The Hydrogenation of Dietary Unsaturated Fatty Acids by the Ruminant[1]

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IN AN EFFORT to explain the relatively high content of stearic acid in "stearic-rich" animal fat Hilditch and his school have postulated that palmityldioleins are first synthesized in the tissues of such animals and then converted to palmitylstearins by a tissue "biohydrogenation" process (1, 2, 3). This hypothesis was supported by the observation that the percentage of fully saturated glycerides in these fats is not very different from that calculated on the basis of "random" distribution. Convincing evidence was adduced from the fact that the palmitic acid content is the normal 25–30% of other animals and that the increase in saturated acids, which may reach 60%, is attributable entirely to the replacement of oleic by stearic acid.

With the exception of some pig fats the "stearic-rich" depot fats are limited to ruminants. The fatty acid composition of ruminant animal depot fat is furthermore much less affected by the nature of dietary fat (4, 5) than that of nonruminants. These facts suggest that the rumen microorganisms may be responsible for the hydrogenation of the dietary fat, which is then digested, absorbed, randomly resynthesized, and deposited.

This hypothesis has been supported by a preliminary study in which linolenic acid was partially hydrogenated *in vitro* by rumen contents (6). The hypothesis was later offered as the explanation for the paradoxical presence of more saturated and less oleic acids in the depot fat of steers fed cottonseed oil than in the controls (7). Later Hartman, Shorland, and McDonald referred to this hypothesis as the explanation for the presence of *trans* monoethenoic acids in ruminant depot fat (8); and Shorland, Weenink, and Johns verified it by feeding pasture grass rich in linolenic acid to sheep and demonstrating its hydrogenation to saturated acids (9).

In order to test the hypothesis in this laboratory four adult goats were placed on an alfalfa meal regimen. Two were fed 10% cottonseed oil in the alfalfa meal and two 10% linseed oil. After being maintained on this ration for 11 weeks the animals were sacrificed six hours after the last meal. The fatty acid composition of samples of back fat and of the contents of the rumen, abomasum, and caecum were compared to that of the feed.

Some technical difficulties were encountered in the extraction of clear colorless fatty acids from the samples. Adsorbents were found selectively to adsorb unsaturated fatty acids as well as pigments. The method finally used was to saponify the entire sample with alcoholic potassium hydroxide. The extract and alcoholic washings were reduced in volume under reduced pressure, and the nonsaponifiables were removed as completely as possible with Skelly B.

The soaps were decomposed with HCl, and the acids were extracted with Skelly B. After removal of the solvent the fatty acids were distilled in a flask designed for the purpose. This consisted of a 25-ml. round bottom flask, the neck of which was fused ½ in. into the bottom of a 50-ml. round bottom flask. The sample was placed in the bottom (25-ml.) flask by means of a dropping pipette and distilled into the 50-ml. flask by means of vacuum and a rotating evaporator. The protruding neck of the bottom flask prevented the distilled acids from returning to the distillation bulb.

This system has a very short distillation path, results in very little loss due to wetting of the glassware, reduces oxidation and destruction by heat to a minimum, and requires very little time.

The fatty acids were determined according to the method of Brice, Swain, Schaeffer, and Ault (10), as corrected (11).

The results of the analyses are given in Table I.

TABLE I
Changes in Fatty Acid Compositions of Rations at Various Levels in the Gastrointestinal Tract of the Goat

Goat no.	Sample	Iodine value	Fatty Acids			
			Oleic	Linoleic	Linolenic	Saturated
			%	%	%	%
Alfalfa-Cottonseed Oil Ration						
....	Feed	100.5	29.4	35.0	4.1	31.5
1	Rumen	47.3	49.3	1.4	0.4	49.0
2	Rumen	40.6	29.0	6.5	1.2	63.5
2	Abomasum	31.3	25.4	3.3	1.0	70.4
2	Caecum	26.0	22.6	3.2	0.1	74.7
2	Back fat	46.6	36.3	7.1	0.6	56.1
Alfalfa-Linseed Oil Ration						
....	Feed	180.6	15.5	14.6	51.5	18.5
1	Rumen	49.0	33.5	3.2	4.9	58.4
2	Rumen	65.5	41.0	5.9	6.7	46.4
2	Caecum	63.4	54.2	2.4	4.0	40.5
1	Back fat	54.7	48.5	4.7	1.2	45.5

Because of the limited amount of material, all analyses are given.

Practically all of the changes took place in the rumen, with some continued hydrogenation taking place between rumen and caecum. The linoleic and linolenic acids of linseed oil were converted to both oleic (including palmitoleic) and saturated acids. This was also true in the rumen of goat number 1 on cottonseed oil, but in goat number 2 there was actually less oleic acid in the stomach and caecum than in the feed.

