AN IN VITRO STUDY OF THE HYDROLYSIS OF SEVERAL FATS AND OILS BY PANCREATIC LIPASE

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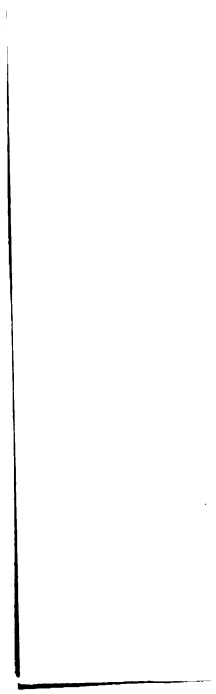

AN IN VITRO STUDY OF THE HYDROLYSIS OF SEVERAL FATS AND OILS BY PANCREATIC LIPASE

by

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A THESIS

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CONTENTS

HISTORICAL REVIEW

Probably the most obvious and seemingly the most important of the intestinal lipases is that supplied by the pancreas. No doubt it was for that reason that Eberle, in 1834, first observed the action of pancreatic lipase upon neutral fat. Notwithstanding, the first worker to make progress in the study of pancreatic lipase was Claude Bernard (1856), who found that pancreatic juice both emulsified and hydrolysed the fat. In those days the action of the pancreatic juice was observed mainly by means of fistulas, either natural or artificial, as in vitro studies had not yet been made.

In 1903 Pottevin reported that pancreatic lipase hydrolysed fats to fatty acid and glycerol. He was also able to show that the reaction was reversible. By using oleic acid homogenized with glycerol and mixing it with a glycerol extract of the pancreas he was able to demonstrate the esterification of about 33% of the oleic acid with glycerol in 50 hours at 38°C.

In 1914 Armstrong and Gosney found equilibrium was reached when 40% of the acid was combined, and in 1936 Artom and Reale reported over 50% esterification. However, most of the more recent studies on pancreatic lipase have been done in an effort to learn more about the hydrolytic action of the enzyme and the factors that favor or hinder that action.

Two of the factors that have received a considerable amount of attention are the optimum pH for lipase activity and the pH at which it is most stable to heat. McGillivray (1) reported the optimal pH to be 8.0, and the optimal thermal stability in the region of pH 6.0.

He also observed that an unpurified extract was more heat stable than a purified, which he explained was due to the protection the impurity gave to the enzyme. Platt and Dawson (2) placed the optimal pH in the region of 7.0 to 8.4, with the explanation that it depended upon the buffer used. Noyes, Sieguira, and Falk (3) more or less confirmed the findings of Platt and Dawson by placing the optimal pH at 7.0. Sabatka and Glick (4) in quite an extensive study on the subject found that the optimal pH lies between 6.7 and 8.2, and that the type and nature of the buffer, as well as the substrate and the enzyme preparation have much to do in determining the optimal pH. Bloor (5) placed the optimal temperature for the enzyme activity at about 40°C. He found that the activity was reduced by ten minutes heating at 45°C. and destroyed at 55°C.

Platt and Dawson (2) believed that the optimal pH may be looked upon as the outcome of two opposing tendencies; with an increase in alkalinity an increase in hydrolytic activity takes place, however, at the higher pH the stability of the enzyme decreases. Glick and King (6) were of the opinion there are at least two opposing tendencies operating when foreign substances are added to a solution containing enzyme and substrate. One tendency is to inhibit the enzyme, due to a combination of the foreign compound with the enzyme to produce an inactive complex; the other, is to effect an activation of the enzyme by making the enzyme and substrate molecule more accessible to each other. They cited as an example of the activation of an enzyme the lowering of the interfacial tension between the enzyme and substrate, an effect produced by bile salts. They maintained that in the case of pancreatic lipase there are very few

inactivating substances. In fact, it was learned that the best inhibiters for liver esterase are not only the best activators for pancreatic lipase but also lower the surface tension the most. In addition, they learned that activation occurs only in an alkaline medium. Consequently, as hydrolysis proceeded, the pH tended to fall as a result of accumulating fatty acids with the consequent slowing of the process. However, Versar and Kuthy (7) explained that bile salts offer an exception to the rule in that the pH does not go down as much as would be expected. They expressed the view that this is due to a tendency of the bile salts to combine with the fatty acids and hence to remove them, which would permit hydrolysis to go on. This view was substantiated by Weinstein and Wynne (8) in their studies on pancreatic lipase.

