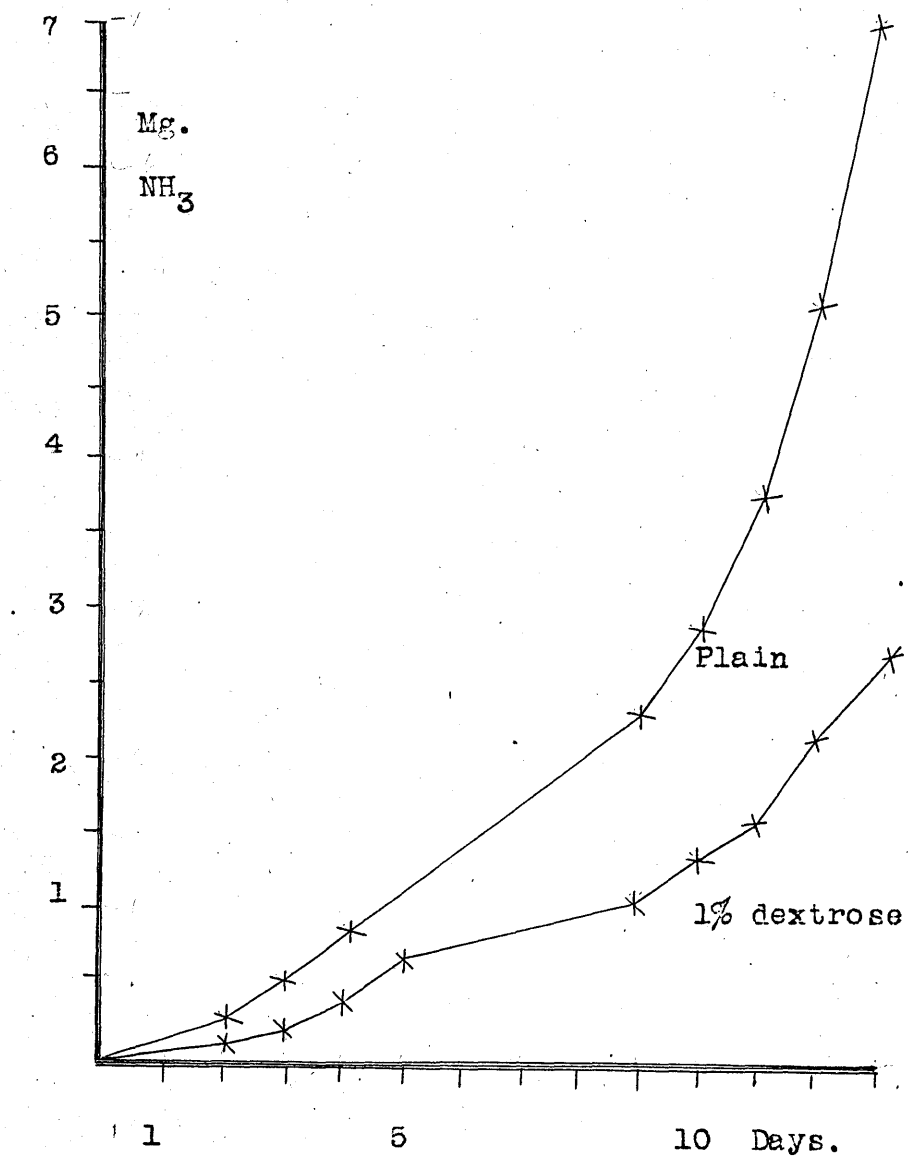
All results expressed in milligrams of ammonia nitrogen per 100 c.c. of culture media.

Uninoculated broth contained 317 mg. of ammonia nitrogen per 100 c.c. broth.

Days	Mg. NH per da.	Mg. total NH	NH total percent.
Dextrose broth (1%)			
2	.3640	.3640	.151
3	.4687	.8327	.262
4	.6686	1.5013	.473
5	.7091	2.2104	.700
9	1.4700	3.6804	1.161
10	.8112	4.4926	1.417
11	.8112	5.3048	1.637
12	1.732	7.0368	2.220
15	2.860	9.8968	3.122
Plain meat infusion broth			
2	.8112	.8112	.256
3	.8112	1.6224	.512
4	1.061	2.6834	.850
5	lost		
9	4.8122	7.4546	2.368
10	1.7542	9.2498	2.911
11	2.860	12.1098	3.820
12	4.065	16.1748	5.102
15	9.300	25.4748	8.036

	Dextrose	Plain
Final NH determination	17.3 mg.	29.0 mg.
Final total N (as NH)	288.2	260.4

Growth Curve for Organism 292.



