representing these selected cells on the oscilloscope display screen can be intensified, resulting in a display photograph shown in Fig. 1, where the ordinate of each point is proportional to absorption and the abscissa to scatter. The actuation pulse also initiates a cell separation, as described below.

The cells suspended in a fluid medium are pumped by pressurization of the sample container at a flow rate of 0.5 ml/min through a flow channel (*2*) shaped as shown in Fig. 1. The cells are observed at the optical axis by the spectrophotometer and flow to the sorter junction in 2 msec. Unselected cells continue to flow out into the outlet container. If a specific cell has optical properties causing an actuation signal, the stepper motor is pulsed, causing the plunger in the syringe to draw 0.03 μl of fluid. This fluid pulse is propagated across the flow channel at the junction, causing fluid flow across the junction for about 3 msec. There is a delay of 2 msec from the time of detection until an appreciable flow occurs across the channel, matching the delay time of the cell to reach the junction from the observation point. In practice the cells flow at varying velocities, owing to the parabolic velocity profile across the channel. The velocity profile of the cells can be measured by imaging the cells upon a grating, and the actuation delay and displacement time can be matched to this profile. The actuation time is made longer than necessary to ensure that the desired cell has been captured in the side channel. Thus, several other cells will accompany the selected cell. We were successful in achieving a final concentration of selected cells of about 1 : 5 from initial concentrations in the range of 1 : 10,000. The selected cells are drawn far enough into the side channel to remain there for the duration of a run. Up to 300 selections can be made during a run of 100,000 or more cells.

After a sample has been processed the main channel is automatically flushed in reverse for cleaning. A Millipore filter is placed on a holder connected by tubing to a radial hole drilled into the barrel of the syringe as shown in Fig. 1. During the wash the plunger of the syringe is withdrawn to just beyond the radial hole so that there is a flow of clean water through the side channel, the syringe, and the Millipore filter. The separated cells are thus flushed out of the side channel and trapped on the Millipore filter. The Millipore filter is then removed, placed on a slide, and stained for visual observation of the cells. The cells are all visible in one low-powered microscopic field, as illustrated in Fig. 2.

The efficiency of separation can be demonstrated by comparing oscilloscopic display patterns of a test sample composed of two types of cells with different photometric properties before and after separation of one of the cell types. This is illustrated in Fig. 3.

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4. We thank Herbert Derman and Leopold G. Koss for helpful suggestions and support, and H. H. Glattli, Issac Klinger, George A. Folchi, and Arnold Halperin for assistance in the design and testing of the apparatus.

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Saturation in Milk and Meat Fats

Abstract. *Meat and milk products from ruminants (cows, goats, sheep, and beef animals) contribute 35 to 40 percent of the fat in the average American diet. Such fat is highly saturated, containing less than about 4 percent polyunsaturated fatty acids. The unsaturated plant lipids (fats) ordinarily consumed by the ruminant are hydrogenated (saturated) in the rumen. Transport and incorporation of this hydrogenated fat into meat and milk follows. Rumen hydrogenation does not take place until the fat is broken down to free fatty acids, thus establishing the fact that lipolysis is an essential feature of the process. Circumvention of this lipolysis may lead to more-unsaturated meat and milk fat.*

In the course of the controversy concerning the relation between fats and heart disease, Americans have been advised "to eat less animal fat" (*1*) and "to increase the intake of unsaturated vegetable oils and other polyunsaturated fats, substituting them for saturated fats wherever possible." The proponents of this argument believe that polyunsaturated fats in the diet tend to establish lower amounts of cholesterol in the blood than the saturated fats do, and that high amounts of cholesterol in the blood are associated with damaging plaque formation in the arteries. The role of dietary fat in human health has been reviewed (*2*).