In 1907 Loeverhart and Peirce (9) advanced the idea that though the pancrease contains mostly lipase and the liver mostly esterase, it is possible that the pancreas also contains esterase and the liver also lipase. In fact, Walverkamp and Griffioen (10) in 1934, reported two types of enzymes in the pancreas which could be demonstrated as separate entities. They also suggested that there may be several additional enzymes in the pancreas each specific for a definite group of esters.

An antithesis of this idea was advanced by Platt and Dawson (2). They believed that the protein structure which has been ascribed to lipase is characteristic of the activating protein associated with the enzyme rather than that of the enzyme itself. They were able to alter the ester hydrolyzing property of the enzyme by changing the accompanying protein. They explained the apparent destruction of

lipase by trypsin as being due, in part, to the removal by hydrolysis of the protein which activates the lipase. The work of Falk (12) rather strongly supports the belief of Platt and Dawson, for Falk was able to show that a pancreatic extract having definite hydrolytic action on ethyl butyrate and olive oil had its action modified by the addition of albumin. The addition of this protein resulted in an increased rate of hydrolysis of olive oil. However, when edestin was added to the same system the action of the enzyme was increased for both ethyl butyrate and olive oil. He concluded that lipase, or ester-hydrolysing action, is due in all probability to a definite chemical grouping, and that this action can be altered by changing the accompanying protein.

Sabatka and Glick (13) observed that in the case of pancreatic lipase only a few percent of the total possible hydrolysis is effected when the reaction between the enzyme and substrate ceases. They found that it was not due to low affinity for enzyme to substrate; neither was it due to the products of hydrolysis, as fatty acids and glycerol, for addition of more enzyme did not produce any more action. This would exclude enzyme destruction; however, when an additional amount of substrate was added new action ensued, and they were able to repeat the phenomenon. They believed this to show that a great part of the substrate disappears from solution without being hydrolysed. Their explanation was that the substrate is bound to inactive areas of colloidal pancreas globulin particles in such a manner that it is not accessible to the active group of the enzyme. If this hypothesis is correct, the combination of large amounts of substrate with inactive areas on the lipase particle or with altogether

inactive globulin particles would eventually diminish the actual concentration of the substrate until action would cease. In contrast to this, they observed that the action between liver esterase and substrate goes on until at least 90% of the substrate has been hydrolysed which they think indicates that there is little, if any, inhibitory effect by cleavage products.

The findings of Murry (14) support those of Sabatka and Glick. However, Murry reasoned that if substances possessing a particular molecular structure, other than that of the natural substrate, are adsorbed at the surface of the enzyme but not activated they will compete for the active center with those which are activated, and hence bring about a reduction in the velocity of reaction of the natural substrate. He learned that a substance containing the carboxyl group competes with the substrate for the enzyme. He was of the opinion that it is by this group that the substrate is normally attacked, which makes the relative non-specificity of the enzyme more understandable.

The ability of lipase to hydrolyze fats has been more or less taken for granted. In fact, pancreatic extracts are known to hydrolyse all ordinary glycerides except possibly stearins. Ball and Matlock (15) believe that the stearins are no exception in principle, but merely are hydrolysed more slowly. They were able to show that tristearin was split so poorly by otherwise powerful preparations of pancreatic lipase that its digestion, when observed at all, was entirely out of line with what might be expected. However, they were able to carry out the hydrolysis of mono-di-or tristearine at a rate quite comparable to that found for other fats, provided high

