physical activity under pleasant hospital circumstances, and to our continual efforts to educate them about their disease. Patients who have gained the most reassurance have unquestionably benefitted the most. Because so many non-specific factors have profoundly affected our patients' clinical status, it is impossible at this time to attribute to our dietary management any specific therapeutic merit. In fact, we consider hazardous any short-term evaluation of a therapeutic regimen in patients with coronary-artery disease. Until it becomes possible to assess the progress of the atheromatous process during life, it will be exceedingly difficult to determine whether a therapeutic measure is effective. It has been gratifying to observe the disappearance of skin xanthomata in patients with hyperlipæmia or hypercholesteræmia, but we have seen no changes in tendon xanthomata despite marked decreases in serum-cholesterol levels as long as two years. It is an open question whether atheromata respond to this regimen as do skin xanthomata, or fail to be appreciably influenced like tendon xanthomata.

It is our present conclusion that recommendations for radical changes in food habits, even by those populations most seriously threatened by atherosclerosis, should await a clearer definition of the specific food factors which control serum-lipide levels. It is entirely possible that an understanding of the mechanisms evoked by these factors will lead to practical measures for control of serum-lipide concentrations. Only then can large-scale epidemiological experiments be planned to determine whether the incidence of atherosclerosis and its complications in the human species will be affected by decreasing the levels of lipides in the serum.

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Progress in the Metabolism of Lipides[1]

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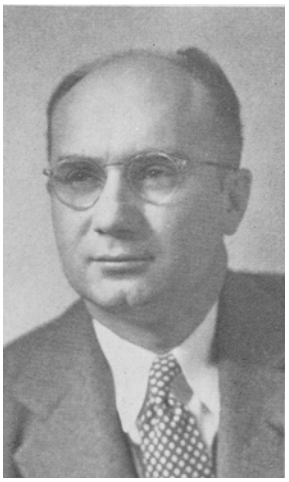
NOT TOO MANY years ago a review of recent advances in fat metabolism would have presented no particular problem. Today the task is a difficult one. Between 75 and 100 papers on fat metabolism were presented at the Federation of American Societies for Experimental Biology held in Chicago during the week of April 15, 1957. The spectrum of the subject matter of these papers was very wide, yet almost all carried very important implications.



Raymond Reiser

Being faced with this dilemma, I have decided to limit myself to about three areas in which I have been personally interested and which will not duplicate what the others on this program will discuss.

Digestion and Absorption

Until very recently it was assumed that a glyceride was either completely hydrolyzed during digestion or it was not hydrolyzed at all. However there have been some revolutionary observations in the study of fat digestion in recent years. These new concepts were anticipated as early as 1935 by Artom and Reale (1), who found that *in vitro* pancreatic fat digestion produced no free glycerol but only fatty acids and mono- and diglycerides. This was confirmed independently 10 years later by Frazer and Sammons (2). In the years between 1945 and 1950 Desnuelle and others (3, 4, 5), in a series of studies, reported that the action of pancreatic lipase on fat produced diglycerides readily and monoglycerides only in the presence of calcium ions, also that the production of free glycerol required calcium ions, bile salts, and a large excess of water.

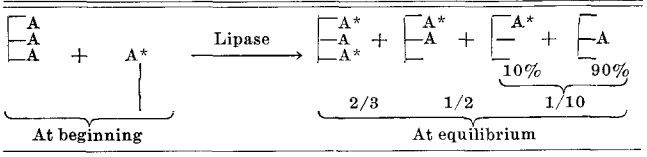
These three groups of authors, in about four or five papers, demonstrated that concepts of all-or-none hypotheses of fat digestion are untenable and that the end product of fat digestion is a partial glyceride; the truth, as usual, lies between the two extremes.

These observations brought up the questions as to which of the three positions of the glyceride molecule are the most resistant and the most susceptible to lipase action and as to whether there is any difference in specificity toward saturated or unsaturated or long- or short-chain acids.

[1] Presented at the Symposium on Fats in Nutrition and Health, Animal meeting, American Oil Chemists' Society, New Orleans, La., April 30, 1957.

