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# A study of the melting points and iodine numbers of the lipoids extracted from psychrophilic mesophilic and thermophilic bacteria

Robert L. Somerville
*The University of Montana*

# A STUDY OF THE MELTING POINTS AND IODINE NUMBERS OF THE LIPOIDS EXTRACTED FROM PSYCHROPHILIC, MESOPHILIC, AND THERMOPHILIC BACTERIA.

by

Robert L. Somerville

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Approved:

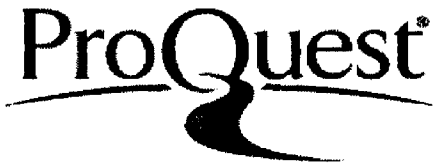
G. Albin Matson

Chairman of Examining Committee

W. J. Bateman

Chairman, Committee of Graduate Study

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DEDICATION

To the Memory of My Mother,

Sarah Somerville

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# A STUDY OF THE MELTING POINTS AND IODINE NUMBERS OF THE LIPOIDS EXTRACTED FROM PSYCHROPHILIC, MESOPHILIC, AND THERMOPHILIC BACTERIA.

## I. INTRODUCTION

The effect that environment has on protoplasm has led many investigators to speculate and investigate as to how environment affects the protoplasm of organisms. Huxley[11] stated that protoplasm is the physical basis of life. Although Wilson[23] points out that our knowledge of the chemical composition, physical state and the structure of protoplasm is in certain respects unsatisfactory, it is nevertheless known that the composition of protoplasm varies within certain limits. The most casual observer is aware of the fact that an environment which is favorable for the development and growth of one type of life is not at all suitable for life of another, that is, the various kinds of life possess different types of protoplasm. Loeb[14] believed that physiologic characteristics are dependent upon the physical or chemical properties of protoplasm. According to this view it is to be expected that animal and plant life which grows optimally under varied environmental conditions must be as varied in its protoplasmic structure.

This view has led several workers to investigate the behavior of the protoplasm of living organisms with respect to

the optimal temperature at which the organisms grow. Mayer's[17] experiments in 1917 led him to attribute the killing effect of heat on protoplasm to the accumulation of acid (possibly carbonic acid) in the tissues, the rate of formation of this acid being commensurate with the rate of metabolism. The theory of Heilbrun[10] is, however, somewhat different. He concluded that the action of heat on the coagulation of protoplasm is due to a primary effect on the emulsified lipoid in the cell, and secondarily by the action of the liquefied fat on the protein. It would indicate from Heilbrun's conclusions that the type of fat present in the cells plays a large part in keeping the protoplasm alive at the optimal growth temperature of the organism. If the fats play a role in the chemical make up of the protoplasm then it is necessary that the organisms synthesize fats that are liquid at the optimal growth temperature.

Ivanow's[12] experiments tend to substantiate this theory. He studied the iodine numbers of the oils extracted from different species of plants of the same genus grown under similar climatic conditions. He found under these conditions a similar content of unsaturated fatty acids in the oil extracted from the various species used. Other experiments conducted by him to show the effect of climate on the fatty acids showed that oils containing glycerides of unsaturated fatty acids with one double bond (oleic, erucic, or ricnic) are indiffer-

ent toward climatic changes, while in the acids with three double bonds ($\alpha$-$\beta$ linolenic acids) the iodine numbers decreased with increasing temperature or with the growth of the plants in more southernly regions. The oils with two double bonds (linoleic type) held an intermediate position.

It is conceivable that certain organisms have the ability to synthesize certain kinds of fats and are thus, because of their specific type of fat synthesis adapted to grow within the temperature range peculiar to those organisms. A temperature range suitable to the optimum growth of one organism may thus be destructive to other organisms having a different type of fat metabolism. On the other hand it may also be possible that the optimum temperature at which an organism grows may greatly influence the kind and amount of lipoids present in the cells. Thus Hempil[8] working on the influence of temperature on the life processes and death of bacteria concluded that the propagation of all bacterial life is necessarily limited by temperatures which may affect the physiological functions of catabolism and metabolism of such unicellular organisms. This is illustrated by the known fact that psychrophilic bacteria are found in water at low temperatures and in cold storage places. They are characterized by a slower rate of growth (lower metabolic rate) and a higher water content. Their optimum temperature ranges be-

tween 5° and 10° C. In comparison, the thermophilic bacteria are found in hot water springs and decaying vegetable matter. They have a lower water content and produce enzymes at temperatures at which other bacteria are destroyed. The optimal growth temperature of the thermophiles is between 50° and 60° C. Another group of bacteria which holds an intermediate position is known as the mesophiles. As the name indicates they are characterized by a medium metabolic rate and water content. They are found in the bodies of warm blooded animals and in the air, and their optimum growth temperature ranges between 30° and 37° C.

