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Lipolytic activity of gram-negative intestinal bacteria

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LIPOLYTIC ACTIVITY OF GRAM-NEGATIVE INTESTINAL BACTERIA

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INTRODUCTION

Some species of bacteria are known to hydrolyze fatty substances, yet comparatively little study of lipolytic organisms has been made. Lipolysis produced by bacteria may be important in nature as an aid in the destruction of organic waste materials; rancidity produced in butter, lard, and other fatty substances, may be caused by bacterial digestion; lipolysis may also be employed as an aid in classifying bacteria, just as are carbohydrate fermentative powers and indications of proteolytic activity.

The purpose of this investigation was to study the lipolytic activity of Gram-negative bacteria, commonly found in the alimentary tract of man and animals in health and in disease. In order to make comparisons of enzymatic activities, tests for indications of proteolytic activity and carbohydrate utilization were made.

HISTORICAL.

Sommaruga[15] in 1894 studied the lipolytic ability of organisms by growing them on solid media in which 2.0 per cent olive oil or other fat was dispersed. He used no indicator, and determined lipolysis by the disappearance of the fat globules from the region surrounding the bacterial growth. In 1901 Eijkman[8] inoculated melted agar and poured it over a thin layer of tallow in a Petri dish. Lipolytic activity produced by the organisms was indicated by zones of clearing in the tallow beneath the colonies.

In 1907 Thorpe[17] demonstrated the general action of Nile-blue sulphate in the staining of fats. He found that the red coloring fraction of the dye was apparently formed only when the dye was dissolved in water. His investigation was followed by that of Smith and White[14] in 1908 who showed that blue coloring portions of the dyes of the oxasine series, when used as a stain for tissue sections containing neutral fat, possessed the property of coloring the fat red.

Sayer, Rahn, and Farrand[15] in 1908 used a litmus agar for the detection of lipolysis produced by bacteria. These workers used a sugar-free broth in which a small amount of butter fat was dispersed in the preparation of the agar. A change in the color of the indicator indicated lipolysis.

In 1920 Boeminghaus[5] studied the color reactions of Nile-blue sulphate with palmitin, stearin, and olein, their corresponding acids, and certain other derivatives. His work demonstrated that intense colors were secured with oleic acid and its ester combinations, the

free acid being colored blue and the combinations red. Palmitic and stearic acids, and palmitin and stearin were only slightly colored. In comparing the intensity of the colors in his work, he noted that oleic acid is an unsaturated acid.

In 1921 Buchanan(4) pointed out that lipolytic organisms, when grown on a solid medium in which a fat was dispersed, produced a lipase which caused the disappearance of the fat from the immediate vicinity of the growth.

In 1926 Waksman and Davison(19) demonstrated the use of the changes produced by freed fatty acids in certain indicators to detect lipolysis, or fat hydrolysis, brought about by bacterial action. Kaufman and Lehman(11) in 1926 studied the action of various stains, including Nile-blue sulphate, on various organic acids, esters, and other materials. They noted the marked effect produced by Nile-blue sulphate on unsaturated fatty acids and unsaturated tri-glycerides, but found that certain compounds without double bonds were also stained.

In 1927 Turner(18) investigated the hydrolysis of fat by bacteria. The medium he used was composed of 1000 ml. of sugar-free meat digest fluid, 5 gms. of di-basic sodium phosphate, and 30 gms. of agar, the final reaction being adjusted to pH 7.6. After dissolving the ingredients in the autoclave, Nile-blue sulphate was added and the medium was then tubed and heated in an Arnold steriliser. A sterile fat emulsion was added to the melted and cooled medium. In 1929 this investigator made a comparison of the relative merits

of various methods for the determination of fat hydrolysis by bacteria, and concluded that the Nile-blue sulphate medium gave remarkable sharpness of differentiation with reference to the amount of lipolysis produced. Nile-blue sulphate, in a concentration of 1-8,000, was found to inhibit the growth of a number of organisms. The flooding of plates after incubation with a Nile-blue sulphate solution was also suggested.

In 1931 Rettie[12] concluded that Nile-blue sulphate contains two coloring fractions. The blue portion is considered soluble in fatty acids and the pink portion soluble in the fat.

Hussong[10] in 1932 modified Turner's method. He used beef infusion agar with a pH of 6.8 to 7.0. He added an alcoholic solution of Nile-blue sulphate in the proportion of 1-10,000 of the medium, and the fat in the proportion of 1-200. An emulsion of the fat was made in 0.5 per cent agar, which was sterilized before it was added to the medium. Cultures were streaked on the surface of the solidified medium. Lipolysis was demonstrated by a change in the color of the fat globules.

In 1933 Berry[2] worked with the test originated by Carnot and Mauban[5] in 1918 for the detection of microbial lipase. The organisms were grown on a solid medium in which a fat was dispersed. When good growth appeared on the plates, they were flooded with a saturated copper sulphate solution. If hydrolysis had taken place, the freed fatty acids reacted with the copper sulphate to give an insoluble blue soap.