FIGURE 1

Action of Lipase on a Mixture of Triglyceride and Free Fatty Acid (6)

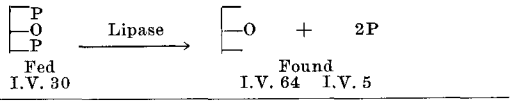


E = the glycerol moiety
A = fatty acid moiety

Borgström (6, 7) made a start on the answer to those questions in 1952 and 1954. He treated a mixture of unlabelled triglyceride and C^{14} labelled fatty acids with pancreatic lipase and, after equilibrium, separated the free fatty acids and the mono-, di-, and triglycerides chromatographically. He found that the triglycerides had only two-thirds of the activity of the original free fatty acid, the diglyceryde one-half, and the monoglyceride one-tenth. He suggested that this could be explained by the mechanism shown in Figure 1, that is, only the fatty acids in the 1 and 3 positions were exchanged and the fatty acid in the 2 position was insensitive to the enzyme.

FIGURE 2

Action of Lipase on 1.3 Dipalmitoyl Olein (8)



P = Palmitoyl, O = Oleoyl, E = glyceryl.

Mattson *et al.* (8) in 1952 went a step farther to demonstrate convincingly that lipase in fact attacks the 1 and 3 or primary alcohol linkages of the glycerol molecule but is relatively inert toward the 2 position. These authors fed a synthetic 1,3 dipalmitoyl, 2 olein and found that the monoglycerides of the intestinal contents had an iodine number of 64 and the free fatty acids an iodine number of only 5. The results of this study are presented graphically in Figure 2.

Finally, within the last year, Mattson and Beck (9) and Savary and Desnuelle (10) have proven beyond equivocation that not only are the 1 and 3 positions the only labile ones but also that there is no specificity with respect to unsaturation and chain length.

Table I gives the iodine values of the di- and monoglycerides and fatty acids which result from the action of lipase on 1,3 dipalmitoyl, 2 olein, and on 1,3 distearoyl, 2 olein and shows that the enzyme does not distinguish between palmitic and stearic acids. Comparison of the products of lipase action on 1,3 dioleoyl, 2 palmitin with the other two, demonstrates that the enzyme also does not distinguish between palmitic or stearic and other acids.[2]

Savary and Desnuelle (10), simultaneously with

TABLE I

Action of Lipase on Triglycerides of Known Structure (9)

Glyceride	Iodine value			
	Acids	Glycerides		
		Mono-	Di-	Tri-
POP	8	61	44	29
SOS	10	56	44	27
OPO	85	2	43	56
OPP	39	8	22	31

POP = 1,3 dipalmitoyl olein
SOS = 1,3 distearoyl, 2 olein
OPO = 1,3 dioleoyl,2 palmitin
OPP = 2,3 dipalmitoyl, 1 olein

TABLE II

Action of Lipase on Triglycerides of Known Structure (10)

Glyceride	Percentage of Oleic Acid in Hydrolysis Products			
	Acids	Glycerides		
		Mono-	Di-	Tri-
POP	5	88	47	32
OPP	32	4	19	35
POO	55	81	75	64

POP = 1,3 dipalmitoyl olein. OPP = 2,3 dipalmitoyl, 1 olein.
POO = 2,3 dioleoyl, 1 palmitin.

Mattson and Beck (9) but using a slightly different approach, also demonstrated that the 1 and 3 positions are the only labile ones and that there is no distinction made between oleic and palmitic acids. From the data presented in this table it may be seen that the percentages of oleic acid in the free fatty acids and in the mono-, di-, and triglycerides conform well with the theoretical values: from OPP the diglycerides would be OP and PP and thus contain 25% oleic acid; from POO the diglycerides would be PO and OO and thus contain 75% oleic acid. These values of course, would be true only if there were no specificity (or distinction) between oleic and palmitic acids, which appears to be the case.

Savary and Desnuelle (10) found that about 75% of the monoglycerides formed were 2-monoglycerides. The 25% which were 1-mono were, in their opinion, caused by direct action of the lipase on the 2 position rather than by migration of the acid from the 2 position. It has been Mattson and Beck's (9) opinion that migration occurs.

The question of which species of glycerides is absorbed is still a matter of controversy. As far back as 1952 we published results of studies in which we fed to rats triglycerides labelled in both the glycerol and fatty acid moieties (11). The lymph fat contained about 60% of the labelled glycerol as well as the labelled fatty acid. Since we were able to demonstrate that any completely hydrolyzed glycerol is not utilized in triglyceride resynthesis, this meant that glycerides were absorbed.