temperature and good dispersion were maintained. They learned that pancreatic lipase hydrolysed all the triglycerides of normal saturated fatty acids, but that they were split at different rates. They found that the fats which are split most rapidly are the ones that have fatty acids occurring midway between acetic acid and stearic acid. Also, there was noted a surprising indifference on the part of the enzyme to the constitution of the alcohol as long as it was of the primary variety. However, the length of the carbon chain, the occurrence of free hydroxyl groups, the presence of a double bond, or of secondary or tertiary carbon atoms may all affect the rate of splitting but do not change the qualitative or quantitative results. The results obtained with secondary propyl stearate, secondary butyl stearate and tertiary butyl stearate were in marked contrast to those of the corresponding esters of n-propyl, n-butyl and n-amyl alcohol. In fact, they believed that the splitting of secondary ester linkage takes place at an almost negligible velocity in comparison with the splitting of a primary ester linkage.

Ball, Matlock and Tucker (16) found that the hydrolysis of the higher but not the lower saturated triglycerides is dependent on temperature and that at a moderate temperature the maximum rate of splitting is exhibited by glycerides containing the straight chain saturated acids of approximately 7 to 10 carbon atoms. However, they explained this does not apply to unsaturated glycerides, for olein behaves as if it contained a C_9 rather than a C_{18} acid.

The work of Ball and Matlock (17) elaborated on this point by showing that the rate of hydrolysis at 40°C. is independent of the substrate concentration over a wide range. However, only the esters

of lower or unsaturated fatty acids are hydrolyzed in the cold. In fact, tristearin undergoes almost no hydrolysis at 20°C., whereas benzyl stearate hydrolyzes at this temperature with fair rapidity. The butyrate continues to hydrolyze rapidly even when the system is frozen.

Ball, Matlock and Tucker (16) carried the hydrolysis of tristearin to practical completion. They showed that what remained after interrupting the hydrolysis of the substrate was almost entirely tristearin. They concluded therefore, that the gross chemical composition of the substrate remains practically unchanged throughout digestion.

Weinstein and Wynne (18) observed that the rate of liberation of acid from diacetin was exactly twice that from monoacetin. However, the rate of liberation of acid from triacetin was only 2.66 times that of monoacetin, suggesting that the middle ester linkage is less readily attacked than the two end linkages. They assumed that the two end linkages in triacetin, as in diacetin, are hydrolyzed at the same rate.

INTRODUCTION TO PROCEDURE

Since pancreatic lipase brings about the hydrolysis of fats into fatty acids and glycerol, and since bile salts are necessary to lower the surface tension and so aid in emulsification and in turn accelerate the action of lipase, a digest consisting of a fat, pancreatin and sodium glycocholate was prepared, and digestion carried out at 37°C.

The object was to study the effect of continued hydrolysis on various fats. Therefore, at intervals of progressively greater length a portion of the digest was removed and the undigested fat extracted with ether, weighed and characterised. Because of the general occurrence of unsaturated fat acids in natural fats and oils, the Iodine Number was also determined on the residual fat. The Reichert-Meissl Number, which gives a measure of the volatile soluble fat acids, was also determined in order to follow the hydrolysis of glycerides containing the short chain acids, particularly in the case of milk fat.

In the preliminary studies, determinations were also made of the free acids formed at various stages by titrating with standard alkali. These results, as well as those obtained in a study of the changes in melting point, proved to be of little value in revealing the course of lipolysis so that these determinations were discontinued.

PROCEDURE

The first digest consisted of 200 ml. of 30% milk fat, 2 grams of pancreatin (0.4%), 1 gram of sodium glycocholate (0.2%), and enough water to give a total volume of 500 ml. The control used for comparison consisted of 200 ml. of 30% milk fat and 300 ml. of water. Digestion was carried out at 37°C. At intervals of 1, 2, 4, and 24 hours, 5 ml. portions were transferred to erlenmeyer flasks, to each of which was added 10 ml. of ethyl alcohol and 5 drops of phenolphthalein. Titration was then carried out with 0.1 N. sodium hydroxide, giving a measure of the amount of fat acids liberated.