Collins and Hammer[6] in 1935, in studying the action of

bacteria on tri-glycerides, observed that butter-fat or olive oil when dispersed in agar containing Nile-blue sulphate were colored bright red. The olive oil did not appear as red as the butter-fat, presumably because of the small size of the globules. These workers noted that with natural fats the disappearance of the globules was not clearly evident in all cases, so they determined hydrolysis by color changes in the globule. They observed also that bacterial hydrolysis of natural fats was not appreciably affected either by the manner in which the materials were dispersed or by the pH of the medium within the limits 6.8 to 7.0.

Again in 1933 Collins and Hammer[7], studying types of lipolysis on this medium, observed that uniform and non-uniform lipolysis were demonstrable with different organisms. They also found that cultures within a species might show considerable variation. Of the 159 lipolytic cultures which they studied, 113 (71 per cent) produced proteolytic changes in milk, and 60 produced rancidity in butter.

Horowitz-Wlassowa and Livschitz[9] in 1933 reported the ability of certain fungi and bacteria to produce lipolysis. The marked increase of the fatty acids was considered evidence of lipolytic activity. They employed both a sodium hydroxide and the Nile-blue sulphate tests on fat-agar plates. In addition to lipolytic activity, these authors discovered the ability of various organisms to destroy fats and oils by oxidation, by means of an enzyme designated as "lipoxydase", in a manner analogous to the effect of light and some acids. This ability to oxidize fatty substances appeared

to be less widespread than lipolytic activity in the microbic realm. The oxidation of fats and oils was recognized by the following changes: formation of peroxide, increase in the acid radical count, hydrogenation, and increase of refractive index. Two tests for the detection of oxidation of fatty substances were used. These tests were the pyrogallic acid test and the so-called "biological test", which consisted in growing the organism on Endo's agar which was without lactose and in which soy oil was emulsified. This "lipoxydase" is not detectable when a nutrient medium is employed.

Stark and Scheib(16) in 1955 worked extensively on fat-splitting bacteria isolated from butter. Of 486 cultures isolated, they found 77 per cent (375 cultures) to be capable of hydrolysing butter-fat. The organisms, present in large enough numbers to be of importance in the spoilage of butter, were Gram-negative rods, Gram-negative micrococci, Gram-positive sarcina, and Gram-positive non-spore-producing rods. Of these groups which occurred in large numbers, only the Gram-negative rods were regularly present in all of the butters tested. Hydrolysis of butter-fat was produced by 97 per cent of these organisms.

These same investigators used the indicator, Nile-blue sulphate, in their medium in a slightly different manner than that of Collins and Hammer(6). The butter-fat was treated with a hot aqueous 5 per cent solution of the Nile-blue sulphate in the proportion of one part of the solution to nine parts of fat. The fat

was allowed to take on the red color, and then was washed free of excess stain with hot water. The stained fat was then added to the nutrient agar, and the plates were ready to be inoculated. Results comparable to those derived by the Collins and Hammer method were obtained on this medium. The change of the red color to the dark blue of the stain, as the fats were hydrolyzed and the resulting fatty acids were liberated, indicated lipolysis.

EXPERIMENTAL.

Organisms Employed.

A preliminary series of organisms was studied in duplicate on the special medium, in order to perfect the method and to verify the results reported by Collins and Hammer(7). This series consisted of the following:

No. *	Organism
51	Staphylococcus aureus
59	Staphylococcus albus
62	Staphylococcus citreus
88	Serratia marcescens
94	Pseudomonas aeruginosa
97	Pseudomonas fluorescens
98	Pseudomonas syncyanea
146	Aerobacter cloacae
153	Proteus vulgaris
166	Salmonella schottmülleri
243	Bacillus subtilis
245	Bacillus mycoides
246	Bacillus cereus

* Note: The numbers refer to the laboratory numbers of the cultures in the Marshall Hall collection.

This series of organisms was chosen to provide a varied selection for preliminary study. According to Collins and Hammer the species of the genus Pseudomonas were lipolytic in varying degrees.

In this series both Gram-positive and Gram-negative organisms were included.

A second series was composed of Gram-negative, lactose fermenting organisms of types commonly associated with the intestinal tract of man and animals. It consisted of stock laboratory strains of the Escherichia and Aerobacter genera, as follows:

Escherichia communior	4 strains
Escherichia acidi-lactici	4 strains
Escherichia neapolitana	2 strains
Aerobacter cloacae	4 strains
Aerobacter oxytocum	1 strain

This series was chosen to compare the reactions of stock strains, which had been carried in the laboratory for some time, with the reactions of freshly isolated strains of known origin.