Leathes and Raper[15] state that, there is reason to believe that all the fats formed in nature are fluid at the temperature at which they are normally formed in the animal and plant. It is pointed out that the fats found in cold-blooded animals are of a lower melting point because of their unsaturated nature, whereas, the fats occurring in warm blooded animals and in tropical plants are more saturated and have a high melting point. If the fats are liquid at the temperature at which they are normally formed in the animal and plant then it is conceivable that they would also be liquid in bacteria. In the psychrophilic bacteria the fats formed would be of a highly unsaturated type so as to be a liquid at their optimal growth temperatures. In comparison the fats found in

the thermophilic bacteria would be of a more saturated type because the organisms have an optimal temperature of between 50° and 60° C. Thus enabling the bacteria to synthesize a more saturated type of fat.

This conclusion is born out by the experiments of Pearson and Raper[20] using Aspergillis niger and Rhizopus nigricans in their experiments. The organisms were grown at different temperatures and the fats extracted. Aspergillis niger grown at 18° C. had an iodine number for its fats of 149, grown at 25° C. the iodine number was 129, and at 35° C. it was 95. Rhizopus nigricans grown at 12°C. the iodine number of the fats was 88 and grown at 25° C. was 78.

Similarly Pigulevski[19] who examined the oils extracted from different species of the same family of plants concluded that the composition of the oils and fats they contained depended upon climatic conditions and possibly nutrition. Thus a cold climate induces in seeds of plants the necessity of accumulating oils exhibiting a high degree of unsaturation, therefore possessing great chemical activity.

Pontillon[18] was of the opinion that the media has something to do with the type of fat synthesized by organisms as well as the temperature. To test the media factor he grew molds on four different types of fat free synthetic media. He concluded from his experiments that the different types of media caused the synthesis of different kinds of fats by the

organisms. He observed that the dry weight of growth in each case was greater after 72 hours, whereas, the weight of the total fats was in each case greatest after 42 hours. The melting point and iodine number of the fatty acids varied with the media and the growth but more with the media. The mean molecular weight of the fatty acids varied with the time and media. From Pontillon's conclusions it would seem necessary to take into consideration the media factor in any experiment designed to study the type of fat synthesized by organisms as well as the temperature factor.

Anderson[1,2,3,4] and Chargaff[5] working with the Human Tubercle bacillus and Anderson and Roberts[6,7] working with the Avian type of Tubercle bacillus grown at 37° and 42° C. respectively didn't find the results that would be expected. The melting points of the saturated fatty acids were the same for both types. However, the iodine number of the liquid fatty acids of the Human type was 33.5 and the iodine number of the liquid fatty acids isolated from the Avian type was 36.84. Judging from the iodine numbers the type of fat present in the Avian type is of a more unsaturated type than that of the Human, although the optimal growth temperature of the Human type is 37° C. While that of the Avian is 42° C. It is possible that the fatty acids of the Avian type are composed of longer chain fatty acids than those of the Human type.

This would take into account the higher iodine number of the Avian type.

Matson[16] conducted experiments on psychrophilic, mesophilic, thermophilic, human and avian tubercle bacteria. The bacteria were grown on the following media; Uschinsky's[21] medium for the mesophile, a modification of Koser's[13] medium for the mesophile, Hansen's[9] medium for the thermophile and Long's[22] medium for the human and avian tubercle bacilli. From his experiments he concluded that: (1) there was an inverse correlation in temperature at which the organisms grew and the iodine number and a direct relation with the melting point, and (2) that the type of fats produced varied with the kind of media used. The results of his experiments are shown in Table I.

TABLE I

Melting points and Iodine numbers of various fractions of fats extracted from different organisms grown at various temperatures.

| EXTRACT | Psychrophile | Mesophile | | Thermophile | Human T.B. | Avian T.B. |
|---|---|---|---|---|---|---|
| | | B.coli 20° | B.coli 37° | | | |
| Temperature of growth | 4°C. | 23-26°C. | 37°C. | 55°C. | 37°C. | 42°C. |
| Melting pt. of Alc-ether extract | -9to-4° | 35-38° | 33-39° | 28-30° 48-50° | 38° | 32-34° |
| Iodine no. of Alc-ether ex. | 49 | 51.6 | 28.6 | 30.1 | 30.9 | 11.9 |
| Melting pt. of fatty acids | 38° | 19° | 43° | 25° | 37° | 35° |
| Iodine no. of fatty acids | 65.6 | 76.6 | 42.6 | 80 | 113.2 | 37.2 |
| Melting pt. of Non-saponifiable | 5-8° | 27° | 35-36° | 42° | 25-43° | 40-42° |
| Iodine no. of Non-saponifiable | Not Tested | Not Tested | 53.2 | 24.6 | 108.5 | 77.6 |

