THE ABSORPTION OF SOLUBLE VOLATILE FATTY ACIDS

by

RAYMOND HICKMAN HUGHES

B. S., Kansas State College of Agriculture
and Applied Science, 1933

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1934

TABLE OF CONTENTS

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to Dr. Edward J. Kinmer for helpful suggestions and aid in conducting this experimental work. The writer wishes to express his appreciation to Dr. E. J. Frick of the Veterinary Department for supplying the experimental animals used and to the Chemistry Department for the use of chemicals and a laboratory in which to conduct the chemical analyses.

INTRODUCTION

Results of practical feeding tests as well as those of scientific experiments show clearly that the composition of adipose tissue can be changed by the kind of fat contained in the feed. When oil-bearing feeds such as peanuts or soy beans are fed to hogs the melting point of the fat in the adipose tissue is lowered to such an extent as to produce a very poor quality of bacon. This lowered melting point might result either from the increase of low melting unsaturated fatty acids or low melting short chain fatty acids. A number of experiments have been conducted to study the possibilities of depositing the various types of fatty acids in adipose tissue.

Maude Powell (1930) admirably summarized the initial investigations in this field as follows: "Early work by Munk, Leich and Winkler, Lebedeff, Lummert, Rosenfeld, Henriques and Hansen, and others, on the effect of ingestion of foreign fats upon the depot fat of animals, showed a striking change in the iodine number of the depot fat, corresponding to the character of the fat in the food. When mutton fat was fed it went down, when linseed oil was fed it went up. The unsaturated acids of the depot fat are evidently influenced by those in the food. Lummert,

Henriques and Hansen, König, Gibbs and Agcooli, and others have found that feeding butter fat or coconut oil results in a lowering of the iodine number of the depot fat." These experiments tend to show that the fatty acids of the food fats do not change their degree of saturation during the process of digestion, absorption, and deposition in the adipose tissue.

As early as 1882 in a study on body fats Lebedeff investigated the influence of feeding fats containing short chain fatty acids on body fats. Instead of using a natural fat, he fed tributyrin, but could find no evidence of the deposition of butyric acid in the depot fat. Leube (1895) found that there was very little, if any, increase in the volatile fatty acids when butter was fed, showing that the shorter chained fatty acids of the butter were not deposited in the adipose tissue. This work was confirmed by Zuntz (1901).

Raper (1913), while studying the function of the liver in the metabolism of fats, found, in an experiment on one dog, that the mean molecular weight of the fatty acids present as glycerides in the lymph of the thoracic duct was much higher than that of the fatty acids in the cocoanut oil fed. The molecular weight was even higher than the theoretical calculated molecular weight in which allowance

was made for the fat normally present in the lymph. He explains this rise in mean molecular weight by saying, "the lower fatty acids of cocoanut oil appear to be absorbed as sodium salts and are not resynthesized into glycerides", and again, "It thus appears as if the lower fatty acids of cocoanut oil do not completely undergo the glyceride synthesis during absorption." Jenkins (1923) showed that short chain volatile acids are not deposited in the animal body, but that the longer chain volatile acids are deposited to some extent.

Rahstein (1929a) could find no evidence of the butyryl radical in the body fat of white rats after feeding sodium butyrate. He believed (1929b) that fats like tributyrin and tricaproin are utilized by the white rat to form a "new fat", the lower fatty acids being used to form a fat more characteristic of the animal's body fat. He also reports data (1930) to show that the feeding of butter and tricaproin does not change the saponification number or Reichert-Meissel number of the depot fat which indicates that the shorter chain fatty acids are not deposited in the adipose tissue.

Davis (1930), carrying this problem a little farther than previous workers, injected tributyrin both subcutaneously and intraperitoneally into white rats and found there

was an increase in the saponification number of the depot fat. This indicates that a fat containing the butyryl radical can be deposited in the adipose tissue if such a fat once gets into the circulatory system. As was the case with investigators before him, he was unable to produce a change in the saponification number of the fat deposits by feeding tributyrin. In his article, Davis cites three possible answers to the question, as yet unanswered, "What is the fate of the short chain volatile acids after they are absorbed by the intestine?" First, the fatty acids might be transformed into longer chain fatty acids; second, they might be oxidized at once and thus spare the other fats permitting them to be stored; and, third, they might be converted into glucose. From his experiments, however, Davis believes that the true fate of the short chain volatile fatty acids is to be found in a combination of the first two possibilities which he presents.

