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THE ABSORBABILITY OF NATURAL AND MODIFIED FATS^a

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Most natural fats have been shown to be readily digested, but in the case of naturally-occurring fats of high melting point and of fats hardened by hydrogenation, the coefficient of digestibility may be reduced. Two major theories have been propounded to explain differences in digestibility: (1) that digestibility is inversely proportional to and dependent upon melting point (3, 9); or (2) that digestibility is limited by the amount of saturated fatty acids of carbon chains 18 or longer (8, 10). Other recent findings deserve consideration: Cheng, Morehouse, and Deuel (2) reported that monostearin was more digestible than either the triglyceride or the free fatty acid and Mattson, Baur, and Beck (12), that long-chain saturated fatty acids were better utilized as diacetin fat than as triglyceride. These results indicate that not only is the chain length of the fatty acids important but also their association within the glyceride structure.

To provide basic information for the development of fats which will meet the Armed Forces' stability requirement of 160° F. these various factors were explored. Preliminary studies of lipid materials formed by interesterification with hexahydric alcohols were also conducted.

METHODS AND MATERIALS

Diets varying only in source of fat were fed to adult male albino rats, 5 to 7 per group, for 15 days and feces collected on the last 5 days for digestibility measurement. In each test a reference cottonseed-oil diet and a fat-free diet were fed. The amount of food offered was constant for all groups in each test and sufficient to permit small weight gains with the reference diet; in most cases consumption was complete.

The diets were composed of 80 parts of a diet base, as shown in Table 1, and 20 parts of test fat. Fats were dissolved in warm ethanol and mixed with the diet base; the diets were then placed on shallow trays and held at room temperature until the alcohol evaporated. This procedure was followed to insure intimate mixing of fat with other diet components. The fat-free diet was treated in the same manner with 80 parts of base added to 30.7 parts of sucrose to yield a diet iso-nitrogenous when equal calories were fed.

Fat content of the diet and of the feces was determined by a modified Association of Official Agricultural Chemists (1) procedure as follows:

To approximately 2 g. of wet feces was added 5 ml. of concentrated hydrochloric acid and digestion carried out at low boiling temperature, with

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These findings were presented, in part, before the American Institute of Nutrition, 1956.

TABLE 1
Composition of diet base for fat test diets

Component	%
Casein	18.26
Methionine.....	0.49
Corn starch.....	37.50
Sucrose.....	36.25
USP Salts #2.....	5.00
Vitamin mixture ¹	1.25
Glycerine.....	1.25

¹ Supplying in milligrams per 100 g. of diet: Thiamine hydrochloride 0.50; riboflavin 0.50; pyridoxine hydrochloride 0.25; nicotinamide 2.00; calcium pantothenate 2.00; inositol 10.00; para-aminobenzoic acid 5.00; biotin 0.01; folic acid 0.10; choline chloride 100.00; vitamin K 0.10; and vitamin B₁₂ 50 µg. Fat-soluble vitamins were given by dropper prior to the collection period and provided 6000 I.U. of vitamin A, 60 I.U. of vitamin D and 5.0 mg. of alpha-tocopherol.

stirring, until all particles disintegrated. The digest was transferred to a Mojonnier flask, the beaker rinsed with a small volume of ethanol, and the mixture cooled. Lipids were extracted by adding 25 ml. of ethyl ether, shaking vigorously, adding 25 ml. of petroleum ether and shaking again. The extraction procedure was repeated three or more times and the ether phases transferred to a weighed crystallizing dish after each extraction. Evaporation was begun immediately in a fume hood and the evaporated sample dried to constant weight at 60-65° C. *in vacuo*. Recovery of 98.5 to 100% was obtained by this method from synthetic mixtures of free fatty acid, soap, neutral fat, and cholesterol. Absorbability was calculated from the formula:

$$\% \text{ Digestibility} = \frac{\text{Fat Intake} - (\text{Fecal Fat} - \text{Fecal Fat of Fat-free Group})}{\text{Fat Intake}} \times 100$$

The term "digestibility" is employed because of its general acceptance although it is recognized that the fecal lipids, particularly when fully hydrogenated fats are fed, contain appreciable amounts of soaps and some free fatty acids which represent end-products of digestion not absorbed. What is measured is a combination of both undigested and unabsorbed materials.