A third series of organisms studied was composed of the freshly isolated strains of the coli-aerogenes group referred to above. This series consisted of the following:

Escherichia	12 strains
Aerobacter	11 strains
Citrobacter	2 strains
Intermediates	26 strains

The strains of Escherichia were isolated from feces, while those of Aerobacter and of the intermediates were isolated from water and from market fish. All of these strains were Gram-negative, lactose fermenting organisms, and were classified according

to their reactions to the Voges-Proskauer, methyl-red, and sodium citrate tests. The reactions of these strains are given:

	Voges-Proskauer	Methyl-red	Sodium citrate
Escherichia	-	+	-
Aerobacter	+	-	+
Citrobacter	-	+	+

Organisms giving results different from those in this table were designated as intermediates.

A fourth series of organisms consisted of stock laboratory strains of the Salmonella, Eberthella, and Shigella genera. These genera were Gram-negative, non-lactose-fermenting organisms of types which are associated with infections of the intestinal tracts of man and animals. This series included:

Salmonella abortivo-equina	1 strain
Salmonella enteritidis	4 strains
Salmonella suipestifer	2 strains
Salmonella paratyphi	3 strains
Salmonella schottmülleri	3 strains
Salmonella pullorum	3 strains
Eberthella typhosa	3 strains
Shigella dysenteriae	3 strains
Shigella paradysenteriae (var. Flexner)	3 strains
Shigella paradysenteriae (var. Hiss)	2 strains

Media and Procedure.

The special medium used in this investigation was that employed by Collins and Hammer[6]. This medium was prepared in two parts, as follows:

Part I. - Nutrient Agar.

Beef extract	3.0 gms.
Peptone	5.0 "
Agar	15.0 "
Distilled water	1000.0 ml.

After the solids were dissolved the reaction of this portion of the medium was adjusted to pH 6.9.

Nile-blue sulphate solution.

Nile-blue sulphate ...	0.1 gm.
Distilled water	100.0 ml.

The aqueous solution of Nile-blue sulphate was added to the nutrient agar in the proportion of 10 ml. to 100 ml. of the agar. This dilution was found to be beyond the bacteriostatic range, yet sufficiently concentrated to produce well stained fat globules. This mixture was tubed in 20 ml. quantities and then sterilised at 15 pounds steam pressure for 20 minutes.

Part II. - Fat solution.

Agar	0.5 gm.
Distilled water	90.0 ml.
Fat (butter or olive oil) ..	10.0 ml.

The fat, either butter-fat (salt-free) or olive oil, was filtered in a heated funnel and added to the melted 0.5 per cent agar solution in the proportion of 10 ml. of fat to 90 ml. of the agar. This mix-

ture was sterilized at 15 pounds for 25 minutes and cooled to the point of solidification. It was then vigorously shaken to obtain an equal dispersion of the fat globules. The percentage of agar employed gave a semi-solid consistency which permitted the mixing of the fat and the agar at the point of solidification. When this emulsion was to be used it was slowly heated to a soft jelly-like consistency so that it might be transferred by means of a pipette.

When the plates were to be poured the nutrient agar (Part I) containing the Nile-blue sulphate was melted in the Arnold sterilizer. While this part of the medium was still hot the fat emulsion (Part II), which had been slowly heated, was added with a sterile pipette in the proportion of 1.0 ml. of the emulsion to 20.0 ml. of the nutrient agar. The plates thus poured were allowed to solidify and were then ready to be inoculated.

The inoculations were made from a suspension of a 48-hour agar slant culture of the desired organism in 10 ml. of sterile physiological saline. Part of the saline solution was added aseptically to the slant and the growth was removed with a sterile needle. The remainder of the saline was added to this suspension and 0.1 ml. of this latter suspension was used as the inoculum. The inoculum was dropped onto the surface of the poured plate and spread over the agar and fat by means of an especially bent sterile glass rod. All organisms, with the exception of

Serratia marcescens, Pseudomonas syncyanea, and Bacillus mycoides in the preliminary work, were incubated at two temperatures, 32° C. and 37.5° C.

All of the organisms studied were observed for their ability to produce lipolysis after two-day and seven-day periods. The plates employing butter-fat and olive oil were made in duplicate, and in all cases where positive results were observed they were found on all plates. Two temperatures of incubation were used in studying all organisms. One set of plates was incubated at 32° C. and another parallel set was incubated at 37.5° C.