Maude Powell (1931) fed butyric acid and caproic acid as glycerides to rats and found none deposited in the body fat. From her work she concludes that fatty acids of eight carbon atoms or less tend to make longer chains while those of ten carbon atoms or more are deposited in considerable amounts unchanged.

These results as well as those of other workers, show quite clearly that the absorption and metabolism of the short chain fatty acids differ in some way from that of the longer chain acids. None of these authors, however, has suggested any reason for this difference. One possible explanation is based upon the different mode of absorption of lipins and non-lipins. The end products of digestion, such as amino acids and monosaccharides, which are water soluble enter the blood after absorption and are carried by the portal blood to the liver. The fats, however, after being resynthesized from glycerol and fatty acids in the intestinal mucosa, are said to enter the lacteals and by way of the thoracic duct are carried to the shoulder region at which point the lymph is emptied into the blood stream. Since butyric, caproic, and caprylic, acids are all soluble in water it is entirely possible that they would follow the path of the non-lipins and thus escape the glycerol synthesis. If this be true, these water soluble fatty acids should not be found in the lymph. It is to test the validity of this assumption that the following investigation was undertaken.

MATERIALS AND METHODS

Dogs were used as experimental animals for this investigation. From the time the animals were obtained until a short time before the operation, they were fed a preliminary diet which consisted either of scraps of lean meat or a specially prepared diet made by mixing ten pounds bran, five pounds cracklings, five pounds stale bread together with water. This mixture was boiled for fifteen minutes and dried in a hot air oven. Three types of fat were fed as the experimental diet: (1) Butter fat. This was selected because it is a common fat containing butyric acid. Butyric acid makes up approximately 5 per cent of butter fat. (2) Crisco. This is a hydrogenated vegetable oil containing practically no short chain fatty acids. 3. Crisco plus 7 per cent Tributyrin. This provides a food fat containing no intermediate water soluble fatty acids.

Two methods were used to feed the butter fat. In one the butter, mixed with lean meat and a pint of cream, was fed to the dogs, while in the other the butter was used as the shortening in corn bread, 15 per cent and 30 per cent on the dry basis, and the corn bread with a pint of cream was fed. Two such meals were fed each dog, one in the evening preceding the operation and the other about four

hours before the dog was anaesthetized.

The Crisco was fed mixed with lean meat. One portion of the fat containing ration was given the dog four and one-half hours before the operation was begun and another portion about two and one-half hours later. The last feeding was to insure the absorption of fat from the intestines as long as it was desired to continue collection of lymph.

Tributyrin, in addition to being harmful to the mucosa of the digestive tract, tastes very bitter and the dogs refused to eat it mixed with their food. It was therefore found necessary to use a stomach tube to introduce the tributyrin into the stomach. To minimize the damage to the mucosa the following method of feeding the experimental ration was used. One hundred grams of lean meat mixed with 40 grams of Crisco were first fed the dog. The stomach tube was then passed, using a speculum to hold the dog's mouth open, and 8 grams of tributyrin mixed with 12 grams of melted Crisco were introduced into the stomach. The dog was then fed another 100 grams of lean meat mixed with 40 grams of Crisco. This feed was given at 7:30 o'clock on the morning of the operation. In a like manner, 200 grams of lean meat and 80 grams of Crisco with 7 grams of tributyrin were fed at 10:00 o'clock. The dog was anaesthe-

tired about noon.

Nembutal was used for the anaesthetic since it offered certain advantages for this type of experiment. In the first place, there was little or no danger of losing the animal from faulty administration of the anaesthetic as might happen with ether. The ease with which it could be administered was another advantage. A single intravenous injection (1 cc. for each 5 lbs. of weight) of nembutal, into the recurrent metatarsal vein would keep the dog anaesthetized for four or five hours. To extend the period of anaesthesia, it was only necessary to supplement the original dose by additional injections whenever the animal showed signs of reviving. The presence of a corneal reflex was used as an indication that the animal was reviving.