Matson found several apparent inconsistencies in his results as will be seen in Table I. In the case of the tubercle bacilli it will be noted that the avian type although it has an optimum growth temperature of 42°C., the alcohol ether fraction melted at 32-34°C. while the Human type melted at 38°C. The melting points of the various fractions isolated from the thermophile are not in direct relationship to the optimum temperature of the organism which is 58°C. One portion of the alcohol-ether fraction, for example, melted at 28-30°C. and another portion of the same fraction melted at 48-50°C. These inconsistencies may be due to the variation among species, that is, one species may be able to synthesize one type of fat while another develops another type of fat. The type of media may have an effect on the type of fat synthesized by the various organisms. Again the temperature factor is important because at one temperature an organism may be able to produce one type of fat while at another it is able to produce another type.

The above results of Matson's[16] as well as the findings of Pontillon[18] suggest that it might be possible to obtain a more comparable series of results if one kind of fat free synthetic media is used for all of the various bacteria. If all other experimental conditions including media are made constant, it should be possible by growing psychrophilic,

mesophilic, and thermophilic bacteria at their respective optimal temperatures, to more accurately determine the type of fat synthesized by the various organisms used. However, since different species of organisms are used it is obvious that the type of fat may not only be influenced by the temperature at which the organisms grow, but the type of fat produced may be a specific organismal function of the various species. It is, therefore, necessary in any experiment designed to study the influence of temperature on the type of fats produced by bacteria, to grow organisms under such conditions as will take into consideration this possible specific organismal peculiarity. This could be done best by growing at different temperatures an organism, for example Bacillus coli, having a wide variation between its minimum and maximum growth temperature.

The purpose of this problem is to study the effect that temperature may have on the type of fat synthesized by bacteria. This can be done by studying the melting points and iodine numbers of the lipoids extracted from psychrophilic, mesophilic, and thermophilic bacteria which have been grown at their optimal temperatures and on the same kind of fat free synthetic media.

## II. EXPERIMENTAL

For a series of experiments to test the effect of temperature on the type of fat synthesized by bacteria, the following bacteria were chosen: a psychrophile, isolated from ice box slime and whose optimum temperature was between four and eight degrees centi-grade, a mesophile represented by Bacillus coli, which was grown at twenty degrees centi-grade and at thirty-seven degrees centi-grade, and a thermophile, strain 1503 University of Illinois, isolated from a manure pile, which was grown at fifty-five degrees centi-grade.

Experiments were run using various fat free media to see on which kind the various bacteria would grow and give the most luxurious growth.[21] It was found that Uschinsky's medium was the best.

USCHINSKY'S MEDIUM

| | |
|---|---|
| Distilled water ..... | 1000 cc. |
| Asparagin ..... | 3.4 gms. |
| Ammonium lactate ..... | 10.0 gms. |
| NaCl ..... | 5.0 gms. |
| $MgSO_4$ ..... | 0.2 gms. |
| $CaCl_2$ ..... | 0.1 gms. |
| $K_2HPO_4$ ..... | 1.0 gms. |
| Glycerine ..... | 40.0 gms. |
| Agar ..... | 20.0 gms. |

Final reaction pH 7.3

After culturing the organisms on the synthetic media mentioned above, on slants in 16 oz. oval bottles, the surface growth was washed off with saline. The growth of the

mesophile and thermophile was harvested after 48 hours and the psychrophile in 5 days. After the organisms had been washed from the surface of the slants they were centrifuged at high speed for thirty minutes. The supernatant fluid was poured off and the organisms resuspended in the extracting fluid which consisted of equal parts of 95% ethyl alcohol and ether. The mixture was put in large glass stoppered bottles. All operations and experiments on the extracts were conducted under an atmosphere of carbon dioxide. After the bottle was filled with bacteria and extracting fluid it was allowed to remain for four weeks and was frequently shaken. An atmosphere of carbon dioxide was kept over the mixture to keep down oxidation. After four weeks the supernatant fluid was decanted and filtered. The residue was washed with ether and these washings were added to the supernatant liquid. The residue was then immediately transferred to chloroform and allowed to stand for four weeks with frequent shakings.

The alcohol-ether extract was treated according to this procedure. The ether and alcohol were distilled off under reduced pressure. The residue of lipoid material which remained suspended in dilute aqueous alcohol was repeatedly shaken with ether in a separatory funnel until the lipoids were extracted. Then the ether extract was dried with an-

hydrous sodium sulfate. This was filtered, concentrated until dry on a steam bath under a current of carbon dioxide, and then dehydrated in a dessicator containing sulfuric acid. The crude alcohol-ether extract thus obtained was weighed and the melting point was determined. The iodine number was determined by the Micro Method of Yasuda.[24]

After the residue had remained in the chloroform for four weeks the supernatant fluid was decanted. More chloroform was added and allowed to stand for one week. These chloroform extracts were then combined and the chloroform distilled off under reduced pressure. This concentrated chloroform extract was evaporated to dryness on a steam bath and dessicated. The extract was weighed, and the melting point and iodine number then determined. The bacterial residue was dehydrated and weighed, then discarded.