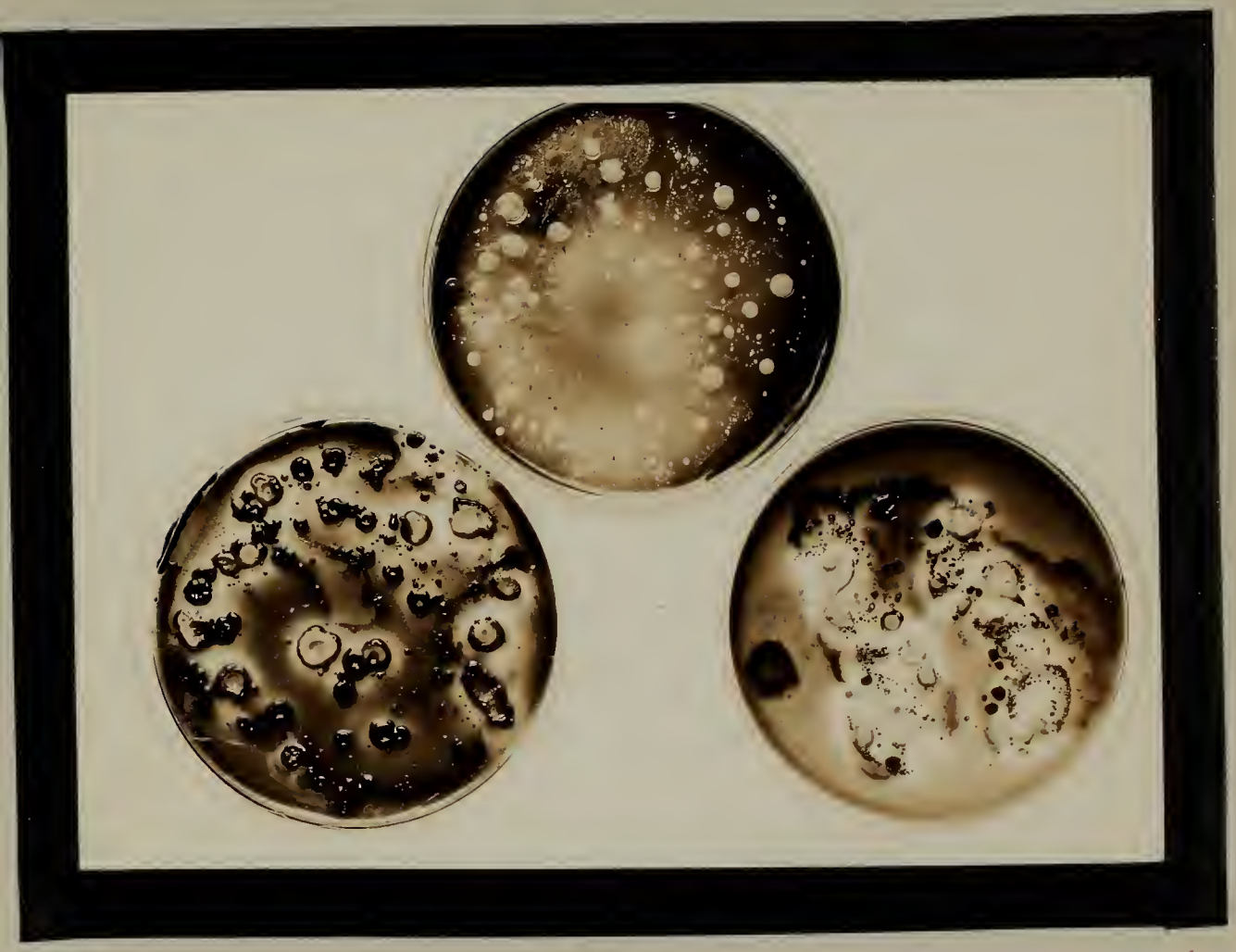
The Reactions on the Nile-blue Sulphate Medium.

The ability of various genera of bacteria to affect lipolysis on the special Nile-blue sulphate medium was shown by their ability to produce hydrolysis of the fat globules. The organisms usually attacked a globule of fat first around the margin, and then hydrolyzed from there toward the center. The hydrolysis was manifested by color changes in the fat globule. The red color gradually disappeared and in its place appeared a dark chalky blue. When the incubation was allowed to continue, the red color was entirely lost, and the dark blue color progressed toward the center of the globule. In some instances after seven days of incubation, a dull grey-white color was observed on the inner edge of the growth or throughout the globule. The nutrient agar remained unchanged, retaining its characteristic dull dark blue color. Colonies appear-

ing on this portion of the medium took up a portion of the blue color, because the dye is soluble in water, and were slow in developing, possibly because of a slight bacteriostatic effect produced by the dye. The organisms hydrolyzing the fat globules grew rather rapidly in the presence of the fat, while their growth on the nutrient agar alone was slow. The growth thus described was the type most commonly found.

Photographs were taken with infra-red lenses so that the red color would be absorbed and the resulting photograph could be more easily interpreted. In figure 1, at the top, is seen an uninoculated plate as a control. The fat globules appear as silver spots of various sizes dispersed over the surface of the plate. On the lower left side of this same figure can be seen a plate containing globules of butter-fat which have been hydrolyzed in varying degrees. The jet-black spots indicate the growth of organisms which have completely hydrolyzed the butter-fat. In the upper left portion of this plate there can be seen a globule which has not been entirely hydrolyzed. The unhydrolyzed fat appears as a small white dot in the center of the growth. In this plate, slightly below the center and toward the left, can be seen the growth in which the grey-white color appeared, surrounded by the dark chalky blue.