ORGANISM NO. 293

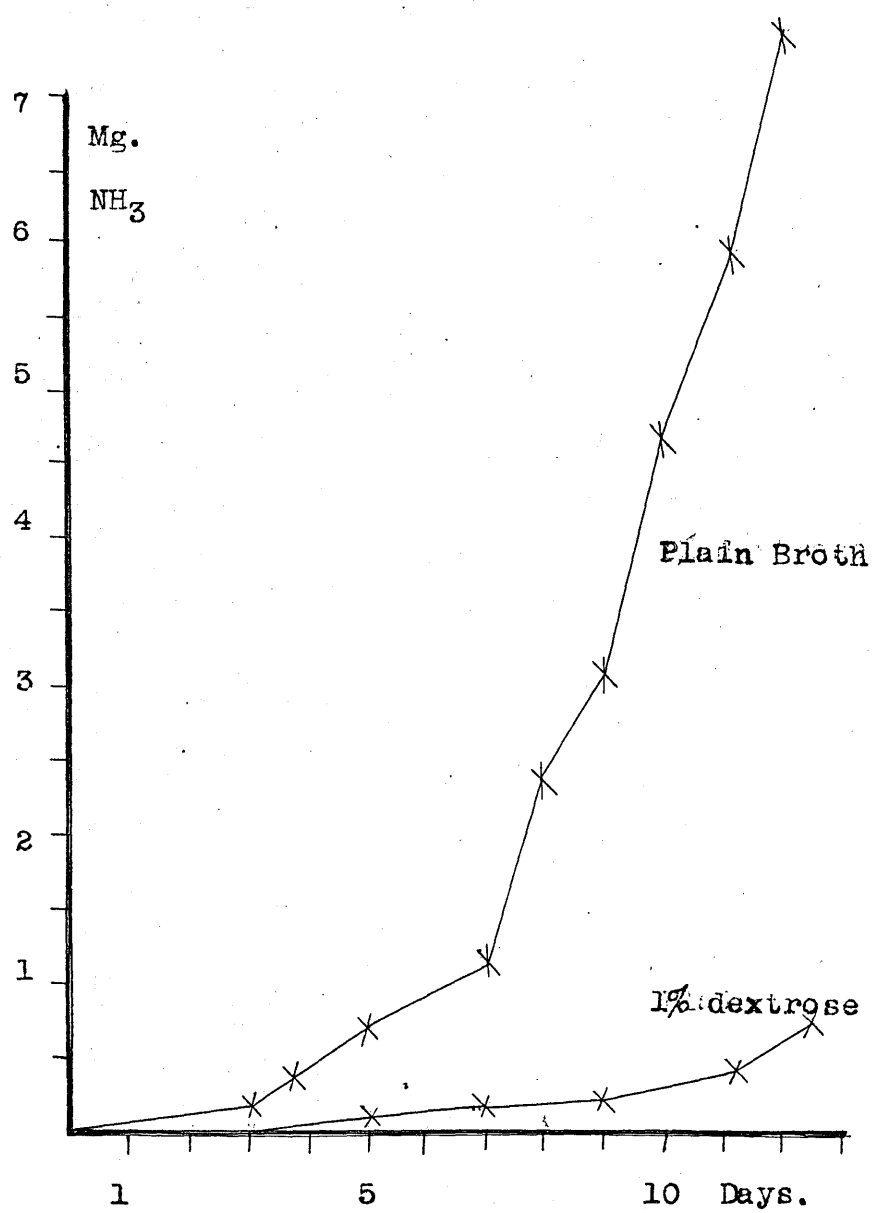
All results expressed in milligrams of ammonia nitrogen per 100 c.c. of culture media.

Uninoculated broth contained 297 8mg of ammonia nitrogen per 100 c.c. broth.

Days	Mg. NH per da	Mg total NH	Total NH percent
Dextrose broth (1%)			
1	.0	.0	.0
2	.0	.0	.0
3	.0378	.0378	.012
4	.151	.1888	.036
5	.1134	.2022	.068
7	.151	.3532	.119
8	.1512	.5044	.169
9	.302	.8064	.274
10	.302	1.1084	.375
11	.302	1.4104	.480
12	.3024	1.7128	.582
Plain meat infusion sugar free broth.			
1	.1134	.1134	.038
2	..1512	.2646	.090
3	.1890	.4536	.152
4	.7530	1.2066	.4050
5	1.0580	2.2646	.794
7	1.1710	3.4356	1.150
8	3.7800	7.2156	2.420
9	3.0240	10.2396	3.130
10	3.7800	14.0196	4.700
11	3.7800	17.7996	5.970
12	5.7800	23.5796	7.910

	DExtrose	Plain
Final NH determination	7.6 mg.	24.6 mg.
Final total N (as NH)	287.4	247.5

Growth Curve for Organism 293.