By means of another group of studies based on the assumption that an insignificant amount of reesterification or interesterification takes place in the intestinal lumen during digestion, we concluded that it is the monoglycerides which are absorbed. This assumption has been challenged by Borgström (12).

Borgström (12) has demonstrated that, after feeding olive oil with labelled palmitic acid, the labelled acid was incorporated into glycerides in the lumen of the intestine. Typical data (13) which were used to determine that a significant degree of re- and interesterification takes place during digestion are given in Table III.

The data represent the average of four experiments with humans. About 45 g. of corn oil mixed with about 3.5 to 8% free palmitic or oleic acid 1-C^{13} were given in a test meal, and the intestinal contents were aspirated 2 to 4 hours afterward.

The authors reason that, since the relative specific activity of the triglycerides was 34% of that of the total fatty acids of the intestinal contents (including that in the triglycerides themselves), 34% of all the

[2] Since this paper was written, a report by P. Savary, J. Flanzy, and P. Desnuelle has appeared in the Biochim. et Biophys. Acta, *24*, 414 (1957), in which this specificity of pancreatic lipase was used to determine the glyceride structure of a number of natural fats. In the pig unsaturated acids were found predominantly in the 1 and 3 positions. In all other fats, animal or vegetable, the unsaturated acids were predominantly in the 2 position.

TABLE III

Humans Fed Various Amounts of Palmitic Acid 1-C^{13} With Corn Oil and the Amount and Relative Activity of Intestinal Contents Examined (13)

	Intestinal Lipides[a]					Fatty acid fed	
	TFA[b]	FFA[b]	MG[b]	DG[b]	TG[b]	Total	Free
Percentage	97.4	46.3	13.3	15.5	22.3	100	6.05
Rel. Sp. Act.	100	183	19	77	34	173	3118

[a] Average values of four experiments.
[b] MG, DG, TG = mono-, di- and triglycerides. TFA = total fatty acids. FFA = free fatty acids.

ingested corn oil fatty acids was exchanged during digestion. By the same reasoning they would say that 77% of the diglyceride and 19% of the monoglyceride bonds were exchanged.

This reasoning is faulty. The relative specific activity of the total fatty acids of the intestinal contents may be used as a measure of the percentage of ester groups exchanged only if there is complete mixing of all the fatty acids. In that case, however, the mono-, di-, and triglycerides and the free fatty acids would all have the same activity, and in that case there would be a 100% ester exchange. Furthermore this would relate only to the glycerides in the lumen at the time and not to the triglycerides ingested. Similar calculations can be made from all experiments of this type (6, 12, 13,14). It is thus obvious that Borgström's experiments (6, 12, 13, 14) do not measure the degree of interesterification in the lumen. Until it is demonstrated that a significant degree of re- or interesterification does occur in the lumen from other considerations such as rapid absorption of monoglycerides and free fatty acids, slow emptying time of the stomach, and the slow reaction time of lipase in the synthesis of triglycerides, we are justified in concluding that very little can take place there.

Our earlier studies were so designed that relatively small amounts of triglyceride could have been absorbed without detection (11). In order to determine if any triglycerides were absorbed, 200 mg. of a mixture of 10% tripalmitin, labelled in both the glycerol and fatty acids, and 90% triunsaturated triglyceride were fed to 200-g. rats (15). If such a mixture were completely hydrolyzed to monoglycerides and free fatty acid and were randomly resynthesized, the resultant fat would contain only 0.1% tripalmitin. Any tripalmitin above 0.1% would be due to lack of hydrolysis.

Table IV contains the data of this study. A total of 84,215 cts./min. of labelled glycerol was ingested in each of two trials. By collecting the lymph for 9 hrs. and extracting and counting the activity of the fatty acids of the lymph trisaturated glycerides, it was found that 75 and 59% of the ingested acid appeared there. If all the labelled palmitic acid of the lymph had appeared as the unhydrolyzed doubly labelled tripalmitin fed, the glycerol of that tripalmitin would have had a total of 63,161 and 49,478 cts./min. in the two trials, respectively. But the actual counts found were only 3.3 and 3.2% of the expected. Therefore not more than 3.3 and 3.2% of the ingested tripalmitin could have been absorbed with no hydrolysis.