The alcohol-ether and chloroform extracts were taken up in ether and combined. To this was added nearly four volumes of acetone. This was allowed to remain over night in the refrigerator. The acetone soluble and acetone insoluble fractions were separated by centrifugation and pouring off the supernatant fluid (acetone soluble). To the acetone insoluble was added about three volumes of acetone and the process repeated. The acetone soluble fractions were combined and concentrated to a very small volume over a steam bath.

Two cc of 50% sodium hydroxide was added to the acetone

soluble fraction and refluxed for one hour. Then two or three cc of 95% alcohol was added and refluxed for two hours more. This experiment was done under an atmosphere of carbon dioxide. Five volumes of water were added to the saponified mixture. This was poured into a separatory funnel and shaken three times with petroleum ether. The ether soluble material was collected as the non-saponifiable fraction. The aqueous layer was then acidified with hydrochloric acid, using litmus paper as the indicator. The acidified solution was then extracted three times with ether. These extracts were united as the saponifiable fraction or fatty acids. The extractions were done under an atmosphere of carbon dioxide to prevent oxidation.

The non-saponifiable and saponifiable fractions thus obtained were concentrated over a steam bath and dehydrated. They were then weighed and the melting points and iodine numbers determined.

The acetone insoluble fractions were taken up in ether, saponified and extracted by the above methods. The melting points and iodine numbers were determined for the non-saponifiable fractions of the acetone insoluble lipoids.

The experimental methods are given in outline form as follows: (See next page.)

## OUTLINE OF EXPERIMENTAL METHODS

**Living Cells ---------- Culture**

Grow cultures at 4°, 20°, 37°, and 55° C. in 16 oz. bottles.
Wash cultures off slants with saline.
Centrifuge.
Place cultures in equal parts of 95% alcohol and U.S.P. ether.
All operations and experiments done under an atmosphere of $CO_2$.
Put mixture in 1 gallon glass stoppered bottles.
Bottles frequently agitated for 4 weeks then allowed to settle.
Supernatant liquid decanted.
Residue filtered on Buchner funnels and washed with ether.

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**Residue**

Immediately transferred to 1 gal. glass stoppered bottles containing chloroform.
Frequently stirred for 4 weeks.
Filtered on Buchner funnels and washed with chloroform.
Residue extracted second time with chloroform for one week.
Insoluble residue filtered off, washed with chloroform and dried.

**Alcohol-ether extract**

Ether distilled off in current of $CO_2$.
Alcohol distilled off under reduced pressure.
Residue of lipoid material remains suspended in dilute aqueous alcohol.
This mixture repeatedly shaken with ether in separatory funnel until lipoids are extracted.

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**Chloroform extract**

Concentrated by distillation.
Evaporated on steam bath.
Dried in dessicator.
Weighed.
Melting point and iodine number determined.

**Residue**

Dried and Weighed

**Ether solution of lipoids**

Dried with anhydrous sodium sulfate.
Filtered.
Concentrated.
Dried in dessicator.
Weighed.
Melting point and iodine number determined.

**Aqueous Alcohol Extract**

Discard

**Saponification**

1. Take up chloroform and alcohol-ether extracts in ether and combine.
2. Pour in four volumes of acetone and allow to stand in refrigerator for 24 hours.
3. Centrifuge.
4. Pour off supernatant fluid which contains acetone soluble.
5. Repeat by taking up in ether the acetone soluble and add

four volumes of acetone.
6. Repeat above procedure.

**Acetone insoluble lipoids**
Take up in ether.
Saponify with 2cc of 50% NaOH for one hour
Add 2cc of 95% alcohol reflux for two hours.
Add 5 volumes water and extract in separatory funnel with petroleum ether.
3 extractions.

**Acetone soluble lipoids**
Combine acetone extracts.
Evaporate acetone and saponify with 2cc of 50% NaOH for one hour.
Add 2cc of 95% alcohol reflux for 2 hours.
Add 5 volumes water and extract in separatory funnel with petroleum ether.
3 extractions.