ORGANISM NO. 294

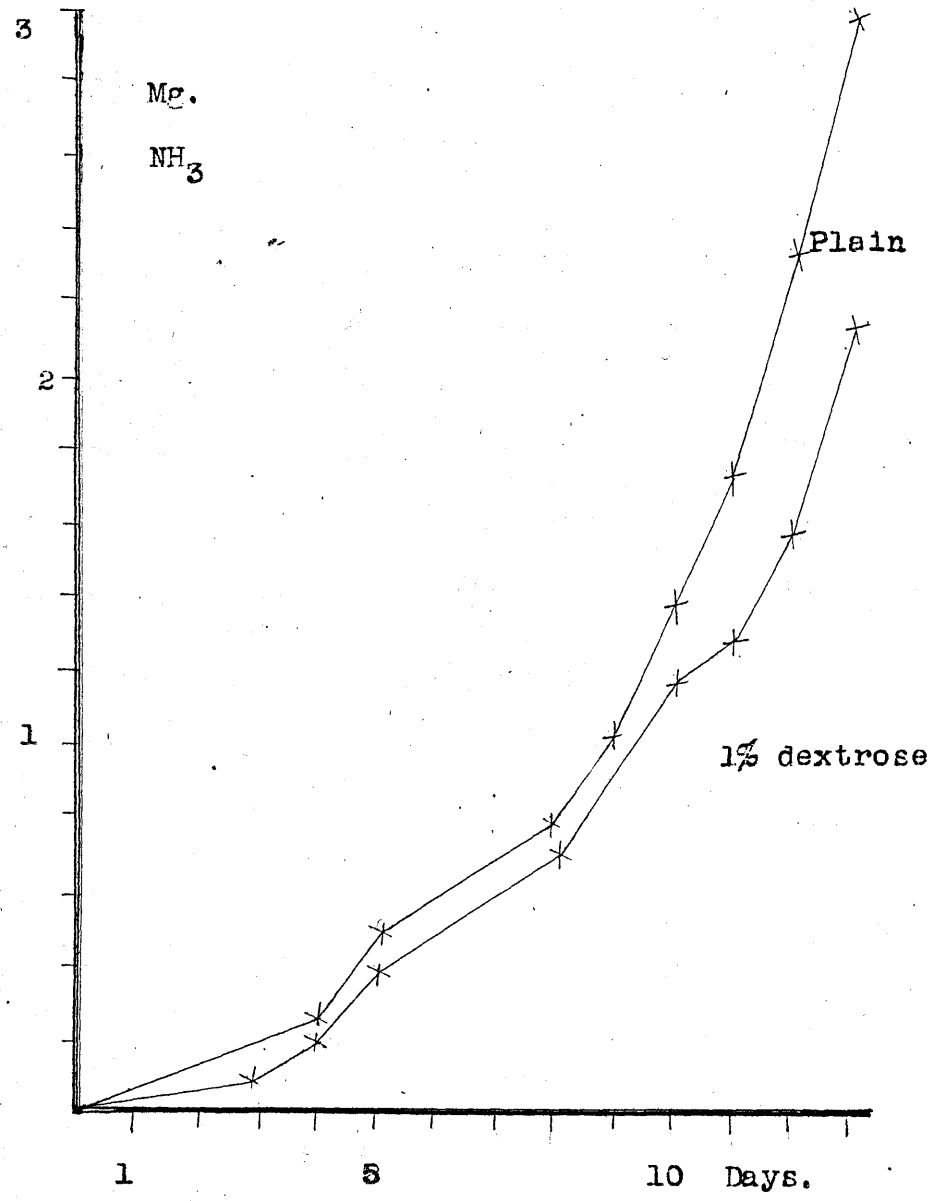
All results expressed in milligrams of ammonia nitrogen per 100 c.c. of culture media.

Uninoculated broth contained 317 mg. of ammonia nitrogen per 100 c.c. broth.

Days	Mg. NH per da.	Mg. total NH	NH total percent.
Dextrose broth (1%)			
3	.2610	.610	.082
4	.4734	.7344	.231
5	.8040	1.3384	.422
8	.9137	2.2521	.710
9	.8040	3.0561	.964
10	.8228	3.8789	1.223
11	.9170	4.8569	1.532
12	.9137	5.7706	1.820
14	1.1290	6.8996	2.175
Plain meat infusion broth, sugar free			
3	.2438	.2438	.080
4	.6360	.8798	.280
5	.7526	1.6324	.515
8	.9348	2.56720	.809
9	.8526	3.4198	1.079
10	.9900	4.4048	1.391
11	1.2030	5.6128	1.773
12	1.9110	7.5238	2.373
14	2.3000	9.8238	3.100

	Dextrose	Plain
Final NH determination	25.16 mg.	44.1 mg.
Final total N (as NH)	284.1	260.4

Growth Curve for Organism 294.