Data were analyzed statistically by the multiple range test of Duncan (4) for the significance of differences among ranked means or by the t-test (5) for the significance of the difference between two means.

Capillary melting point, iodine number, and saponification value of the fats were determined (1). Seven natural fats—corn, soybean, cottonseed, coconut and palm oils, butterfat and lard—representing wide variation in melting point, saturation, and chain length were studied. Fully hydrogenated fats were prepared from these same materials. Characteristics of the natural and hydrogenated products are presented in Table 2.

Characteristics of the modified lards tested are listed in Table 3. The modifications were (1) butyration to the extent of one fatty acid equivalent, (2) interesterification with glycerol to form monoglycerides, and (3) interesterification with mannitol to replace glycerol, forming mixed mannitol esters. A simple mixture of hydrogenated lard and tributyrin compounded to yield approximately the same over-all fatty acid content served as a control for the butyated lard. Hydrogenated lard monoglyceride was compared with glyceryl monostearate of higher melting point; and the mannitol esters with the normal glycerides from which they were made.

RESULTS AND DISCUSSION

Natural fats were found to be 97.0 to 99.7% digestible and these coefficients bore no relationship to melting point, saturation or chain length of the constituent fatty acids. The values obtained are given in Table 4,

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TABLE 2
Characteristics of natural and hydrogenated fats

Fat	Capillary melting point ° C.	Iodine number	Saponification value	Saturated fatty acids C18 and above ¹ % by weight
<i>Natural fats</i>				
Butterfat.....	35.4	31	224	12.1
Coconut oil.....	25.4	10	237	2.7
Corn oil.....	< 0	125	192	4.2
Cottonseed oil.....	< 0	112	196	2.4
Lard.....	45.1	66	195	10.0
Palm oil.....	36.5	53	198	4.7
Soybean oil.....	< 0	126	193	3.3
<i>Hydrogenated fats</i>				
Butterfat.....	49.8	< 1	224	33.8
Coconut oil.....	45.2	< 1	253	12.2
Corn oil.....	68.0	3	190	90.1
Cottonseed oil.....	61.6	1	195	72.3
Lard.....	61.2	< 1	195	73.4
Palm oil.....	59.4	1	198	57.3
Soybean oil.....	67.2	5	193	82.1

¹ Obtained from Hilditch (7).

TABLE 3
Characteristics of modified lards

Product	Capillary melting point ° C.	Iodine number	Saponification value
Butyrate hydrogenated lard (13.4% by weight).....	52.9	< 1	236
Simple mixture of hydrogenated lard and tributyrin (81:19).....	59.0	< 1	237
Monoglycerides of hydrogenated lard.....	67.6	13	160
Glycerol monostearate.....	74.7	4	160
Mannitol esters of lard.....	30.7	63	192
Mannitol esters of hydrogenated lard.....	53.1	< 1	176

together with the statistical comparison of differences between means. Groups of means enclosed within a bracket do not differ from one another. In general, digestibility of the natural fats could not be clearly differentiated.

A similar comparison of the fully hydrogenated fats is shown in Table 4. Hydrogenated coconut oil was 98.5% digestible, significantly different from any other fat. Hydrogenated butterfat was superior to all fats except hydrogenated coconut, being 61.0% digestible. At the 1% significance level, hydrogenated corn oil differed from hydrogenated palm and hydrogenated cottonseed oils, but not from hydrogenated soybean oil or hydrogenated lard.

Representative body weight data are presented graphically in Figure 1. The highly digestible fats permitted adequate gains in body weight after adjustment to the change from stock diet. When a poorly digested fat was fed, the decreased caloric yield was reflected by lack of growth or small losses of body weight. Major losses of weight, indicative of toxicity of the test materials, were not encountered.