TABLE IV

The Absorption of Unhydrolyzed Tripalmitin (15)

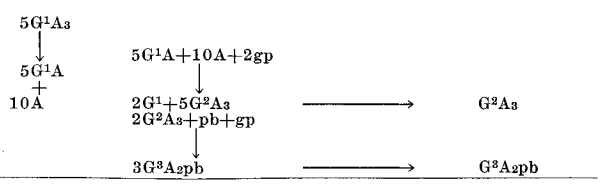
Period	Ingested tripalmitin glycerol	Ingested palmitic acid absorbed	Lymph tripalmitin glycerol		Absorbed unhydrolyzed tripalmitin
			(a)	Found	
	cts./min.[b]	%	*cts./min.*	*cts./min.*	%
1	84,215	75	63,161	2,100	3.3
2	84,215	59	49,478	1,566	3.2

[a] Theoretical if all the absorbed acids had been unhydrolyzed.
[b] Mg. x cts./min./mg.

As the result of our studies (11, 15, 16,17) and of the recent work of others, we have constructed the following scheme for the mechanism of fat absorption:

FIGURE 3

Mechanism of Fat Digestion and Absorption



G^1, G^2, and G^3 have relative specific activities of 100, 60, and 40, respectively.
gp = glycerol precursor.
pb = phosphorylated base.

Triglycerides are transformed in the intestine to monoglycerides and fatty acids. These are absorbed into the mucosa, where two molecules of glycerol precursor compete successfully with five molecules of monoglycerides, resulting in a loss of two molecules of the original glycerol. Most of the newly synthesized triglyceride passes to the lymph. Some however combines in the ratio of 2:1:1 with a phosphoryl base, such as choline phosphate, and glycerol precursor to produce three molecules of phospholipide, which passes to the lymph.

There are a number of practical factors which must be considered when one discusses the degree of digestibility of a fat but are usually neglected. Thus in discussing the digestibility of stearic acid, it is necessary to take into consideration the chemical form in which it is ingested.

TABLE V

Digestibility of Different Glycerides of Stearic Acid (18)

Lipide	Digestibility (%)
Stearic acid	18–27
Stearin	28–42
Monostearin	74–84
Monooleoyl-distearin	70–74

This table presents the data of Scribante (18), a student of Favarger who found that under the same conditions stearic acid is 18–27% absorbed, tristearin 29–42%, monostearin 74–84%, and mono-oleoyl- distearin 70–74%.

Since melting point is an important factor in the digestibility of a fat, its digestibility will vary with its crystalline form. Thus tripalmitin exists in three crystalline forms. The α form melts at 44°, the β' form at 56°, and the β form at 66°. We have fed a tripalmitin which appeared to be mostly β' form to swine at the 20% level and obtained only 20% absorption.

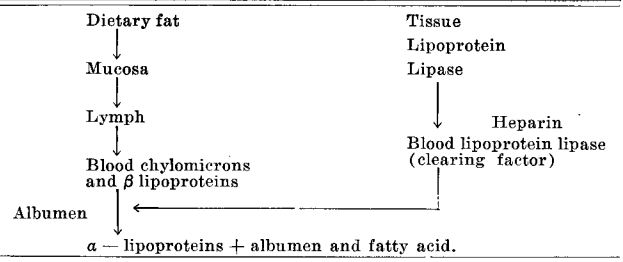
Clearing Factor

After fat leaves the mucosa by whatever mechanism it is absorbed and resynthesized, it makes its appearance in the mesenteric lymph vessels, by which it is carried to the cysterna chyla and thence to the blood at the junction of the subclavian and jugular veins

in the neck. There has been much speculation concerning the function of this roundabout method for absorbed fat reaching the blood and by-passing the liver. One older theory is that newly absorbed fat appears to be hemolytic, and this roundabout transport is to dilute it before adding it to the blood. In carnivorous animals, including man, fat makes its appearance in the blood as small droplets known as chylomicrons, and much work has been done on fat absorption by studying the degree and extent of the lactescence these chylomicrons give to blood serum or plasma, or by actually plotting the chylomicron count. The peak of the chylomicron count may be reached in 3 to 10 hrs., depending on the size of the fat meal, the other constituents of the meal, and the individual variation between animals.