The plate on the lower right side of figure 1 was poured with an olive oil emulsion. The results were comparable with those of the butter emulsion, varying amounts of hydrolysis appearing in



Control

Butter-fat emulsion

Olive oil emulsion

Figure 1. Showing the more predominant type of lipolysis.

different globules. In this plate there can be seen the loss of the blue color of the dye in the nutrient agar around many of the hydrolyzed globules. To a smaller degree this loss of color can be seen in the butter emulsion plate. This loss is thought to be due to a reduction of the Nile-blue sulphate. However, this action upon the dye was unimportant in comparison with the hydrolysis of the fats, and was not significant in this investigation.

The plates in figure 1 show the more prevalent type of hydrolysis as it was found in the experiments. In figure 2, however, can be seen plates showing a different type of hydrolysis. The upper plate served as a control, the lower two plates having been poured with an emulsion of butter-fat and an emulsion of olive oil respectively. The action of the growth and the resulting hydrolysis are comparable on these two plates. However, the growth and subsequent hydrolysis appearing on the lower right plate is more clearly defined and can easily be described. The globules are surrounded by the growth of the organism. This growth is spread from the margin of the globule onto the nutrient agar, and in toward the center of the globule. The color changes, being slower, do not appear in the same state of development as they do in the previously described instance in figure 1. A diffusion of the grey-white color with the red is the main characteristic of this second type of hydrolysis. Throughout this growth small spots of the dark chalky blue color appear in concentric rings as shown on the plates in figure 2. The diffusion of the grey-white and red colors is dry

Control

Butter-fat emulsion

Olive oil emulsion

Figure 2. Showing the less predominant type of lipolysis.

while the chalky blue spots are very moist. The growth and hydrolysis as described, and as shown in figure 2, were found to be the much slower type, and appeared in only a very few instances. If such plates were incubated for seven to fourteen days the red color of the fat globules disappeared, showing complete hydrolysis.

In the plates showing this second type of hydrolysis, the loss of color in the nutrient agar around the globules which have been overgrown by the organisms can be seen, as it is in the first and more common type of lipolysis shown in figure 1.

Gelatin Liquefaction and Hydrogen Sulphide Production as Indicators of Proteolysis.

Studies of gelatin liquefaction and hydrogen sulphide production by all of the organisms employed were made in nutrient gelatin and in basic lead acetate agar. The nutrient gelatin was prepared according to the seventh edition of "Standard Methods of Water Analysis"(1). The basic lead acetate agar was prepared as follows:

I. Nutrient agar.

Beef extract	5.0 gms.
Peptone	5.0 "
Agar	10.0 "
Distilled water	900.0 ml.

II. Lead acetate solution.

Basic lead acetate	0.5 gm.
Distilled water	100.0 ml.

The beef extract, peptone, and distilled water were heated to dissolve the solids, and the reaction of this solution was adjusted to pH 7.2. The agar was then added and dissolved. Lost weight was

made up by the addition of hot distilled water. The lead acetate solution was then added to the nutrient agar and the resulting mixture was tubed in 10 ml. quantities and sterilized at 15 pounds steam pressure for 20 minutes.

Both the nutrient gelatin and the basic lead acetate agar were inoculated by the stab method. The nutrient gelatin was incubated at room temperature for seven days and then read for liquefaction. Liquefaction of gelatin is an indication of possible proteolytic activity on the part of the organisms. Non-liquefaction of gelatin definitely indicates the lack of proteolytic power of the organisms. The basic lead acetate agar was incubated at 37.5° C. for five days and then read for blackening along the line of inoculation. The blackening in this medium is due to the production of hydrogen sulphide and its subsequent linkage with the lead acetate to form lead sulphide. While the production of hydrogen sulphide in the lead acetate medium does not result from proteolytic activity, it does act as an indicator of the possible ability of the organism to attack proteins, in that the production of hydrogen sulphide is a characteristic of proteolytic organisms. Negative results in the lead acetate agar may be considered as indicative of the lack of proteolytic power because the bacterial digestion of protein is characterized by the evolution of hydrogen sulphide.

Carbohydrate Utilization.

The various carbohydrate media were made using nutrient broth prepared according to the seventh edition of "Standard Methods of Water Analysis[1] as a base. The reaction was adjusted to pH 6.9, and 5 ml. of a 0.5 per cent alcoholic solution of brom thymol blue indicator were added to a liter of medium. The carbohydrates were added in amounts that would yield 0.5 per cent concentrations. The media were tubed in Durham fermentation tubes and sterilized at 15 pounds for 15 minutes. They were inoculated in the usual manner. After incubation for 48 hours, the tubes were read for fermentation and change in reaction. The carbohydrates employed in this study were:

Monosaccharide - dextrose, levulose

Disaccharide - lactose, sucrose

Trisaccharide - raffinose

Polysaccharide - starch

Pentose - xylose

Glucoside - salicin, aesculin

Alcohol - mannitol, glycerol

DISCUSSION.