ORGANISM NO. 295

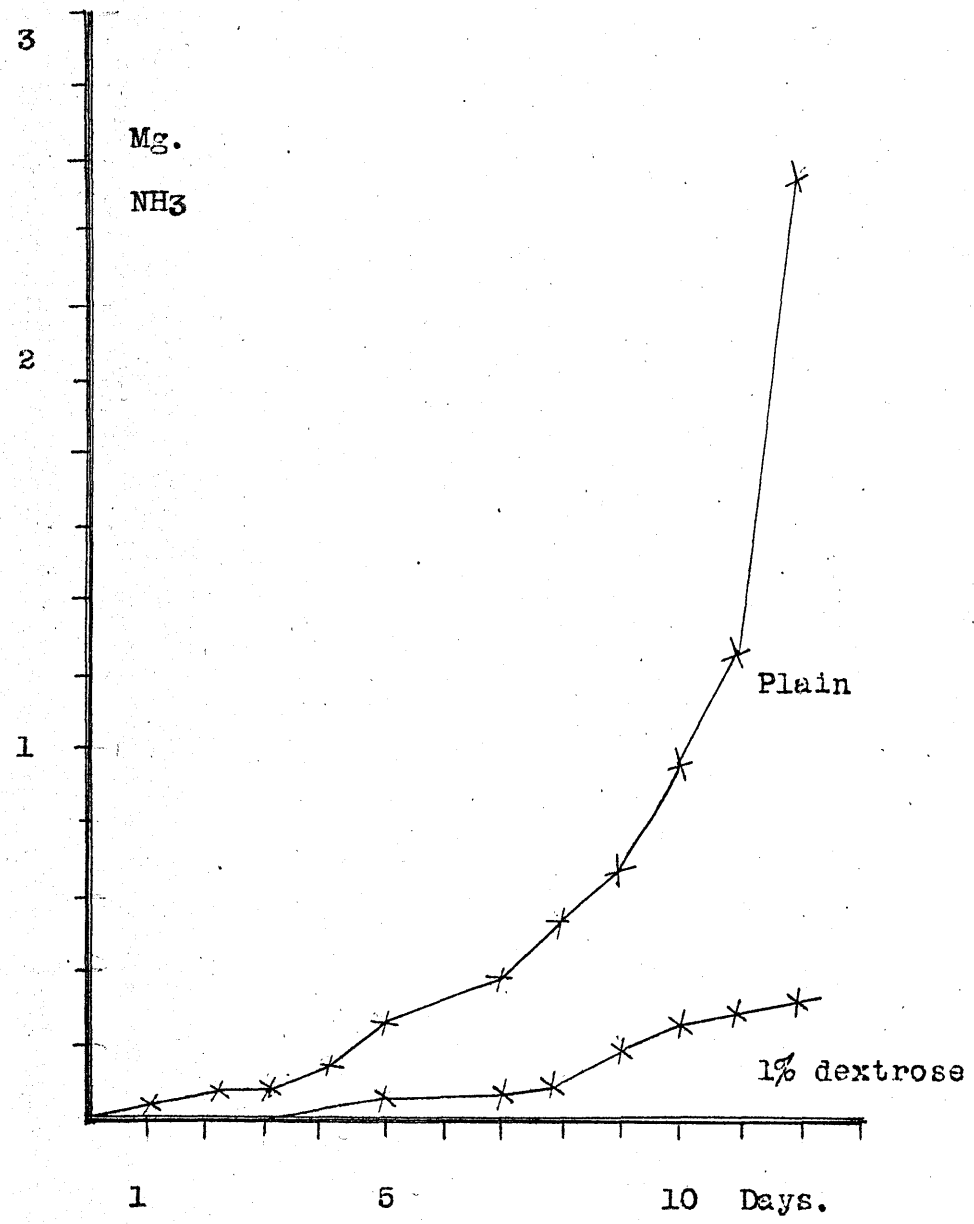
All results expressed in milligrams of ammonia nitrogen per 100 c.c. of culture media.

Uninoculated broth contained 297.8 mg. of ammonia nitrogen per 100 c.c. of broth.