TABLE 4
Digestibility of natural and hydrogenated fats

Fat	% Digestibility		
<i>Natural fats</i>			
Coconut oil.....	.01 ¹	{ 99.7 ± 0.07 ² }	.05 ¹
Soybean oil.....		{ { 99.0 ± 0.13 } }	
Corn oil.....		{ { 98.6 ± 0.12 } }	
Lard.....		{ { 98.0 ± 0.16 } }	
Cottonseed oil.....		{ { 97.9 ± 0.02 } }	
Butterfat.....		{ { 97.8 ± 0.62 } }	
Palm oil.....		{ { 97.0 ± 0.27 } }	
<i>Hydrogenated fats</i>			
Coconut oil.....	.01	98.5 ± 0.42	.05
Butterfat.....		61.0 ± 2.17	
Palm oil.....		{ 23.8 ± 3.04 }	
Cottonseed oil.....		{ 22.8 ± 2.37 }	
Soybean oil.....		{ { 17.2 ± 0.96 } }	
Lard.....		{ { 15.2 ± 1.58 } }	
Corn oil.....		{ { 12.0 ± 3.08 } }	

¹ Means enclosed within brackets do not differ at the indicated confidence level.
² Standard error.

A plot of the percentage digestibility of hydrogenated fats against melting point, illustrated in Figure 2, showed an inverse linear relationship with a correlation coefficient of -0.941 . It should be noted, however, that the slope and definition of the line rely on the extreme values and that the data scatter in the very high melting point range. A better correlation ($r = +0.999$) was obtained when digestibility was plotted against saponification value, as in Figure 3. Digestibility varied directly with saponification value, or inversely, then, with the chain length of the fatty acids. Digestibility varied in curvilinear fashion with the calculated

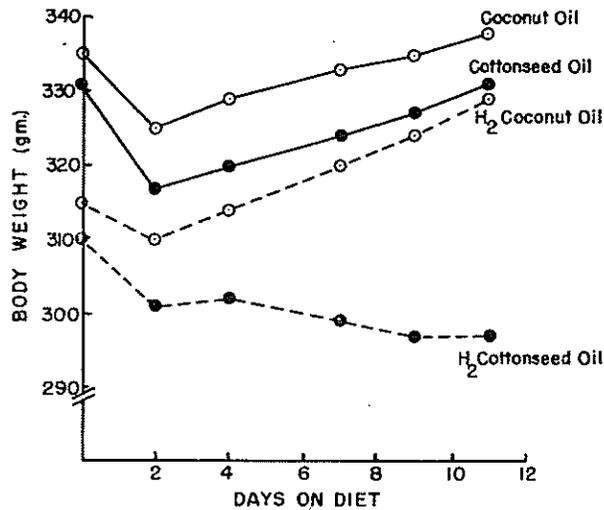


Figure 1. Representative body weight data.

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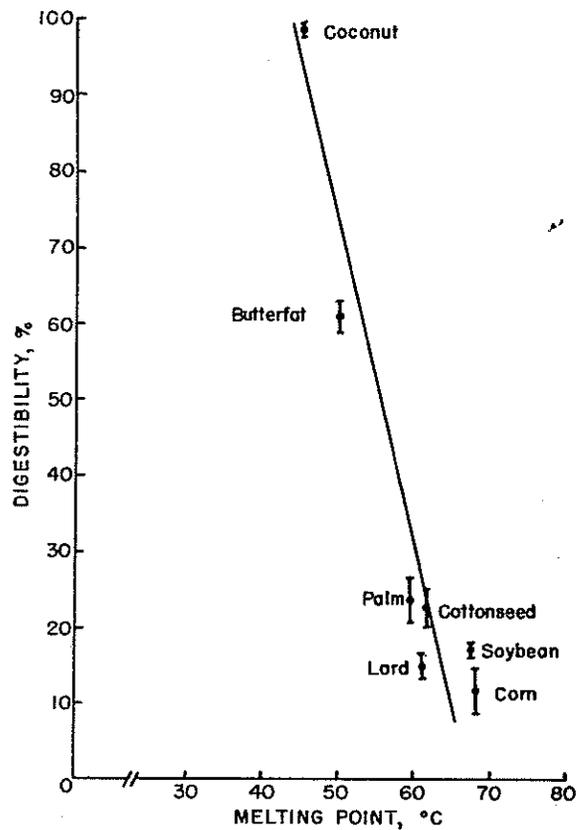