The results of the study of the preliminary series are shown in table 1. This series served as a means of studying the method of detecting fat digestion by bacteria. Lipolysis was evident with certain strains of the Pseudomonas and with the Proteus genera. None of the other organisms in the series, including some Gram-positive organisms, showed lipolysis. Since this series was included for the purpose of studying the method, it will not be considered further in the discussion.

In table 2 can be seen the results derived with the stock laboratory strains of the Escherichia-Aerobacter group. None of the organisms in this series showed lipolysis.

Table 3 shows the results with the freshly isolated strains of the Escherichia-Aerobacter group. This series includes strains of the Escherichia, Aerobacter, and Citrobacter genera, and strains of intermediates of the group. These organisms were isolated from feces, water, and fish fillets. Fifty-one strains were included in this series. Their distribution according to classification, and the prevalence of lipolytic activity among them, are shown in the following tabulated form:

Genus	No. studied	No. lipolytic
Escherichia	12	2
Aerobacter	11	2
Citrobacter	2	1
Intermediate	26	13

Among these strains, there was no apparent correlation between lipolysis and the reactions employed for classification: namely, the Voges-Proskauer, the methyl-red, and the sodium citrate tests.

It will be noted that fifty per cent of the intermediates gave positive lipolysis, while less than ten per cent of either the Escherichia or Aerobacter genera were positive. This suggests that with the study of many more intermediate strains, lipolysis might be employed as an aid in their classification. Lipolysis did not appear to be sufficiently prevalent among the Escherichia and Aerobacter genera to be of any great significance, while the number of the Citrobacter genus studied was not sufficiently large to justify an opinion.

In table 4 are given the results of the studies of the organisms which are pathogenic in the alimentary tract of man and of animals. No lipolysis was evident in this series of organisms. This series included strains of the Salmonella, Eberthella, and Shigella genera.

In considering any possible correlation of lipolysis with hydrogen sulphide production and with gelatin liquefaction, only the eighteen strains showing lipolysis were considered. The results may be found in table 5. A summary follows:

Strains producing hydrogen sulphide,	10
Strains liquefying gelatin,	13
Strains giving both reactions,	8

It will be seen that one or the other of the reactions occurred with more than half of the strains, and that slightly less than half gave both reactions. Only three of the strains gave neither of the reactions. No definite relationship is apparent between these reactions and lipolysis. However, the results suggest the possibility of a significant correlation of these types of enzymatic action among the intermediate strains of the coli-aerogenes group. If such a correlation should be proven to exist, it could also be of assistance in the systematic study of these organisms.

In correlating carbohydrate utilization with lipolytic activity the eighteen lipolytic strains were again employed as shown in table 6. Of these organisms two produced acid and gas in all of the eleven carbohydrates employed, nine produced acid and gas in all but one of the carbohydrates, and one produced acid and gas in all but two of the carbohydrates. Two organisms produced no gas in any of the carbohydrates, and one produced gas in only one of the carbohydrates. Fifteen of the positively lipolytic organisms were found to be actively fermentative for most of the carbohydrates. Note should be taken of the fact that only five organisms produced both acid and gas in starch, the percentage of gas in each instance being very low. The ability of these lipolytic organisms to utilize carbohydrates was found to be quite marked, and may possibly be correlated in their enzymatic activities previously considered. It appears that these eighteen lipolytic strains possess widely distributed enzymatic power.

Bacterial lipolysis in nature may be an important factor in the natural decomposition of plant and animal tissues. An interesting fact is that all lipolytic organisms were isolated from feces, water, and fish fillets. All but one of the eight cultures isolated from the fish fillets showed indications of proteolytic activity, and also actively broke down both of the fats used in this investigation. This fact suggests that the proteolytic decay of fish tissue may be accompanied by the lipolytic decomposition of fish oils in some, or all, cases of spoilage of fish.

Table 1.

Preliminary Study of Lipolysis at 48 Hours of Incubation.

Organism	Culture No.	32° C.		37.5° C.	
		Butter	Olive Oil	Butter	Olive Oil
Staph. aureus	51	-	-	-	-
Staph. albus	59	-	-	-	-
Staph. citreus	62	-	-	-	-
Ser. marcescens	88	-	-	-	-
Ps. aeruginosa	94	+	+	+	+
Ps. fluorescens	97	-	-	-	-
Ps. syncyanea	98	+	+	+	+
Aero. cloacae	146	-	-	-	-
Prot. vulgaris	153	+	+	+	+
S. schottmülleri	166	-	-	-	-
B. subtilis	243	-	-	-	-
B. mycoides	245	-	-	-	-
B. cereus	246	-	-	-	-

The + sign denotes lipolysis.

Note: Readings at seven days were identical with those in the table, except that growth was more advanced and lipolysis of the fat globules was more complete.