Days	Mg. NH per da.	Mg. total NH	NH total percent.
1	.0	.0	.0
2	.0	.0	.0
3	.0765	.0765	.025
4	.0	.0765	.025
5	.0	.0765	.051
7	.0765	.2297	.071
8	.0765	.4185	.140
9	.1980	.7209	.242
10	.3024	.8729	.290
11	.1513	.9494	.319
12	.0765	.9494	.319
Plain meat infusion sugar free broth			
1	.0765	.0765	.025
2	.1134	.1899	.064
3	.1512	.3111	.103
4	.2268	.5379	.180
5	.3780	.9159	.304
7	.3024	1.2183	.409
8	.4156	1.6339	.582
9	.4156	2.0495	.688
10	.5440	2.2935	.770
11	1.5160	3.8095	1.280
12	3.8500	7.6595	2.570

	Dextrose	Plain
Final NH determination	15.2 mg.	20.8 mg
Final total N (as NH)	280.8	267.4

Growth Curve for Organism 295.



ORGANISM NO. 296

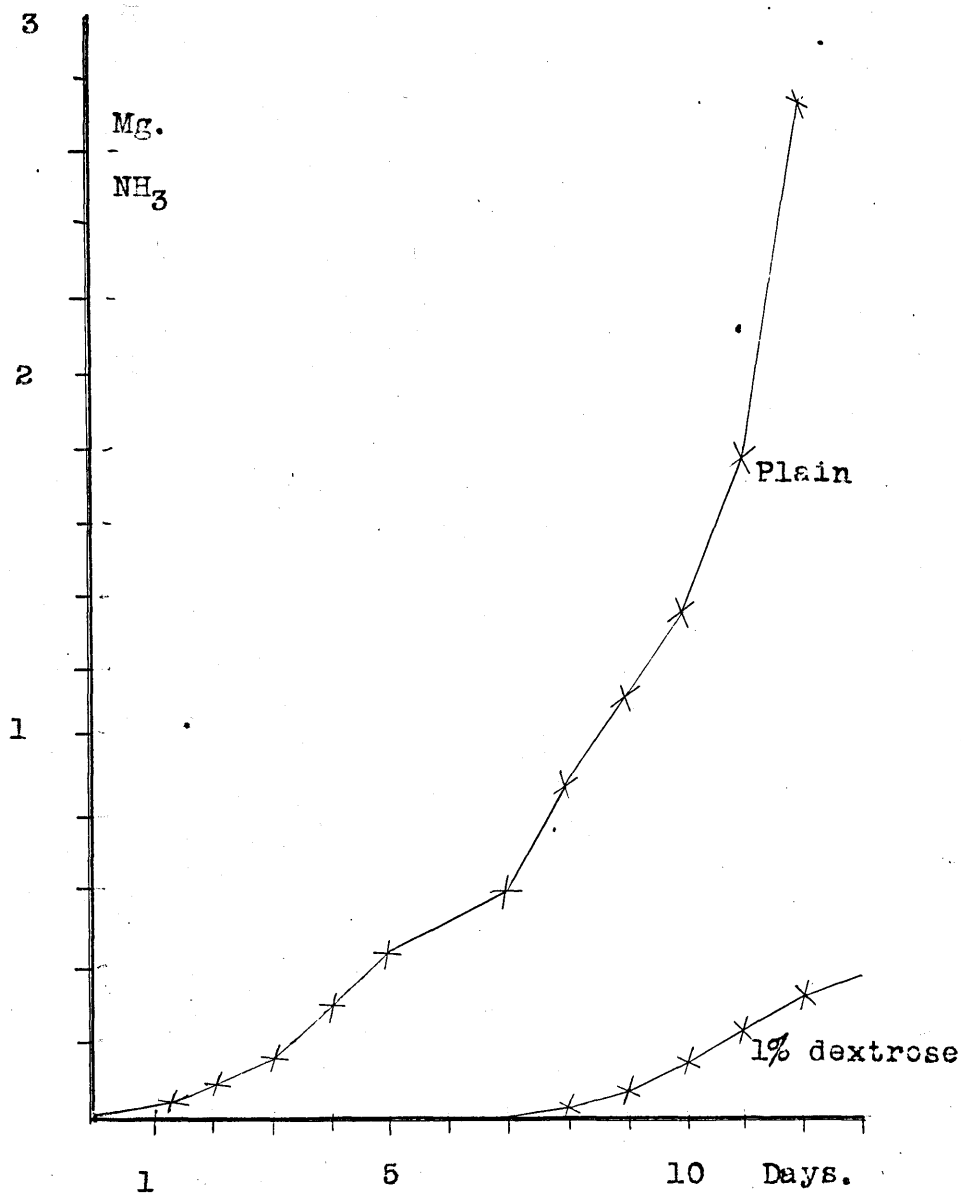
All results expressed in milligrams of ammonia nitrogen per 100 c.c. of culture media.