Figure 2. Relationship between melting point and digestibility of hydrogenated fats.

stearic acid content of the hydrogenated fats, the slope of the line changing at about 55% stearic acid. (See Figure 4). Extension of the curve to 100% stearic acid content resulted in a predicted value of about 11% digestibility for tristearin which agrees well with the reported value of 10.6% (2). These various relationships suggest that the digestibility of stearic acid is not a constant and that its digestibility is a function of the other fatty acids contained in the glyceride structure. They also indicate that the relationship of digestibility and of melting point is coincidental, rather than causal, as melting point is determined by the component fatty acids of the fat. Verification was obtained in the studies of modified lards.

Butyrated hydrogenated lard was 47.7% digestible and the mixture of hydrogenated lard and tributyrin, 34.3%—a definite improvement over the 13.9% of the starting material. (See Table 5.) When digestibility of the hydrogenated lard fractions was calculated, assuming the butyryl groups to be 100% absorbable, it became apparent that mixing with tributyrin did not significantly improve absorption of the lard fatty acids—18.3% as compared with 13.9%—whereas butyration was distinctly beneficial, resulting in 35.4% absorption. Comparable results were obtained by Mattil and Higgins (11), who concluded that the “solvent” action of oleic acid

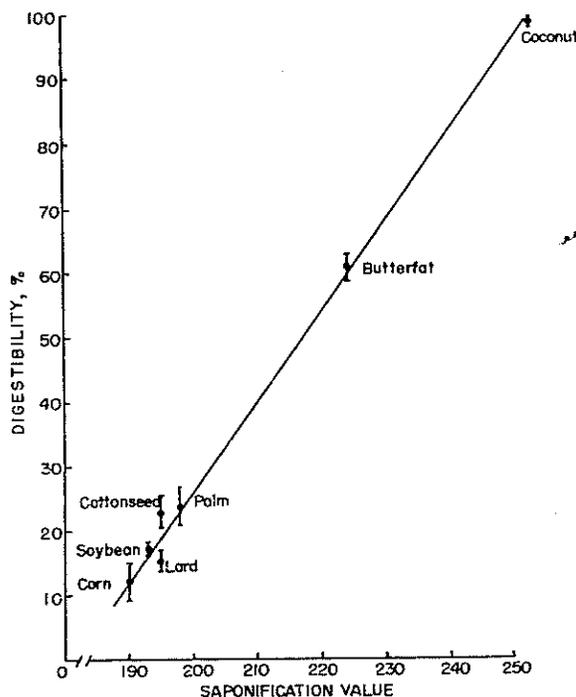


Figure 3. Relationship between saponification value and digestibility of hydrogenated fats.

for stearic acid was markedly greater with mixed glycerides than with mixtures of triglycerides.

The monoglycerides of hydrogenated lard exhibited the same digestibility, 33.9%, as the lard fraction of the butyrate product. As shown in Table 6, stearic acid was equally as well absorbed from glyceryl monostearate, although its melting point was 7° C. higher than that of the lard monoglycerides.

Experimental results indicate that there is a maximum absorption value for stearic acid. This limit appears to be 30 to 40%.

The digestibility of glyceride lard was 98.6% and that of its mannitol esters, 95.5%. (See Table 7). This difference is statistically, if not practically, significant. No difference was noted between hydrogenated lard and the mannitol esters of hydrogenated lard, with digestibility values of

TABLE 5
Digestibility of butyrate lard

Fat	% Digestibility	
	Total	Lard fraction
Butyrate hydrogenate lard.....	47.7 ± 3.9 ¹ } ²	35.4 ± 4.8
Hydrogenated lard + tributyrin.....	34.3 ± 4.6]	18.3 ± 3.9 } ²
Hydrogenated lard.....	13.9 ± 1.2	13.9 ± 1.2]

¹ Standard error.

² Means within brackets do not differ at the 5% level of significance.