Table 2.

Lipolysis by Stock Laboratory Strains of the Escherichia-Aerobacter Group.

Organism	Culture No.	32° C.		37.5° C.	
		Butter	Olive Oil	Butter	Olive Oil
Esch. communior	128	-	-	-	-
" "	129	-	-	-	-
" "	130	-	-	-	-
" "	131	-	-	-	-
" acidi-lactici	134	-	-	-	-
" "	135	-	-	-	-
" "	136	-	-	-	-
" "	137	-	-	-	-
" neapolitana	139	-	-	-	-
" "	140	-	-	-	-
Aero. cloacae	147	-	-	-	-
" "	148	-	-	-	-
" "	149	-	-	-	-
" "	150	-	-	-	-
" oxytocum	151	-	-	-	-

Note: Readings at seven days were identical with those in the table.

Table 5.

Lipolysis by Freshly Isolated Bacteria of the Escherichia-Aerobacter Group at 48 Hours of Incubation.

Organism	Culture No.	32° C.		37.5° C.	
		Butter	Olive Oil	Butter	Olive Oil
Esch. coli	1	-	-	-	-
" "	2	-	-	-	-
" "	3	-	-	-	-
" "	4	-	-	-	-
" "	5	-	-	-	-
" "	6	-	-	-	-
" "	7	-	-	-	-
" "	8	-	-	-	-
" "	9	-	-	-	-
" "	10	-	-	-	-
Aero. aerogenes	101	-	-	-	-
" "	102	-	-	-	-
" "	103	-	-	-	-
" "	104	-	-	-	-
" "	105	-	-	-	-
" "	106	-	-	-	-
" "	107	+	+	+	+
" "	108	-	-	-	-
" "	109	-	-	-	-
" "	110	-	-	-	-

Table 5. (Continued)

Organism	Culture No.	32° C.		37.5° C.	
		Butter	Olive Oil	Butter	Olive Oil
Intermediate	201	-	-	-	-
"	202	-	-	-	-
"	203	-	-	-	-
"	205	-	-	-	-
"	206	-	-	-	-
"	207	-	-	-	-
"	208	+	+	+	+
"	209	-	-	-	-
"	210	-	-	-	-
"	212	-	-	-	-
"	213	+	+	+	+
"	214	+	+	+	+
"	215	+	+	+	+
"	216	+	+	+	+
"	217	+	+	+	+
"	218	+	+	+	+
"	219	-	-	-	-
"	220	+	+	+	+
"	221	-	-	-	-
"	222	-	-	-	-
"	223	+	+	+	+
"	224	+	+	+	+
"	225	-	-	-	-

Table 5. (Continued)

Organism	Culture No.	32° C.		37.5° C.	
		Butter	Olive Oil	Butter	Olive Oil
Esch. coli	44	+	+	+	+
Intermediate	46	+	+	+	+
"	47	+	+	+	+
Citrobacter	59	-	-	-	-
"	60	+	-	-	+-
Intermediate	130	+	+	+	+
Aero. aerogenes	133	+	+	+	+
Esch. coli	134	+	+	+	+

Note 1: Readings at seven days were identical with those in the table, except that growth was more advanced and lipolysis of the fat globules was more complete.

Note 2: The Escherichia strains were isolated from feces, and the Aerobacter, Intermediate, and Citrobacter strains were isolated from water and fish fillets.

Table 4.

Lipolysis by Strains of Genera Usually Pathogenic to the Intestinal Tract of Man or Animal.

Organism	Culture No.	32° C.		37.5° C.	
		Butter	Olive Oil	Butter	Olive Oil
Sal. abortivo-equina	155	-	-	-	-
" enteritidis	156	-	-	-	-
" "	157	-	-	-	-
" "	158	-	-	-	-
" suipestifer	159	-	-	-	-
" "	160	-	-	-	-
" paratyphi	162	-	-	-	-
" "	163	-	-	-	-
" "	164	-	-	-	-
" schottmulleri	167	-	-	-	-
" "	168	-	-	-	-
" enteritidis	169	-	-	-	-
" schottmulleri	170	-	-	-	-
" pullorum	178	-	-	-	-
" "	179	-	-	-	-
" "	180	-	-	-	-
Eber. typhosa	189	-	-	-	-
" "	190	-	-	-	-
" "	191	-	-	-	-

Table 4. (Continued)

Organism	Culture No.	32° C.		37.5° C.	
		Butter	Olive Oil	Butter	Olive Oil
Shig. dysenteriae	192	-	-	-	-
" "	192a	-	-	-	-
" "	193	-	-	-	-
" paradysenteriae (var. Flex.)	194	-	-	-	-
" "	195	-	-	-	-
" "	195a	-	-	-	-
" paradysenteriae (var. Hiss)	196	-	-	-	-
" "	197	-	-	-	-

Note: Readings at seven days were identical with those in the table.

Table 5.