Uninoculated broth contained 297.8 mg. of ammonia nitrogen per 100 c.c. broth.

Days	mg. NH per da.	Mg. total NH	NH total percent
Dextrose broth (1%)			
1	.0	.0	.0
2	.0	.0	.0
3	.0	.0	.0
4	.0	.0	.0
5	.0378	.0378	.013
7	.0	.0378	.013
8	.0378	.0756	.025
9	.1134	.1890	.064
10	.2494	.4484	.150
11	.2190	.6674	.224
12	.3280	.9954	.334
Plain meat infusion sugar free			
1	.151	.151	.050
2	.151	.302	.101
3	.181	.483	.165
4	.415	.898	.301
5	.464	1.362	.457
7	.453	1.815	.609
8	.706	2.521	.876
9	.792	3.313	1.116
10	.815	4.128	1.358
11	1.210	5.338	1.791
12	2.780	8.118	2.724

	Dextrose	Plain
Final NH determination	25.7 mg.	36.8 mg.
Final total N (as NH)	269.13	249.48

Growth Curve for Organism 296.



ORGANISM NO. 297

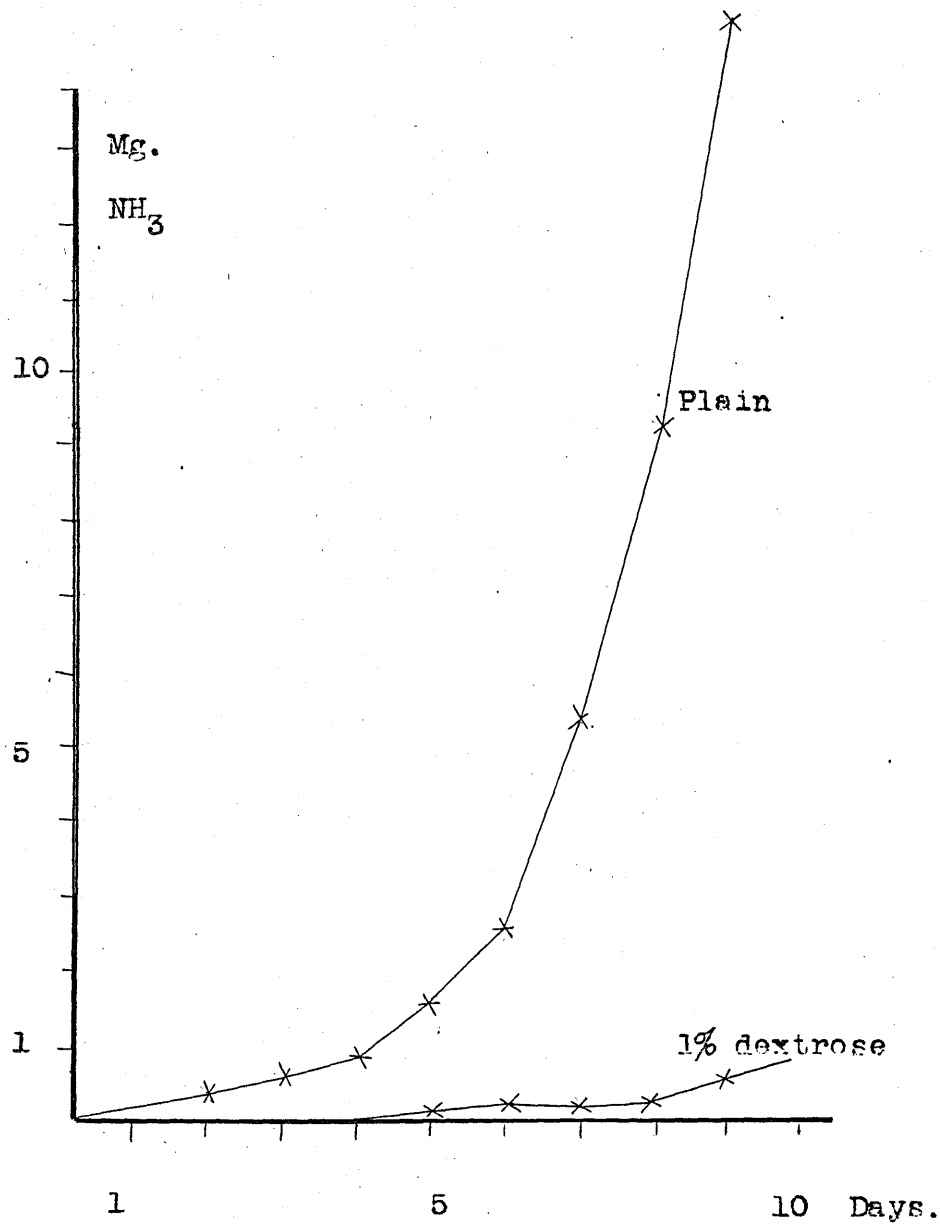
All results expressed in milligrams of ammonia nitrogen per 100 c.c. of culture media.