The back fat shows some influence of the diet, not so much in the levels of the linoleic and linolenic acids as in the amounts of oleic and saturated. Even though these were adult animals on experiment only 11 weeks, the back fat of the goats on the two diets shows a distinct difference. The back fat of the cottonseed fed animals contained more saturated and less oleic than those fed linseed oil.

Discussion

There are a number of possible alternative interpretations of the observation of the present study. It is conceivable that unsaturated fatty acids are pref-

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erentially adsorbed from the rumen, or are preferentially passed from the rumen, leaving the saturated. These possibilities are most unlikely. The rumen is incapable of absorbing long chain fatty acids, and the resolution of a solution of saturated and unsaturated acids in a churning milieu such as a rumen must be considered impossible.

Another alternative is that the unsaturated acids are oxidized to short chain acids and these are resynthesized to saturated acids. Since the synthesis of saturated fatty acids from acetate is a proven phenomenon, this may take place to some degree, but to the author's knowledge nowhere in all the rather voluminous literature on the short chain acids of the rumen has this been observed to occur. On the contrary, short chain acids are absorbed to a considerable degree directly from the rumen.

There is the possibility that the unsaturated acids are oxidized to short chain acids in the rumen and absorbed there, leaving the saturated acids. However Shorland has shown that the saturated acids in the rumen are mainly stearic. The saturated acids of linseed and cottonseed oil are mainly palmitic.

The interpretation that the dietary linoleic and linolenic acids of the goat are converted in the rumen to oleic and saturated acids explains the high level of stearic acid in the tissues of ruminant animals and the relative lack of influence of dietary fatty acids on the depot fat of these animals. Previous work in this laboratory (7) has shown that the inclusion of 5% of cottonseed oil in an otherwise low fat ration of fattening steers resulted in a fat of higher saturated and lower oleic acid than the controls. Linoleic and linolenic acids were not affected. This paradox was explained by the hypothesis that the exogenous fat, hydrogenated in the rumen, contained more saturated and less oleic than endogenously produced fat. The present data substantiate this interpretation. Endogenous animal fat produced on "fat-free" rations is about 35% saturated and about 65% or more oleic and palmitoleic (12, 13, 14). Rumen fat, after ingestion of cottonseed oil, contained between 50 and 60% saturated acids and only 30 to 50% monoethenoid acid. Thus the addition of cottonseed oil to a low fat cattle ration actually would paradoxically increase the level of the saturated acid and reduce the level of the oleic (monoethenoid) acid. Others have also found that the ingestion or rather large quantities of soybean and other unsaturated fats to steers for 260 days had little influence on the iodine number or firmness of the depot fats (5). No analysis was made of the fatty acid composition.

Summary

Goats were fed alfalfa meal containing 10% cottonseed or linseed oil. After 11 weeks the fatty acids of rumen, stomach, and caecum contents were compared to those of the feed.

It was found that the high levels of linoleic and linolenic acids of the feed were reduced to very low levels in the rumen, with comparable increases in the saturated acids. Monoethenoid acids were increased after linseed oil ingestion and in one animal after cottonseed oil ingestion.

The ratio of monoethenoid to saturated acids in the rumen fat was lower than in the endogenous fat of nonruminant animals. This explains the paradox of the low ratio in the depot fat of ruminants even after the ingestion of highly unsaturated fats.

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Concurrent Oxidation of Accumulated Hydroperoxides in the Autoxidation of Methyl Linoleate[1,2]

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UNDER MILD CONDITIONS OF OXIDATION the yield of hydroperoxides in the initial stages of the autoxidation of methyl linoleate is believed to be virtually quantitative (4, 8, 10, 12, 16), that is, secondary oxidation of hydroperoxides has been considered to be relatively unimportant in the early stages of the reaction. In fact, Bolland (7) suggested that hydroperoxides formed in the autoxidation of ethyl linoleate would be less likely to undergo oxidative attack than ethyl linoleate because less resonance energy would be made available on radical formation.

Notwithstanding, there are a number of observations peculiar to the autoxidation of methyl linoleate that remain unexplained. Notable among these is the amount of diene conjugation formed during the reaction. The average value of about 23,000 for the molecular extinction coefficient for the peroxides formed in the autoxidation of methyl linoleate is much lower than that determined for *cis, trans* diene conjugated methyl octadecadienoate (14, 17). This cannot be explained on the basis of our present knowledge unless one takes the unattractive view that nonconjugated hydroperoxides are produced. Formation of nonconjugated hydroperoxides has not been demonstrated in the autoxidation of methyl linoleate, and from thermodynamic and other considerations it appears unlikely that they are produced in an amount sufficient to account for the low molecular extinction (4, 21, 22).

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