FIGURE 4

Mode of Action of Clearing Factor



The mechanism of the removal of the chylomicrons has never been settled. It has been thought that the reticuloendothelium probably plays a major role and that the chylomicrons become solubilized by being converted to lipoproteins. It has been repeatedly shown that a clear serum may have as much fat as a milky serum. It is quite possible that much of the fat is converted to lipoproteins and solubilized as it passes through the intestinal mucosa.

Although chylomicrons are visible in the dark field microscope, recent work indicates that they are also lipoproteins belonging to a group known as low-density β-lipoproteins. These low-density β-lipoproteins have been implicated as a "causative factor of atherosclerosis," and the mechanism of their removal from blood has received considerable attention.

In 1943 Hahn (19) observed that when heparin is injected into an animal, there is a rapid clearing of alimentary lipemia. This did not attract much attention at first, but between 1952 and the present there has been a constantly increasing interest in the subject because of its implications in atherosclerosis.

In summary, the work of the past three years has resulted in the following concept of this so-called clearing factor or lipoprotein lipase. Ingested fat appears in the blood in the form of various-sized lipoproteins. The fat attached to the larger and lighter-weight ones, the β-lipoproteins, and the chylomicrons are acted on by lipoprotein lipase, which originates in the tissues and is excreted into the blood under the stimulus of heparin or a heparin-like substance. In the presence of albumen, which acts as a fatty acid acceptor, the triglycerides are hydrolyzed and are transported as the albumen fatty acid compound.

Workers in this field show significant differences between as little as .0025 meq. of acid and attribute this to fatty acid. As little as 0.01 mg. of a nonfatty organic acid of molecular weight of 100 present in the extracts would be equivalent to this difference. In other trials the "glycerol" released by periodate oxidation was measured. Many compounds are oxidized by periodate, and it is quite possible that traces of the products of carbohydrate or fat metabolism in blood could result in the values found.

FIGURE 5

Synthesis of Triglycerides (20)

$$\text{Phosphatidic acid} \xrightarrow[\text{mitochrondria}]{\text{rat liver}} \text{d,}\beta\text{ diglyceride} + H_3PO_4$$

$$\text{D,}\alpha\text{,}\beta\text{ diglyceride} + \text{cytidine diphosphate choline} \xrightarrow[\text{of chicken liver}]{\text{Mitochrondria}} \text{lecithin}$$

$$\text{D,}\alpha\text{,}\beta\text{ diglyceride} + \text{palmitoyl CoA} \xrightarrow[\text{mitochrondria}]{\text{chicken liver}} \text{triglycerides} + \text{CoASH}$$

Even if we assume that 1 meq./L of unesterified fatty acid does appear in the blood, this is a very small amount (about 28 mg./100 ml.) and, unless it is turned over extremely fast, cannot account for all the fat transported from adipose tissue for metabolic purposes or the removal from the blood of all dietary fat. This may be possible, but it is unlikely and should be demonstrable by labelling techniques.

Another factor which makes the concept that lipoprotein lipase is the major mechanism for solubilizing chylomicrons unlikely is the fact that lipoprotein lipase acts only on lipides which are already combined with protein. It thus gets into the picture late as a clearing mechanism since the formation of lipoproteins is itself the best clearing mechanism.

The whole subject of blood clearing as a factor in atherosclerosis is itself on rather uncertain ground since the rat and the dog, which have pronounced alimentary lipemia, are resistant to atherosclerosis while the rabbit and pig, which are susceptible to atherosclerosis, are resistant to lipemia.

Resynthesis

A subject that has intrigued biochemists interested in fat metabolism is the synthesis of triglycerides, either after the absorption of fatty acids or from nonfat precursors. We are still very much in the dark about this matter. It is clear that free glycerol is not the precursor of glyceride glycerol, at least for the resynthesis of triglycerides from absorbed fatty acids. However Weiss and Kennedy (20) have recently shown that the same system which synthesizes lecithin from D,α,β diglycerides, and cytidine diphosphate choline will also synthesize triglycerides. from D,α,β diglycerides and palmitoyl CoA.

This interesting observation revives the concept that phospholipides are intermediate in triglyceride synthesis since the diglyceride was obtained from phosphatidic acid.