At the same intervals, portions of the digest and control were transferred to 500 ml. separatory funnels to which were added 5 ml. of 20% sodium hydroxide and 20 ml. of 95% ethyl alcohol. After shaking the contents thoroughly, the unhydrolyzed fat was then extracted with one 100 ml. portion of ether and subsequently with two additional 50 ml. portions of ether. The combined ether extract was then washed with water until free from alkali (phenolphthalein was used as the indicator). The alkali-free ether extract was dried by adding 20-30 grams of anhydrous sodium sulfate, slowly agitating for 15 minutes and then decanting the ether extract. The sodium sulfate was washed with 50 ml. of ether and the ether wash added to the main ether extract. The dry ether solution was then placed under reduced pressure to remove the ether, and leave the extracted fat. Both extracted fat samples were weighed and the Iodine Number and Reichert-Meissl Number determined.

The Hanus Method used to determine the Iodine Number was carried out as follows: about 0.5 grams of the fat was dissolved in 10 ml.

of chloroform and placed in an iodine flask. Then 25 ml. of Hanus iodine solution was added, the flask stoppered and allowed to stand for 30 minutes. The stopper was then removed and 20 ml. of potassium iodide solution (3.0 grams of potassium iodide) poured into it, followed with 100 ml. of water and titrated immediately with standardized sodium thiosulfate solution (approximately 0.1 N.). When the solution became pale yellow, 2 ml. of starch solution (1-200) was added and titration continued until the disappearance of the blue color. By substituting the amount of thiosulfate solution used minus the amount used for the blank, into the following formula, the Iodine Number can be calculated:

$$\frac{\text{ml. of thiosulfate} \times \text{normality of thiosulfate} \times \text{m.e. of } I_2 \times 100}{\text{weight of sample}} = \text{Iodine Number}$$

The procedure used for the Reichert-Meissl Number was as follows: to 5 grams of fat in a flask 2 ml. of potassium hydroxide (1-1) and 10 ml. of 95% alcohol were added. The contents were refluxed on a steam bath for 25 minutes, after which the alcohol was evaporated off. Then 10h ml. of recently boiled distilled water cooled to 50°C. was added to the flask and warmed until a clear solution of soap was obtained. The solution was cooled to 60°C., 8 ml. of sulfuric acid (1/4) added and refluxing continued until the fat acids had completely separated as an oily layer. Subsequently 110 ml. was distilled in as nearly 30 minutes as possible and the distillate filtered and 100 ml. of it titrated with 0.1 N. sodium hydroxide using phenolphthalein as the indicator. The Reichert-Meissl Number is calculated by using the following formula:

$$\frac{\text{ml. of 0.1 N. sodium hydroxide} \times 5 \times 11}{\text{weight of sample} \times 10} = \text{Reichert-Meissl Number}$$

The results of the first digestion may be found in Table I.

For the second series, four different digests were set up, each being the same as the first except that the amount of pancreatin used was respectively 0.4%, 0.8%, 1.6%, and 3.2%. Digestion, sampling and extraction of samples were carried out in the same manner as before. The Iodine Number and Reichert-Meissl Number were determined as previously outlined. In addition, the melting point of the extracted fat was determined as follows: a small sample of the fat was melted at as low a temperature as possible, and drawn up into several thin-walled capillary tubes about 3 cm. long. They were then placed in a refrigerator for not less than 12 hours. One of the tubes was attached to a thermometer in such a manner that the fat was as close as possible to the bulb of the thermometer. The thermometer was supported so that its bulb was immersed in water in a wide mouth test tube, which in turn rested in the neck of a round-bottomed flask also containing water. The water was heated gradually until the fat melted. The temperature at which the fat became transparent was taken as the final melting point of the fat.

The results of the second digestion may be found in Table II.

In a third series, four different digests were set up, each being the same as the first, except that the amount of sodium glycocholate used was respectively 0.2%, 0.4%, 0.8%, and 1.6%. Digestion, sampling, extraction and analysis were carried out in the same manner as before.

The results of the third digestion study may be found in Table III.