After the animal was anaesthetized, the thoracic lymph duct was exposed for cannulation. In dogs, this duct usually empties into the venous circulation dorsally at the junction of the internal and external jugular veins. Consequently, an incision was made in the neck region a little to the left of the medial line, exposing the left external jugular vein. It was found to be advantageous in the process of cannulation to have this incision commence above the clavicle from the jugular notch of the sternum along the medial margin of the sternocleidomastoideus muscle and

to extend about six inches anteriorly. The external jugular was then followed down to its junction with the internal jugular vein, recognizable as a broad triangular enlargement. If the thoracic duct entered normally, the lymph was seen as a whitish fluid surging into the junction during diastole. The thoracic duct is surrounded by a layer of connective tissue which must be dissected away before the duct can be seen. The thoracic duct can then be identified as a thin white duct extending anterio-medially. Dissection was commenced on the dorsal side of the junction and the tissue dissected away from the thoracic duct for a distance of two to two and one-half centimeters. The thoracic duct, which was rarely more than one or one and one-half millimeters in diameter when distended, was then ready for cannulation.

One method of procedure for cannulation which was followed was taken from the laboratory outline of the University of Chicago. Following this method, a small hemostat was clamped onto the duct near its entrance into the venous system. This resulted in an increase in the size of the duct due to the increased pressure of the lymph. Another small hemostat was then placed onto the duct as far from the junction as possible. The hemostat which was placed below the point of incision prevented the flow of the lymph from the duct while the cannula was being inserted. The duct was

cut half way across by a diagonal incision. The index finger was then placed under the duct and the cannula was inserted. After the cannula was tied in place, the hemostats were removed and the lymph allowed to flow from the cannula.

The other method of cannulation used was one demonstrated to the author by Dr. Mann of Mayo Foundation. In this method, the lymph was not held back by hemostats, but was allowed to flow freely from the duct during the time the cannula was being inserted. As in the previous method, the duct was snipped at an angle with a pair of scissors. To facilitate the process of cannulation a seeker was inserted into the opening. A cannula, moistened in sodium citrate solution, was then inserted beside the seeker and the seeker removed. The cannula was then made secure by tying it in place.

If the cannulation was successful a small rubber tube was attached to the end of the cannula from which the lymph was collected. In two cases (dogs 2 and 3) in which the cannula could not be inserted the dogs were placed in such a position that the lymph would run into the collecting dishes. Surprisingly little contamination from tissue was obtained as shown by the fact that the lymph was not greatly discolored and the analysis of the lymph collected in

this manner did not differ significantly from that collected by means of a cannula.

Chemical Analysis

After collection, the lymph was usually dried in an oven at 100° C. and then kept in a desiccator until the extraction was made. However, in a few cases, the lymph was placed on a hot water bath and a current of air was passed over it for more rapid evaporation. The first step in the analysis was to extract the dried lymph with anhydrous ether, in either a Soxhlet or Bailey Walker extractor. When the Soxhlet extractor was used, the extraction was made directly into a weighed 300 cc. Erlymer flask so that transfer of the fat was not necessary for the subsequent analysis.

Since the purpose of this experiment was to determine the presence or absence of water soluble volatile fatty acids in the lymph of the thoracic duct, the most appropriate chemical determination seemed to be the Reichert-Meissel number. This chemical measurement is defined as the number of cubic centimeters of N/10 alkali required to neutralize the water soluble volatile fatty acids in five grams of fat. The method for obtaining this value is of necessity an emperical one since it is impossible to make

a quantitative separation of fatty acids by fractional distillation. In running such an emperical analysis, therefore, the specifications for the construction of the apparatus and the distillation must be rigidly followed to get comparable results.

When as much as 4.0 to 5.0 grams of fat were obtained from the dried lymph the Reichert-Meissel number was determined by the official method (1930). Accordingly, the weighed fat was saponified in a 300 cc. flask with 20 cc. of glycerol soda, after which 135 cc. of carbon dioxide free distilled water was added. Six centimeters of sulfuric acid was next added to liberate the fatty acids. In addition, two pieces of pumice stone were added to prevent bumping, and the whole mixture was distilled. The distillation was made in a distilling apparatus constructed in accordance with the official method at such a rate that 110 cc. of the liquid was distilled in thirty minutes. The distillate was cooled for 15 minutes at 15° C. in order to allow the insoluble fatty acids to solidify. It was then filtered. One hundred centimeters of this filtrate was titrated with N/10 NaOH from which the amount of soluble volatile fatty acids in the entire 110 cc. of distillate was calculated by multiplying by 1.1.

$$(1.1 = \frac{\text{110 cc. (original sample)}}{\text{100 cc. (sample titrated)}})$$

If the amount of ether extract was not exactly 5 grams, the amount of soluble volatile fatty acids which would have been distilled over from exactly five grams of fat was calculated.