**Water layer**
Acidify with HCl and extract 3 times with petroleum ether

**Ether Layer (Unsaponifiable)**
Evaporate
Dessicate
Weigh
Melting pt.
Iodine number determined

**Water layer**
Acidify with HCl and extract 3 times with petroleum ether

**Ether layer (Unsaponifiable)**
Evaporate
Dessicate
Weigh
Melting pt.
Iodine number determined.

**Water layer (Discard)**

**Ether layer (Saponifiable) or (Fatty acids)**
Evaporate
Dessicate
Weigh
Melting pt.
Iodine number determined.

**Water layer (Discard)**

**Ether layer (Saponifiable) or (Fatty acids)**
Evaporate
Dessicate
Weigh
Melting pt.
Iodine number determined.

## III. RESULTS

The results of the experiments run on the lipoids extracted from the psychrophilic, mesophilic, and thermophilic bacteria are shown in the following tables. Table II shows the results of the melting points and iodine numbers of the lipoids extracted from the psychrophile.

### TABLE II

Melting points and iodine numbers of various fractions extracted from the psychrophile grown at 4°C.

| Fraction | Weight | Melting point | Iodine No. |
|---|---|---|---|
| Alcohol-ether | 3.6079 gms. | Cleared at 25° Melted at 30° | 64.13 |
| Chloroform | 379.9 mgs. | Cleared at 38° Melted at 40° | 40.17 |
| Acetone Soluble Fatty Acids | 781.4 mgs. | 10-11° | 83.09 |
| Non-saponifiable | 389.7 mgs. | 30-31° | 47.05 |
| Acetone-insoluble Fatty acids | 92.6 mgs. | Cleared at 22° Melted at 23° | 53.48 |
| Non-saponifiable | 596 mgs. | 27-28° | 68.27 |
| Bacterial residue | 38.2964 gms. | | |

In the above table it will be noted that the alcohol-ether extract, which was a dark brown waxy material and tenacious at room temperature, cleared to a dark brown mass at 25° C. and melted at 30° C. The iodine number (64.13) was rather high due to the complex nature of the extract. The chloroform extraction yielded a small amount of a light brown

sticky lipoid which cleared to a semi-transparent mass at 38° C. and melted at 40° C. The iodine number (40.17) was comparatively low showing that the lipoid was partly saturated. The fatty acids, isolated from the acetone soluble fraction, were dark brown in color, having a syrupy consistency at room temperature. The iodine number (83.09) indicates a high degree of unsaturation of the fatty acids. The non-saponifiable fraction of the acetone soluble was a cream colored lipoid which was soft and sticky at room temperature it melted at 30-31° C. The iodine number was low (47.05) in comparison to that of the fatty acids showing that the lipoid was more saturated. In the acetone insoluble fraction the mass of fatty acids was a dark brown lipoid which was semi-solid at room temperature and it cleared to a dark brown liquid at 22° and melted at 23° C. The iodine number (53.48) showed that the fatty acids were unsaturated. The non-saponifiable fraction was a light cream colored fat which was crystalline at room temperature. It melted at 27-28° C. and the iodine number (68.27) was rather high showing that the fat was unsaturated.

The results of the mesophile, Bacillus coli, grown at 20° C. are shown in Table III.

The alcohol-ether extract of B. coli grown at 20° C. had a melting point of 34-35° C. The iodine number (23.16) was

TABLE III

Melting points and iodine numbers of the various fractions extracted from the mesophile, B. coli, grown at 20°C.

| Fraction | Weight | Melting Point | Iodine No. |
|---|---|---|---|
| Alcohol-ether | 829.3 mgs. | 34-35° | 23.16 |
| Chloroform | 169.7 mgs. | 22-22.5° | 41.24 |
| Acetone soluble Fatty Acids | 284.3 mgs. | 17-18° | 124.94 |
| Non-saponifiable | 155.5 mgs. | Cleared at 47° Melted at 48° | 45.07 |
| Acetone-insoluble Fatty Acids | 77.7 mgs. | 22-22.5° | 62.58 |
| Non-saponifiable | 43.3 mgs. | 33-34° | 29.45 |
| Bacterial residue | 25.557 gms. | | |

low showing that the lipoids had a high degree of saturation. The chloroform extraction produced an amber colored fat which was semi-solid and semi-transparent at room temperature. The melting point was 22-22.5° C. and the iodine number (41.24) was high in comparison to that of the alcohol-ether extract. This showed that the lipoids of the chloroform extract were more unsaturated than those of the alcohol-ether extract.

The acetone-soluble fraction yielded upon saponification fatty acids that had a very high iodine number (124.94) and a low melting point (17-18° C.). The high iodine number indicates a high degree of unsaturation consequently a low melting point. It was a brown colored lipoid which was a liquid at room temperature. The non-saponifiable fraction on the other hand had a high melting point (47-48° C.) and a low

iodine number (45.07) showing that the lipoid is partially saturated. It was a light brown solid at room temperature.

The fatty acids of the acetone insoluble fraction were amber colored and solid at room temperature. They melted at 22-22.5°C. and had an iodine number (52.58) which indicated they were unsaturated to some extent. The non-saponifiable fraction showed a very low iodine number (29.45) with a comparatively high melting point (33-34° C.) which shows that the lipoid was partially saturated. It was also an amber colored lipoid which was solid at room temperature.