Since milk fat, a fat of animal origin, had been used for the digestions carried out so far, three fats of vegetable origin,

corn oil, olive oil, and crisco, a hydrogenated vegetable oil, were selected for further study. Butter fat being included in this group for comparison. In each case, the digestion was carried out in the presence of 0.8% pancreatin and 0.4% sodium glycocholate.

It was desirable to have the fats in a well dispersed state to facilitate digestion. This was accomplished by the following means: two grams of gum arabic and two grams of sodium glycocholate were added to 275 ml. of water and warmed. To this warm solution were added 2 ml. of oleic acid, 5 ml. of 0.1 N. sodium hydroxide and 60 grams of fat. This was well mixed and repeatedly passed through a Canfield emulsifier until a good emulsion was obtained. To the emulsion was added 4 grams of pancreatin with stirring and enough water to give a total volume of 500 ml. The emulsified fats were sampled and the residual fat extracted in the usual manner. The pH of the digest was obtained each time just before sampling. The Iodine Number and Reichert-Meissl Number were determined on each of the extracted fat residues.

The results of this digestion study may be found in Table IV.

It is apparent from the results shown in Table IV that there were fairly uniform conditions with respect to the pH of the digests except in the case of butter fat. The low pH of the butter fat was obviously due to the rapid release of soluble acids and could be expected to affect the rate of hydrolysis of butter fat. It would therefore be of interest to know how the digestion would be changed if the digests were held at a pH of 7.0.

Accordingly the same fats were used (corn oil, olive oil, crisco, and butter) altho it should be noted that they were of different origin than the ones used in the previous experiments.

The digests were set up in the same manner except that the pH was kept at 7.0 throughout the digestion. This was accomplished by regularly checking the pH of the digest with a pH meter and adding 1.0 N. sodium hydroxide as needed. It might be noted that very little alkali was required after the first two hours. Sampling and the extraction of samples were carried out in the usual manner and the Iodine Number and Reichert-Meissl Number determined as before.

The results of this portion of the study may be found in Table V.

DISCUSSION

From Table I it will be noted that during the first hour hydrolysis of the fat took place at a rapid rate, but as time increased the rate of hydrolysis decreased. It is also evident that as hydrolysis increased there was a concomitant increase in the amount of free fatty acid formed as indicated by titration with 0.1 N. sodium hydroxide.

There was a slight increase in the Iodine Number as digestion progressed. This indicates that the hydrolysis of unsaturated fatty acids did not keep apace with that of the saturated fatty acids. The relative high content of saturated acids of low molecular weight in butter fat would make such a result reasonable. Nevertheless, in subsequent studies both slight increases and decreases were observed.

At the end of the first hour of digestion the Reichert-Meissl Number of the fat sample was found to be much lower than in the control and continued to decrease progressively as digestion continued. It is apparent that the esters of the volatile fatty acids were more rapidly hydrolysed than those of the non-volatile acids—as found by Ball, Matlock, and Tucker (16).

The results summarized in Table II indicate that the amount of hydrolysis increased with increasing amounts of pancreatin.

Examination of the Iodine Numbers again seems to indicate that the proportion of unsaturated fatty acids in the residual fat remained fairly uniform throughout the 24 hour period regardless of the amount of pancreatin used.

The Reichert-Meissl Numbers show that the hydrolysis of the esters of the more volatile fatty acids increased most with increasing amounts of pancreatin during the first hour.

The variations although slight in the melting point of the residual fat at various stages indicates a consistent increase due to the preferential hydrolysis of the glycerides containing the short chain fatty acids. The increases are also consistent with the more rapid hydrolysis at the higher concentrations of pancreatin.

With increasing amounts of sodium glycocholate, the rate of hydrolysis was increased.

The Iodine Number values indicate that at the higher concentrations of sodium glycocholate there was increased liberation of unsaturated fatty acids. A similar result was observed in the case of the hydrolysis of the glycerides containing the lower saturated fatty acids.

From Table IV one can see that the fat least readily hydrolyzed under the conditions prevailing in this part of the study was butter. This can easily be explained on the basis of the rapid decrease in pH as soon as pancreatin was added to the digest. In the case of corn oil, olive oil, and crisco, the pH remained appreciably higher throughout the 24 hour digestion period. Consequently conditions were more favorable for the hydrolysis of these fats.