Correlation of Lipolysis with Indicators of Possible Proteolytic Activity.

The Voges-Proskauer, methyl-red and sodium citrate reactions are given for reference.

Organism	Culture No.	Lipolysis	Gel. Liq.	H_2S	V.-P.	M.-R.	S.C.
Aero. aerogenes	107	+	-	-	+	-	+
Esch. coli	44	+	+	+	+	-	-
Intermediate	46	+	+	+	+	+	-
"	47	+	+	-	+	+	-
Citrobacter	60	+	+	+	-	+	-
Intermediate	130	+	+	+	+	+	+
Aero. aerogenes	135	+	+	+	+	-	+
Esch. coli	154	+	+	+	-	+	-
Intermediate	208	+	+	-	+	-	+
"	213	+	+	-	+	+	+
"	214	+	-	-	-	-	+
"	215	+	-	-	-	-	+
"	216	+	+	-	-	-	+
"	217	+	+	-	+	-	+
"	218	+	+	+	-	-	+
"	220	+	-	+	-	-	+
"	223	+	-	+	-	+	+
"	224	+	+	+	-	-	+

Note: Gel. Liq. = gelatin liquefaction.
H_2S = hydrogen sulphide production.
V.-P. = Voges-Proskauer.
M.-R. = methyl-red.
S.C. = sodium citrate.

Table 6.

Carbohydrate Utilization by the Lipolytic Bacteria Employed in the Study.

Organism	Dextrose	Levulose	Lactose	Sucrose	Raffinose	Starch	Xylose	Salicin	Esculin	Mannitol	Glycerol
III 107	A.G.	A.G.	A.G.	A.G.	K.R.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.
" 208	A.	A.	K.R.	A.	K.R.	K.R.	K.R.	A.	A.	A.	A.
" 213	A.G.	A.G.	A.G.	A.G.	K.	A.G.	A.G.	K.	A.	A.G.	A.G.
" 214	A.G.	A.G.	A.G.	A.G.	A.G.	A.	A.G.	A.G.	A.G.	A.G.	A.G.
" 215	A.G.	A.G.	A.G.	A.G.	A.G.	A.	A.G.	A.G.	A.G.	A.G.	A.G.
" 216	A.G.	A.G.	A.G.	A.G.	A.G.	A.	A.G.	A.G.	A.G.	A.G.	A.G.
" 217	A.	A.	K.R.	A.	K.R.	K.R.	A.	A.	A.	A.	A.
" 218	A.G.	A.G.	A.G.	K.R.	A.G.	K.R.	A.G.	K.R.	K.R.	A.G.	A.G.
" 220	A.G.	A.G.	A.G.	A.G.	A.G.	A.	A.G.	A.G.	A.G.	A.G.	A.G.
" 223	A.G.	A.G.	A.G.	A.G.	A.G.	A.	A.G.	A.G.	A.G.	A.G.	A.G.
" 224	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.
" 44	A.G.	A.G.	A.G.	A.G.	A.G.	K.R.	A.G.	A.G.	A.G.	A.G.	A.G.
" 46	A.G.	A.G.	A.G.	A.G.	A.G.	K.R.	A.G.	A.G.	A.G.	A.G.	A.G.
" 47	A.G.	A.G.	A.G.	A.G.	A.G.	K.R.	A.G.	A.G.	A.G.	A.G.	A.G.
" 60	A.G.	A.G.	A.G.	A.G.	K.	A.G.	A.G.	A.G.	A.	A.G.	A.G.
" 130	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.	A.G.
" 133	A.G.	S.A.	S.A.	S.A.	K.	S.A.	A.	S.A.	S.A.	S.A.	S.A.
" 134	A.G.	A.G.	A.G.	A.G.	A.	K.R.	A.G.	A.G.	S.A.	A.G.	A.G.

A. = Acid
G. = Gas
S. = Slight
K. = Alkaline
R. = Probable reversion

Incubation:
48 hrs. - 32° C.
48 hrs. - 37.5° C.

SUMMARY

1. The lipolytic activity of several genera of bacteria was studied, using Nile-blue sulphate as an indicator. The work of previously reported investigations was verified, and original studies were made with various strains of the Escherichia-Aerobacter group of bacteria, and with other Gram-negative intestinal bacteria.

2. Twenty per cent of all organisms studied were found to be lipolytic. Lipolysis was slightly prevalent in the Escherichia coli and Aerobacter aerogenes strains, while fifty per cent of the intermediate strains of this group were lipolytic. None of the closely allied intestinal organisms were found to produce lipolysis.

3. Eight-three per cent of the lipolytic organisms showed indications of possible proteolytic activity.

4. Eighty-three per cent of the lipolytic organisms fermented carbohydrate substances to a marked degree.

5. The possibility of using lipolytic activity as a means of classification is indicated. It is suggested that further work along this line be pursued.

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We, the undersigned, members of Mr. Harvey's thesis committee, approve this thesis.

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