The results of the mesophile, B. coli, grown at 37°C. are shown in Table IV. The mesophile was grown at 20°C. and at 37°C. so as to afford a check on whether or not the temperature had an effect on the type of fats produced by the bacteria.

TABLE IV

The melting points and iodine numbers of the lipoids extracted from the mesophile, B. coli, grown at 37°C.

| Fraction | Weight | Melting point | Iodine No. |
|---|---|---|---|
| Alcohol-ether | 2.309 gms. | 37-37.5° | 23.50 |
| Chloroform | 414.7 mgs. | Cleared at 39° Melted at 43.5° | 44.33 |
| Acetone soluble Fatty Acids | Not tested | Not tested | Not tested |
| Non-saponifiable | 754 mgs. | Cleared at 30° Melted at 32-33° | 52.38 |
| Acetone-insoluble Fatty Acids | 74 mgs. | Cleared at 14° C. Melted at 20° | 79.03 |
| Non-saponifiable | 138.3 mgs. | Cleared at 20° Melted at 23-24° | 58.76 |
| Bacterial residue | 44.4248 gms. | | |

It will be noted in the preceding table that the alcohol-ether extract had a melting point (37-37.5°) of exactly the same as that of the temperature at which it was grown. The iodine number (23.50) was low, however, which is to be expected on such a complex mixture as is present in the alcohol-ether extract. It was a light brown lipoid which was hard at room temperature. The chloroform extract was a light brown lipoid which cleared to a transparent mass at 39°C. and melted at 43.5°C. The iodine number (44.33) was considerably higher than that of the alcohol-ether extract showing that it was composed of shorter chain fatty acids than the alcohol-ether extract.

Due to an accident in the laboratory the flask that contained the fatty acids isolated from the acetone-soluble fraction, was broken and the fatty acids lost. The non-saponifiable fraction, however, was saved and the melting point was found to be 32-33°C. The iodine number (52.38) was high indicating a high degree of unsaturation. It was an amber colored lipoid which was soft at room temperature.

The acetone-insoluble fraction yielded upon saponification fatty acids which were light yellow in color and soft at room temperature. They changed to a transparent mass at 14°C. and melted at 20-20.5°C. The iodine number (79.03) was correspondingly high showing the unsaturated nature of the fatty acids. The non-saponifiable fraction had a rather high iodine

number (58.76) and a low melting point 23-24°C. which showed that the lipoids present were unsaturated to some extent. It was a light yellow transparent rather soft fat at room temperature.

Table V. gives the results of the thermophile which was grown at 55°C.

TABLE V.

The melting points and iodine numbers of the various lipoids extracted from the thermophile, strain 1503, grown at 55°C.

| Fraction | Weight | Melting point | Iodine no. |
|---|---|---|---|
| Alcohol ether | 439.2 mgs. | 67-68° | 36.76 |
| Chloroform | 351.3 mgs. | 56-57° | 25.53 |
| Acetone soluble Fatty Acids | 87 mgs. | Cleared at 30° Melted at 31° | 38.88 |
| Non-saponifiable | 85 mgs. | 33-33.5° | 31.57 |
| Acetone-insoluble Fatty Acids | 71.6 mgs. | 38.5-39° | 46.44 |
| Non-saponifiable | 49.5 mgs. | 49° | 20.98 |
| Bacterial residue | 19.6877 gms. | | |

The alcohol-ether extract of the thermophile had a very high melting point (67-68°C.), and a rather low iodine number (36.76) which indicates that the fat was highly saturated. It was a dark brown colored solid at room temperature. The chloroform extraction yielded a lipoid which has a melting point of 56-57°C. which was relatively close to that of the optimum temperature for the organism which is 55°C. The iodine number (25.53) was again low showing the high degree of saturation

of the lipoid. It was an amber colored fat which was sticky at room temperature.

The acetone soluble fraction yielded fatty acids that changed to an amber colored mass at 30°C. and melted at 31°C. It had a comparatively low iodine number (38.88) which would indicate a short chain fatty acid. The non-saponifiable fraction had a melting point of 33-33.5°C. and a low iodine number (31.57) which shows that it is composed of lipoids which are saturated to some extent. They were amber colored and sticky at room temperature.

The mass of fatty acids isolated from the acetone insoluble fraction was an amber colored lipoid which was solid at room temperature. This mass melted at 38.5-39°C. and had a correspondingly low iodine number (20.98) which showed that it was highly saturated in nature. The lipoids were amber in color and solid at room temperature.

Table VI is given to show the comparison of the melting points and iodine numbers of the various fractions of the lipoids extracted from the psychrophilic, mesophilic and thermophilic bacteria.