If less than 4.0 grams of fat were extracted, the analysis was made according to a modification of the official method reported by Millig (1930). Following this method, enough vegetable oil (Crisco) with a previously determined Reichert-Meissel number was added to the lymph fat to make it up to approximately five grams. The Reichert-Meissel number of the mixture was determined as described above and the result was then corrected to give the Reichert-Meissel number of 5 grams of lymph fat by use of the formula:

$$R_s = \frac{(R_m - \frac{R_c}{5} \times \text{Wt. of Crisco})}{\text{Weight of Sample}} \times 5$$

Where R_s is the R.M. No. of the sample, R_m is the R.M. No. of the mixture, and R_c is the R.M. No. of the Crisco.

RESULTS

The results of the chemical analysis are shown in Table 1.

Due to the small amount of fat obtainable, it was impossible to run duplicate determinations, with the exception of dog number nine. It is fortunate, however, that all the mistakes which result from the emperical nature of this determination tend to increase the value instead of lower it. Therefore, the results can be considered valid although not checked by duplicates. The variance in the individual results, with the exception of dogs seven and eight, is not greater than that which would be expected under normal conditions.

In the case of dogs seven and eight it is impossible to explain exactly what was the cause of the high Reichert-Meissel number. It will be noted, however, that these determinations were made upon 0.5534 and 1.6670 gram samples and, therefore, that any errors made would be multiplied by nine and three respectively. It is very probable that these results are too high, but, granting their correctness, the results as they stand would only show that the Reichert-Meissel number of the thoracic lymph fat has a large value range even when no short chain fatty acids are in the food.

Table 1. Summary Table of Data

		Amount of fat collected (gm.)	Reichert-Meissel No.	Ave
1	40 per cent cream Butter Lean meat	0.35		
2	20 per cent cream Butter Lean meat	5.1966	2.25*	
3	Corn bread Cream	0.9316	4.65	
4	Corn bread Cream	1.1784	3.2	3.4
5	20 per cent cream Corn bread	2.5859	3.8	
6	Crisco Lean meat	1.1792	3.45	
7	Crisco Lean meat	0.5634	9.0	
8	Crisco Lean meat	1.6670	7.0	4.7
9	Crisco Lean meat	14.0	(a) 2.0 (b) 2.3	
10	Crisco Tributyrin Lean meat	4.4664	1.6	1.6

Reichert-Meissel number of Crisco - 0.4
Reichert-Meissel number of Butter - 25.0
Reichert-Meissel number of Crisco and 7 per cent Tributyrin - 34.0

*Samples 1 and 2 were combined.

The average of the determination upon the lymph fat from dogs whose experimental ration contained butter was 3.4. If the fat present in the thoracic duct was of the same composition as the food fat the Reichert-Meissel number would have been 25 to 30, the R.M. No. of butter. Likewise, in the case of the dog fed tributyrin, the Reichert-Meissel number would have been 34.0, the Reichert-Meissel number of a 7 per cent tributyrin and crisco mixture, if the extracted fat would have had the same composition as the fat fed. The average Reichert-Meissel number of the fat extracted from the thoracic lymph of dogs fed crisco alone was higher than that obtained from the food fat (the Reichert-Meissel number of Crisco is 0.4). This increase is probably due to the fat normally present in the lymph.

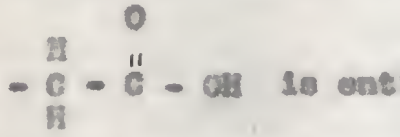
This shows conclusively, and only, that soluble volatile fatty acids are not present as glycerides in the fat contained in the lymph of the thoracic lymph duct.

DISCUSSION

A fatty acid is made up of two parts -- one a nonlipin carboxyl group and the other a lipin carbon chain. The solubility of the fatty acid is determined by the interrelation of these two factors. The lowest molecular weight

fatty acid to be present in the free form in the body is acetic acid which has the formula:

This acid is infinitely soluble in water because the carboxyl group is much more non-lipin in character than the single carbon is lipin. For a similar reason butyric acid

 is entirely miscible in water.

Caproic acid which is the next even chain acid in the series is soluble to the extent of 975 mg. in 100 cc. of H_2O at 20° C. Figure 1 shows the transition of the solubility from caproic acid to lauric acid which is insoluble in H_2O.