This still leaves the synthesis of the diglycerides unknown, and certainly it is the synthesis of the mono-, then the diglyceride, that is the real problem. This work of Weiss and Kennedy, interesting as it is, still leaves the root of the problem to be solved.

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Newer Concepts of the Role of Essential Fatty Acids[1]

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THE RECENT INTEREST in the possible role of unsaturated fatty acids in the regulation of serum cholesterol levels has stimulated a great deal of research to determine whether a relationship between essential fatty acids and cholesterol metabolism exists and, if so, to understand what this relationship is.

Roslyn Alfin-Slater

The connection between essential fatty acids and cholesterol is not a new idea but was suggested as early as 1923, when Bloor (6) in his studies of unsaturated fatty acids in the plasma of various species of animals found that these unsaturated fatty acids existed mainly in combination with cholesterol. Bloor (7) and other workers (10, 20) extended these observations and found that the unsaturated fatty acid ester—cholesterol linoleate—was the chief ester of cholesterol in the plasma. Linoleic acid cannot be synthesized by the animal body, but it is necessary for growth and maintenance of normal body processes and is therefore called "essential." Three fatty acids are classified in this way: linoleic acid, linolenic acid, and arachidonic acid.

The functions of essential fatty acids are not as yet completely known. Essential fatty acids are necessary for growth (15), reproduction, and lactation (16, 17, 18, 25) in the rat. The lack of essential fatty acids in the diets of rats causes them to be more susceptible to X-irradiation injury (11, 14). The absence of essential fatty acids from the diet also produces a deficiency syndrome characterized by capillary fragility (21), increased skin permeability (26), a typical eye condition, scaliness of the paws and tail (9), alopecia, and a plateau in weight. Leveling off in weight and growth is caused by reduction in the number of bone proliferating cells (5). As an example, normally in the proximal head of the tibia (Figure 1) there is a wide section composed of columnar cells in which cell division occurs. This cell division is responsible for the growth of the bone. In the fat-free animals this area is markedly reduced. In addition, in the fat-free animal at the diaphyseal border there is a thin layer of bone sealing off the epiphyseal plate, and in the diaphysis there is a loss of bone cells, which is replaced by fat globules.

In 1953 a further result of EFA deficiency was reported from this laboratory (1). Male rats had been placed on a diet of 16–20 weeks, adequate in all respects but deficient in fat and therefore deficient in essential fatty acids.[2] The rationale was that the rats were able to synthesize any fat they require, with the exception of essential fatty acids, from the two-carbon fragment which is formed as a result of carbohydrate and protein metabolism. On autopsy these rats were found to have abnormal deposits of cholesterol in certain tissues of the body (Table I), increased amounts of cholesterol in liver and adrenal, and slightly decreased amounts in the plasma. The liver was fatty in appearance. Histological sections of the liver confirmed the analytical results and showed abnormal deposits of fat and a depletion of glycogen. Sections of the adrenal also showed increased deposition of fat, but a decrease in the area of the cortex (5).

The most striking effect of EFA deficiency was noticed in the gonadal tissue. Degeneration of spermatic development was a common alteration observed in EFA-deficient animals. In the epididymis the lumens of the ducts are filled with mature sperms in the control animals, but there is almost a complete loss of sperms in the lumen of the ducts of the EFA-deficient animals. In the testes themselves a depletion of EFA produces a degeneration of the tubules and a loss of maturation of the primary spermatogonial cells (5).

[1] Contribution No. 438 from the Harry J. Deuel Jr. laboratory.

[2] The fat-free diet consisted of 20.0% casein, 70.7% sucrose, 4.0% salt mix, 4.0% cellulose and fat-soluble and water-soluble vitamins in adequate amounts. When fat was added to the diet, it was done at the expense of carbohydrate.

TABLE I

The Effect of a Diet Deficient in Fat on Cholesterol Levels in Various Organs of the Rat

Diet	Exp. No.	No. of rats	Mg. cholesterol/g.		Mg. cholesterol per 100 ml.
			Liver	Adrenal	Plasma
12.5% fat	1	10	2.04	35.4	65.6
	2	9	2.08	35.3	63.2
Fat-free (Vitamin-test casein)	3	8	3.15	48.9	38.4
	4	7	4.06	50.4	50.4
Fat-free (Commercial casein)	5	10	4.72	49.3	41.1
	6	9	4.24	46.2	44.9