It can be seen from Table VI that the fats synthesized by the psychrophile would be liquid at the optimum temperature of a psychrophile which is between 5 and 10°C. In the case of the fatty acids of the acetone soluble fraction the

TABLE VI

The results of the melting points and iodine numbers of the lipoids extracted from the psychrophilic, mesophilic, and thermophilic bacteria.

| Fraction | Psychrophile | Mesophile | | Thermophile |
|---|---|---|---|---|
| | | B. coli 20° | B. coli 37° | |
| Temperature of growth | 4°C. | 20°C. | 37°C. | 55°C. |
| Melting point of Alc-ether ex. | Cleared at 25° Melted at 30° | 34-35° | 37-37.5° | 67-68° |
| Iodine No. of Alc-ether ex. | 64.13 | 23.16 | 23.50 | 36.76 |
| Melting pt. of Chloroformex. | 38-40° | 22-22.5° | Cleared 39° Melted 43.5° | 56-57° |
| Iodine No. of Chloroform ex. | 40.17 | 41.24 | 44.33 | 25.53 |
| Acetone Soluble Melting point of Fatty Acids | 10-11° | 17-18° | Not tested | 30-31° |
| Iodine No. of Fatty Acids. | 83.09 | 124.94 | Not tested | 38.88 |
| Melting pt. of Non-saponifiable | 30-31° | Cleared 47° Melted 48° | Cleared 30° Melted 32-33° | 33-33.5° |
| Iodine No. of Non-saponifiable | 47.05 | 45.07 | 52.38 | 31.57 |
| Acetone insoluble Melting point of Fatty Acids | Cleared 22° Melted 30° | 22-22.5° | Cleared 14° Melted 20° | 38.5-39° |
| Iodine number of Fatty Acids | 53.48 | 62.58 | 79.03 | 46.44 |
| Melting point of Non-saponifiable | 27-28° | 33-34° | Cleared 20° Melted 23-24° | 49° |
| Iodine No. of Non-saponifiable | 68.27 | 29.45 | 58.76 | 20.98 |

melting point was 10-11°C. and the iodine number 83.09. The iodine numbers of the other fractions are relatively high also showing that the fats produced are highly unsaturated.

The mesophile, represented by B. coli, shows some very striking results. Bacillus coli was grown at 20° and at 37° C.

to see what effect the temperature may have on the type of fat produced by the bacteria. It will be noted in the case of the alcohol-ether extracts, of the B. coli grown at 20° C. produced fats that melted at 34-35°C. while the B. coli grown at 37° C. produced fats that melted at 37-37.5°C. The iodine numbers were 23.16 and 23.50 at 20° and 37° C. respectively. This similarity in iodine numbers indicates that the fats have approximately the same degree of saturation but the melting points show that there is a difference in the number of carbon atoms present in the molecules of fat. A fat having short chain fatty acids has a lower melting point than the fats having longer chain fatty acids when the iodine numbers are approximately the same as the melting point. In the chloroform extracts a wider variation is shown in the melting points 22-22.5° C. for the B. coli at 20° C. and 43.5° for the B. coli at 37° C., but here again the iodine numbers are the same, 41.24 and 44.33 respectively. This is again due to the number of carbon atoms present in the molecules of fat.

The acetone soluble fatty acids of the B. coli grown at 20° C. are highly unsaturated as indicated by the iodine number which was 124.94. The melting point is quite low as it was 17-18° C. In the case of the fatty acids isolated from the acetone-insoluble fraction it is just the opposite to what one would expect, as the melting point of the fatty acids of the B. coli, grown at 20° C. was 22-22.5° C. while those of

the B. coli grown at 37° was 20° C. The iodine number for the B. coli at 20° was 62.58 while that of the B. coli grown at 37° C. was 79.03. This indicates that the fatty acids of the B. coli grown at 37° C. is of a more unsaturated nature than those of the B. coli grown at 20° C. The non-saponifiable fraction of the acetone-insoluble fraction of B. coli grown at 20° gave a melting point of 33-34° C. while that of the B. coli at 37° C. was 23-24° C. There was a large variation in iodine numbers which were 29.45 and 58.76 at 20° and 37° C. respectively. It would seem that the fats that were synthesized by the B. coli at 20° C. had less carbon atoms present in their molecules than those of the B. coli at 37° C.

In the results of the experiments on the thermophile the melting point of the alcohol-ether extract was 67-68° C. while the iodine number (36.76) was low enough to show the degree of saturation of the complex mixture of fats. The chloroform extract had an iodine number of 25.53 and a melting point of 56-57° C. The iodine number indicates the degree of saturation of the fat which was high. In the case of the non-saponifiable fraction of the acetone-soluble extract the melting point was 33-33.5° C. and the iodine number was 31.57. This shows that there were some long chain fatty acids present. The iodine number of the non-saponifiable fraction of the acetone-insoluble was 20.98 showing that the fat was highly saturated.