Uninoculated broth contains 269.5 mg. of ammonia nitrogen per 100 c.c. broth.

Days	Mg. NH per da.	Mg. total NH	Total NH percent
1	..000	.000	.000
2	.000	.000	.000
3	.077	.077	.028
4	.154	.131	.048
5	.154	.258	.106
6	.231	.516	.191
7	.231	.746	.280
8	..315	1.261	.430
9	.539	1.800	.667
Plain meat infusion broth, sugar free.			
1	.462	.462	.171
2	.539	1.001	.372
3	.703	1.704	.631
4	1.617	2.321	.860
5	2.079	4.400	1.630
6	2.772	7.172	2.656
7	7.636	14.808	5.500
8	10.010	24.828	9.190

	Dextrose	Plain
Final NH determination	12.32 mg.	24.64 mg.
Final total N (as NH)	254.10 mg.	190.00 mg.

Growth Curve for Organism 297.



Discussion

The method of aeration used not only prevented any loss of ammonia by volatilization during incubation but also gave an unusual supply of oxygen. And while in the slightly alkaline reaction given by the calcium carbonate and the gentle aeration all the ammonia liberated was not carried over as formed, the results are relative and the ammonia held in solution was recovered in the final determination which was in a much stronger alkaline solution and fast aeration.

The affinity of ammonia for water has been given by Kendall as being sufficiently great to prevent any loss by volatilization from a broth culture having a fairly large surface exposed to the atmosphere as in culture flasks. It would seem that at least with a heavy growing organism the ammonia lost to the atmosphere would be appreciable, especially in plain broth, and after several days incubation, as a great part of the ammonia was carried over in a very slow air current. The variations in the final ammonia determinations can be accounted for by the differences in the amounts of intermediate products in the solution, the decomposition of which could be brought about by the fairly strong alkali and vigorous agitation used in the final aeration.

It will be noticed from the growth curves that no point is shown at which there is a marked increase or

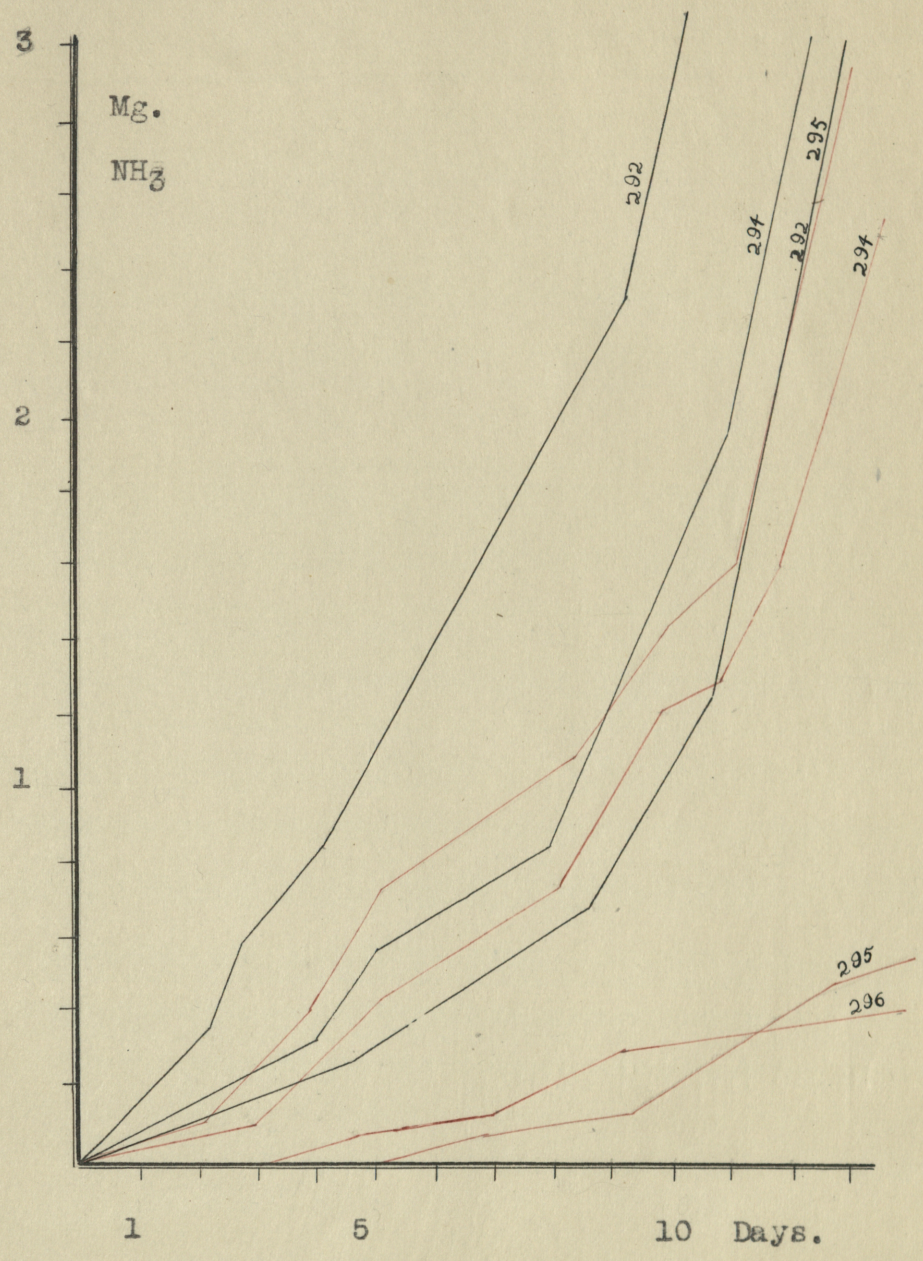
decrease in the ammonia production. Due to the affinity of the ammonia for the broth, the first part of the curve shows no rapid rise, the ammonia being held in solution. And as the age of the culture increased the broth became more saturated until finally probably all of the ammonia liberated by the organisms was carried over, thus the point of decrease in growth which might be expected to be present is shown as giving off as much ammonia as the period of rapid growth.