Only in the case of corn oil did the Iodine Number decrease as hydrolysis progressed. This would indicate a somewhat more rapid liberation of linoleic acid which is the most abundant fatty acid in this oil. Butter does not follow the pattern of either corn oil, olive oil, or crisco in that the Iodine Number seems to change but

little, if any, with continued hydrolysis. In contrast, the Iodine Number was found to increase throughout the digestion period in the case of olive oil and crisco. A more critical study of the digestion products would be necessary to explain these differences.

Although small changes were observed in the Reichert-Meissl Numbers of corn oil, olive oil, and crisco, the values are perhaps too small to have real significance.

The data in Table V shows clearly that maintaining a pH of 7.0 during the digestion affected the hydrolysis of corn oil, olive oil, and crisco but slightly during the 24 hour period, but markedly influenced hydrolysis of butter fat. It is of interest to note that butter fat showed the greatest amount of digestion, followed by crisco, olive oil, and corn oil.

SUMMARY

A study of the course of fat digestion was carried out in which residual fat was extracted and partially characterized at various stages of the digestion period. The following conclusions may be made:

1. With increased amounts of pancreatin, both the rate and extent of hydrolysis were increased during a 24 hour period.

2. A similar effect although of smaller magnitude was noted when the concentration of sodium glycocholate was increased.

3. In a comparative digestion study with uncontrolled pH the following order was established with respect to the maximum rate and extent of hydrolysis: crisco, olive oil, corn oil, and butter fat.

4. When a pH of 7.0 was maintained throughout the digestion period, butter fat was found to head the list followed by crisco, olive oil, and corn oil.

5. The Iodine Numbers show in the case of milk fat that with increased amounts of sodium glycocholate the rate of hydrolysis of the unsaturated fatty acids was increased, but little change was noted when the amount of Pancreatin was increased.

6. The Reichert-Meissl Numbers show in the case of milk fat that the hydrolysis of the esters of the more volatile fatty acids increased with increasing amounts of pancreatin as well as sodium glycocholate.

7. There was a noticeable drop in the Iodine Number as corn oil is hydrolysed which would indicate an increased rate in the liberation of linoleic acid.

RESULTS OF FIRST DIGESTION

TABLE I

CONTROL (No Pancreatin or Sodium Glycocholate)

TIME IN HOURS	0	1	2	4	24
WT.	12.00	11.98	11.97	11.95	11.93
NaOH USED	0.50	0.49	0.50	0.49	0.52
I. N.	38.10	38.00	38.20	38.30	38.60
R. M.	26.00	25.90	26.30	26.00	25.60

DIGEST

TIME IN HOURS	0	1	2	4	24
WT.	12.00	9.94	9.54	6.74	6.53
NaOH USED	0.50	5.40	9.90	12.70	14.40
I. N.	38.10	38.70	39.50	40.10	40.90
R. M.	26.00	16.65	14.50	14.05	11.80

RESULTS OF DIGESTION BY INCREASING THE PERCENT OF PANCREATIN

TABLE II

CONC. OF PANCREATIN (0.4%)

TIME IN HOURS	0	1	2	4	24
WT.	12.000	9.164	8.377	7.963	7.566
M. P.	32.00	32.0	34.0	35.0	36.0
I. N.	38.10	39.60	40.10	41.40	41.80
R. M.	26.00	17.80	9.80	9.10	8.50

CONC. OF PANCREATIN (0.8%)

TIME IN HOURS	0	1	2	4	24
WT.	12.000	8.970	8.021	6.225	6.530
M. P.	32.00	32.0	35.00	36.0	36.00
I. N.	38.10	39.30	39.30	40.50	40.20
R. M.	26.00	16.50	9.00	8.46	8.00

CONC. OF PANCREATIN (1.6%)