## IV DISCUSSION

A study of the melting points and iodine numbers of the lipoids extracted from the various bacteria used in the experiments of this problem revealed that the temperature at which the organisms grew is an important factor in the synthesis of fats by the organisms. The reason for this conclusion is that although the same type of media was used for all the various bacteria, nevertheless, they continued to grow and synthesize fats that were liquid at their optimum growth temperatures. B. coli, was grown at 20° C. and at 37° C. and it continued to produce fats that were liquid at the temperatures at which this organism was grown. In the case of B. coli at 20° C. the fats as a whole had melting points that were low enough to justify this conclusion. The iodine numbers showed that the fats were of an unsaturated type. On the other hand B. coli grown at 37° C. the melting points of the fats showed that they were liquid at 37° C. The iodine numbers indicated that a more saturated type of fat was present. The psychrophile was grown at 4° C. and the results indicated by the iodine numbers of the fats present that they were of such a degree of unsaturation that they were liquid at that temperature. The melting points also indicated that they were of a short chain type which would also bear out the fact that they were liquid at 4° C. The thermophile on the other hand was grown at 55° C. and the melt-

ing points showed that they were of a longer chain type and therefore would have a higher melting point. The iodine numbers indicated that they were of a more saturated type. All of this evidence tends to show that the temperature does have an effect on the type of fat produced.

Pigulevski[19] concluded from his results that the composition of fats synthesized by the organisms he used depended upon climatic conditions and possibly nutrition. If the term climatic conditions is taken to mean temperature then the results of this problem are in accordance with his conclusions. Due to the fact that the mesophile, B. coli, was grown at two temperatures and it produced a different type of fat at each temperature shows that the type of fat synthesized by the organism is to a great extent due to the temperature.

Comparing the results of the experiments run by Batson[16] with the results of the experiments of this problem it will be noted in the case of the mesophile, that he obtained similar results to those of this problem. He used a modification of Koser's[13] medium for B. coli, grown at the two temperatures and found that the type of fats synthesized was different. The mesophile of this problem was the same organism and it was grown on Ushcinsky's[21] medium at the two temperatures. However, the results showed the same differences in the fats produced. In the case of the psychrophile Batson used the same media to

grow the organism on as was used in this problem. There is a slight difference in the results of the two sets of experiments but this may be due to the fact that different organisms were used by him from that of this problem. The thermophile that Matson used was a different species and it was grown on Hansen's[9] medium, while the one used in this problem was grown on Uschinsky's[21] medium. Again there is a slight difference in the results but this may be due to the fact that two different types of media were used and two species of organisms. It is a known fact that organisms within a species or genus vary in their metabolism. Ivanow[12] came to that conclusion with his experiments on plants of the different species of the same genus. According to Ivanow's conclusions it would be possible to explain the variation in the iodine numbers and melting points of the psychrophile and thermophile of the two problems by the fact that the organisms did vary in their metabolism and synthesized fats that were best suited for their environment.

Again Hampil[8] concluded that the temperature may effect the physiological functions of catabolism and metabolism of such unicellular organisms as bacteria. It would seem that the results of this problem would tend to be in accordance with this theory of Hampil's in so far as the fat metabolism is concerned, as the temperature at which the organisms grew caused the production of that type of fat which was best suited

for the organism to survive its environment.

Finally the results of this problem show that the temperature at which the organisms grow optimally is an important factor in influencing the type of fat synthesized by the bacteria.

## V SUMMARY AND CONCLUSIONS

1. The melting points and iodine numbers of the lipoids extracted from the psychrophilic, mesophilic, and thermophilic bacteria have been studied.
2. The temperature does have an effect on the type of fat synthesized by the bacteria.
3. There is a direct relationship between the melting point of the fats and the optimal temperature at which the organisms grow. In other words the fats produced by the psychrophiles grown at 4° C. are of a low enough melting point to be liquid at the optimal growth temperature. The same is true for the mesophile which was grown at 20° and 37° C. and at each temperature the melting points of the fats were sufficiently close to the optimal temperature for the fats to be liquid. Again the thermophile grown at 55° C. had fats whose melting points showed a direct relationship to the optimal growth temperature.
4. The iodine numbers vary inversely with the temperature at which the organisms grow. The iodine numbers of the psychrophile bacteria showed that a sufficiently unsaturated type of

fat was produced so as to be liquid at the optimal growth temperature of the organism. The same is true of the mesophile grown at 20° C. In the case of the mesophile grown at 37° C. the iodine numbers showed that a more highly saturated fat was produced but that it was a liquid at the optimal growth temperature. The same is true of the thermophile grown at 55° C.

5. The literature pertaining to the problem has been reviewed.

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