The slight amount of ammonia always produced in the dextrose broth is due to the fact that enough protein is always broken down to satisfy the protein needs of the bacteria. The acid reaction which would soon form in dextrose broth and bind the ammonia in solution is neutralized to a great extent by the calcium carbonate although no doubt some of the ammonia is also used for this purpose.

The growth curves for these seven strains of streptococci show some interesting variations. For comparison and in order to make the variations more obvious, representative growth curves have been plotted together, using different colors for the growth in plain and dextrose broth. While two curves may very well represent the NH_3 evolved in plain broth, at least four are needed to show the growth in dextrose media. From these curves it is very evidnet that there are various degrees in the

sparing action of the carbohydrates for protein, the four organisms showing first, a very pronounced sparing action for 295, slightly less for 296, poor sparing action for 292 and scarcely no sparing action for 294. Reference has been made to the work of Berman and Rettger in which they show exceptions to the carbohydrate sparing action for certain slow growing organisms and variations between different groups, but no work has been found showing this variation within a group as in these strains of streptococci

Comparative Curves.



Summary and Conclusions

1. This work confirms in part the findings of Foster in his study of the Biochemistry of Hemolytic Streptococci. It has usually been thought that carbohydrates exert a sparing action upon the protein and Foster's work confirms this statement. Variations in the sparing action between different groups has been found by Kendall and others, but variations between members of the same group have not been noted. These seven strains show a very wide variation, from very pronounced sparing action to scarcely no sparing action at all.

2. A noticeable difference is seen in the growth curves given by this method of aeration than in those obtained by Kendall because of the affinity of the NH_3 for water, especially during the first part of incubation.

3. It would seem that in metabolism work precautions against loss of metabolic products through volatilization would be worth while and that an appreciable error would result from disregarding this loss.

4. All strains grew much heavier and lived longer in calcium carbonate broth, and this media was found to bealkaline enough for the ammonia to be liberated.

5. Fresh meat infusion broth was found to be much more desirable than beef extract for the cultivation of these organisms

6. The amount of ammonia carried over depended on the speed of aeration; and the rate was necessarily slow to prevent excessive frothing. But this aeration was fast enough to carry over sufficient ammonia to show the sparing action of carbohydrates for protein

7. The advantages of this method may be summarized as follows: No loss of NH_3 by volatilization; no chemical agents other than the ingredients of the media and in the liberation of NH_3, thus preventing the possibility of breaking down intermediate products through chemical means; an unusual supply of oxygen; the entire volume of broth used for each determination; elimination of the necessity of opening the flasks to remove samples for analysis, thus decreasing the chances of contamination and changes in the volume of the broth; simplicity of the process.

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