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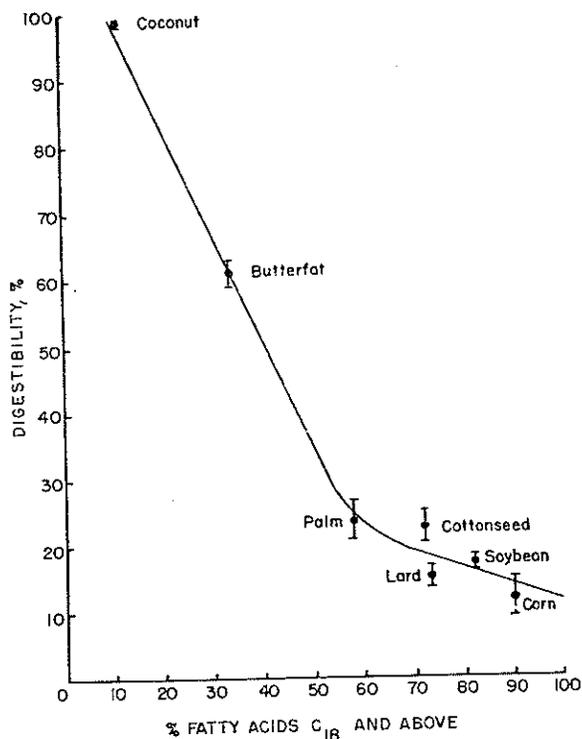


Figure 4. Relationship between content of long-chain fatty acids and digestibility of hydrogenated fats.

19.3% and 18.9%, respectively. The esters of mannitol are seemingly as susceptible to lipolysis as those of glycerol and their absorption a function of the fatty acid composition as in the glycerides. Mannitol esters of olive oil, a by-product of glycerine production, were studied by Halliburton, Drummond, and Cannon (6) and found to be utilized to practically the same extent as olive oil, 95.8% as opposed to 96.6%. The small reductions in digestibility which have been noted may be due to poorer absorption of the mannitol than of glycerol.

SUMMARY AND CONCLUSIONS

Mature rats were fed a series of natural and modified fats in order to determine the relationships among melting point, saturation, chain length, structure and digestibility. Twenty per cent fat was incorporated into a

TABLE 6
Digestibility of monoglycerides

Fat	% Digestibility
Monoglycerides of hydrogenated lard.....	33.9 ± 3.0^1 ²
Glyceryl monostearate.....	31.8 ± 3.9]
Hydrogenated lard.....	13.9 ± 1.2

¹ Standard error.

² Means within brackets do not differ at the 5% level of significance.

TABLE 7
Digestibility of mannitol-lard esters

Fat	% Digestibility
Lard.....	98.6 ± 0.21 ¹
Mannitol-lard esters.....	95.5 ± 0.34
Hydrogenated lard.....	19.3 ± 1.29] ²
Mannitol-hydrogenated lard esters.....	18.9 ± 2.04]

¹ Standard error.

² Means within brackets do not differ at the 5% significance level.

purified diet fed for two weeks. Feces were collected during the last five days for fat analysis.

Natural fats included in the study were: cottonseed, soybean, corn, coconut and palm oils, lard and butterfat. Digestibility was not related to the characteristics cited. When these same fats were fully hydrogenated, digestibility varied inversely in linear fashion with the chain length of the constituent fatty acids and in curvilinear fashion with the amount of saturated acids C₁₈ and above. The general inverse linear relation between digestibility and melting point is ascribed to the relationship which exists between the melting point and the amounts of component fatty acids.

The monoglyceride of hydrogenated lard was found to be more digestible than the original triglyceride. Substitution of one-third of the fatty acid radicals by butyryl groups was equally effective in raising digestibility; while simple mixture of tributyrin with hydrogenated lard showed no effect.

Digestibility of mannitol esters of lards was similar to that of the glyceride lards from which they were made, indicating that digestibility was a function of the constituent fatty acids.

It is concluded that digestibility is primarily dependent upon the amounts and chain length of the saturated fatty acids and their arrangement within the glyceride structure.

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