It is interesting to note that caprylic acid which is reported by Maude Powell to be deposited in small amounts in the adipose tissue has a low degree of solubility (70 mg. per 100 cc. H_2O) while capric acid which she reports to be the shortest chain fatty acid to be deposited unchanged is practically insoluble, having a solubility of 3 mg. per 100 cc. H_2O. This agreement substantiates the theory advanced by this thesis. Another substantiating fact for the theory that the soluble fatty acids are not

absorbed by the lymphatic vessels is that acetic acid, which as butyric acid is infinitely soluble in water, is known to be absorbed by the blood stream.

These two points in favor of the theory are further strengthened by the physical chemistry of absorption. The fats undergo a greater part of their digestion in the small intestines. A fat is an esterification product between the tri-hydroxy alcohol, glycerine, and fatty acids. To be digested the fats, after entering the intestines must be prepared so that the lipase can exert its effect. This is done by bile salts which reduce the interfacial tension between water and the fat particle. This permits emulsification, thus allowing the water soluble enzyme lipase to act upon the fats which are lipins. The resulting products of the action of lipase upon fats are glycerol and fatty acids. These two compounds then travel through the intestine wall. The long chain fatty acids, are next identified in the lacteals having been resynthesized into fats while the short chain fatty acids are next identified in the liver. Since fats are broken up and for a time are present as glycerol and fatty acids, it is probable that at this time the short chain fatty acids, being water soluble and in this respect similar to amino acids and monosaccharides, would be absorbed by the blood and would thus escape the