TIME IN HOURS	0	1	2	4	24
WT.	12.000	7.298	7.123	5.330	5.014
M. P.	32.00	34.0	35.0	36.0	36.0
I. N.	38.10	39.10	39.40	40.90	39.10
R. M.	26.00	13.40	7.22	8.02	7.22

CONC. OF PANCREATIN (3.2%)

TIME IN HOURS	0	1	2	4	24
WT.	12.000	7.035	6.053	4.293	3.813
M. P.	32.00	34.0	35.0	36.0	36.0
I. N.	38.10	39.00	39.00	39.40	38.50
R. M.	26.00	13.00	8.55	8.35	7.02

RESULTS OF DIGESTION BY INCREASING THE PERCENT OF SODIUM GLYCOCHOLATE

TABLE III

CONC. OF SODIUM GLYCOCHOLATE (0.2%)

TIME IN HOURS	0	1	2	4	24
WT.	12.000	9.824	9.464	6.550	6.420
I. N.	38.10	43.60	43.40	43.10	42.50
R. M.	26.00	19.00	15.75	11.60	11.30

CONC. OF SODIUM GLYCOCHOLATE (0.4%)

TIME IN HOURS	0	1	2	4	24
WT.	12.000	8.272	8.103	6.544	6.081
I. N.	38.10	41.60	41.40	39.10	38.60
R. M.	26.00	16.75	13.40	9.35	9.00

CONC. OF SODIUM GLYCOCHOLATE (0.8%)

TIME IN HOURS	0	1	2	4	24
WT.	12.000	7.806	7.444	5.849	5.803
I. N.	38.10	41.20	40.60	37.80	37.20
R. M.	26.00	14.15	12.60	9.00	8.85

CONC. OF SODIUM GLYCOCHOLATE (1.6%)

TIME IN HOURS	0	1	2	4	24
WT.	12.000	7.168	6.244	5.490	5.231
I. N.	38.10	39.90	40.50	37.30	37.00
R. M.	26.00	13.00	12.00	8.20	8.20

DIGESTION RESULTS OF A FEW FATS

TABLE IV

CORN OIL

TIME IN HOURS	0	1	4	24
WT.	12.000	7.874	5.030	4.176
I. N.	-	126.50	126.50	118.10
R. M.	-	0.94	0.95	1.49
pH of Digest	5.8	5.8	6.0	6.0

OLIVE OIL

TIME IN HOURS	0	1	4	24
WT.	12.000	7.037	5.509	4.008
I. N.	-	80.01	86.50	87.50
R. M.	-	0.94	1.09	1.73
pH of Digest	5.8	5.8	5.7	5.7

CRISCO

TIME IN HOURS	0	1	4	24
WT.	12.000	5.646	4.582	3.630
I. N.	-	70.02	76.20	78.10
R. M.	-	1.25	0.95	0.76
pH of Digest	6.0	5.9	5.7	5.7

BUTTER

TIME IN HOURS	0	1	4	24
WT.	12.000	7.977	7.596	6.608
I. N.	-	37.50	38.00	38.90
R. M.	-	19.60	18.50	12.90
pH of Digest	4.6	4.4	4.4	4.4

DIGESTION RESULTS OF A FEW FATS
WITH pH MAINTAINED AT 7.0

TABLE V

CORN OIL

TIME IN HOURS	0	1	4	24
WT.	12.000	7.243	4.982	4.123
I. N.	128.00	126.00	125.00	116.50
R. M.	0.94	0.96	0.97	1.65

OLIVE OIL

TIME IN HOURS	0	1	4	24
WT.	12.000	6.646	4.213	3.874
I. N.	75.00	81.80	87.50	89.80
R. M.	0.71	0.95	1.58	1.92

CRISCO

TIME IN HOURS	0	1	4	24
WT.	12.000	5.201	4.192	3.472
I. N.	78.78	84.02	88.21	90.34
R. M.	1.02	0.92	0.84	0.72

BUTTER

TIME IN HOURS	0	1	4	24
WT.	12.000	4.168	3.291	2.134
I. N.	35.70	38.10	38.90	39.50
R. M.	26.80	18.40	16.80	11.20

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