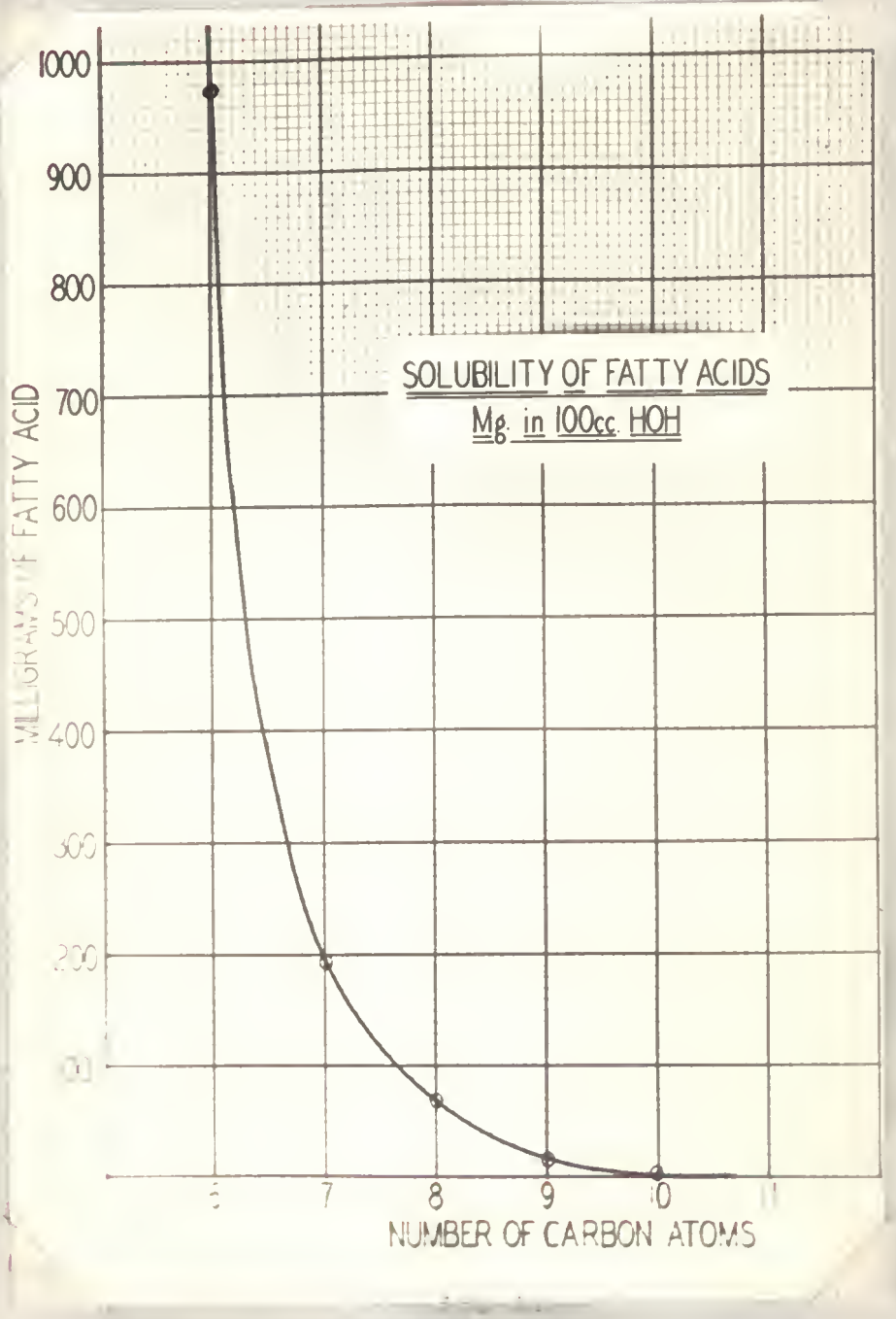

SOLUBILITY OF FATTY ACIDS
Mg. in 100cc. HOH
MILLIGRAMS OF FATTY ACID
1000
900
800
700
600
500
400
300
200
100
7
8
9
10
NUMBER OF CARBON ATOMS

Fig. 1.

glyceride synthesis. Further investigation should show the presence of the short chain fatty acids fed in the diet, in some form in the portal blood.

SUMMARY

The results of this experiment show that soluble volatile fatty acids when fed as a fat to dogs are not present in the thoracic lymph as glycerides.

REFERENCES

Davis, Russell E.
Metabolism of tributyrin.
Jour. Biol. Chem. 88:67-75. 1930.

Eckstein, Henry C.
The influence of diet upon the body fat of the white rat.
Jour. Biol. Chem. 91:613-628. 1929a.

Eckstein, Henry C.
The influence of the ingestion of tricaproin on the body fat of the white rat.
Jour. Biol. Chem. 84:353-357. 1929b.

Eckstein, Henry C.
The influence of the ingestion of butter fat on body fat of the white rat.
Proc. Soc. Exp. Biol. and Med. 27:419-421. 1930.

Gibbs, H. D. and Agnoli, A.
Lard from wild and domestic Philippine hogs and the changes in the constants produced by feeding Corpora cake.
Philippine Jour. Sci. 5:33-43. 1910. Sec. A.

Jenkins, John Clifford
The effect of fats fed in the feed on the fats deposited in the animal tissue.
Unpublished thesis, Kansas State College. 33 p. 1925.

König, J. and Schluckerbier, J.
Ueber den Einfluss des Futterfettes auf das Körperfett bei schweinen mit besonderer Berücksichtigung des verbleibs des Phytosterins. (Upon the influence of food fat upon the body fat in the case of swine with special emphasis upon point of deposition of the Phytosterins.)
Z. Untersuch. Nahrungs-u. Genussmit. 15:841-861. 1908.

Lebedeff, A.
Woraus Bildet sich das Fett in Fällen der akuten Fettbildung? Experimenteller Beitrag zur Kenntniss der Leber- und Milch-fette. (From what is the fat formed in cases of intensive fat formation? Experimental contribution toward the knowledge of the hepatic and milk fat.)
Arch. ges. Physiol. 31:11-59. 1883.

Leube, W. V.
Ueber subcutane Ernährung. (Subcutaneous Alimentation.)
Verhandl. Kong. Inn. Med. 13:418-432. 1895.

Millig, Adam
Die Bestimmung der Reichert-Meitzl mit kleinen Fettmengen. (The determination of the Reichert-Meissel number with small quantities of fats.)
Z. Untersuch. Lebensm. 60:318-320. 1930.

Official and tentative method of analysis of the association of official agricultural chemists. 3rd ed.
Washington, D. C. Assoc. Off. Ag. Chem. p. 322-323.

Powell, Maude
The metabolism of tricaprylin and trilaurin.
Jour. Biol. Chem. 89:547-552. 1930.

Powell, Maude
The metabolism of tricaprin.
Jour. Biol. Chem. 95:43-45. 1931.

Raper, H. S.
Experiments bearing on the functions of the liver in metabolism of fats I.
Jour. Biol. Chem. 14:117-134. 1913.

Zuntz, N.
Ueber die Herkunft der flüchtigen Fettsäuren in der Butter. (Concerning the origin of volatile fatty acids in butter.)
Arch. Physiol. 382-383. 1900.
(Quoted from Z. Untersuch. Nahrungs-u Genussmit. 4:126-127. 1901.)