Why are fats saturated and can they be made less so? About 35 to 40 percent of the fat consumed in the average American diet is derived from meat and milk produced by ruminants (*3*); these products include milk, cream, butter, cheese, ice cream, and other dairy products (19.5 to 27 percent), as well as beef, veal, lamb, and mutton (14 percent). The polyunsaturated fatty acids in these foods seldom exceed 4 percent of the fat fraction, whereas in vegetable oils they predominate (*4*).

Lipids in plant materials fed to ruminants contain large quantities of polyunsaturated acids, and these acids become saturated in the rumen of the animal as a result of microbial action (*5*). This action changes the polyunsaturated fatty acids of the feed substantially into stearic acid which becomes a dominant factor in fat synthesis by the animal. Because the fatty acids in plant lipids occur mainly in esterified form and because the principal resultant of rumen digestion is free stearic acid, hydrolysis of the ester may be a prerequisite for hydrogenation of the fatty acid. Rumen hydrogenation of phytol is facilitated by its hydrolysis from the chlorophyll molecule (*6*). If hydrolysis is required, protection of fed lipids from rumen lipase should permit the polyunsaturated lipids to be metabolized from the lower gut in much the manner of a monogastric animal such as man. For example, Insull *et al.* (*7*) induced an increase in the linoleate (polyunsaturate) content of human milk fat from 5 to 45 percent by including corn oil in the mother's diet.

We analyzed the major lipid classes in rumen ingesta to determine their relative saturation. We extracted lipids (*6*) from ingesta recovered from the rumens of milking Holstein cows of the University herd. These lipids were then separated by chromatography on a silicic acid column into polar lipids (phospholipids and glycolipids), free fatty acids, and neutral lipids (glycerides and sterol esters). The purification steps were followed by thin-layer chromatography. Samples of the lipid classes were methylated and analyzed

Table 1. Composition (percentage by weight) of the lipids of feed and rumen ingesta from that feed. From 15 g of hay, grain, and corn silage (1:1:2.1), 0.219 g of lipid was recovered; from 100 ml of ingesta, 0.375 g of lipid was recovered.

Lipid fraction	Feed	Rumen
Neutral	61.4	28.3
Free fatty acid	20.0	40.5
Polar	18.6	31.2

by gas-liquid chromatography for fatty acid composition (*8*).

The average fatty-acid composition of the major classes of rumen lipids for five lactating cows was determined. Saturation, expressed as the sum of the acids with no double bonds, averaged 85.0 percent for the free fatty acids, 47.4 percent for the neutral lipids, and 62.3 percent for the polar lipids. An average of 69.3 percent stearic acid in the free fatty-acid fraction was mainly responsible for its high degree of saturation. While these data suggested that hydrogenation of the fatty acids does not proceed until they are hydrolyzed from the glycerolipids, a more satisfactory evaluation could have been made if data on the lipids in feed had also been available for comparison.

To define this situation more precisely, the proportions of hay, grain, and corn silage consumed by one of the cows was evaluated over a period of several days during November; no pasture was provided. A feed sample (calculated to be 1.0 part of hay, 1.0 of grain, and 2.1 of silage) was finely ground in a blender and extracted to recover lipids by the same method as that used for ingesta. At the same time, a sample of ingesta from the rumen was obtained and extracted. The lipid fractions from the feed and ingesta samples were quantitated (Table 1) and analyzed for fatty-acid composition (Table 2). These typical data support the concept that fatty acids must be free in the rumen before they are hydrogenated. The neutral lipids of the feed and rumen ingesta are highly unsaturated and very similar in fatty acid composition, while the free fatty acids of the rumen are very highly saturated. The data on weight composition suggest that the neutral lipids of feed are hydrolyzed and that they thus contribute to the free fatty-acid fraction of lipids of the rumen ingesta. The polar lipids of the feed also appear to be hydrolyzed rather than hydrogenated intact because the polar lipids of the rumen, although more saturated, do not contain much more stearic acid than the feed polar lipids. The high concentration of palmitic acid (16:0) in the polar lipids of rumen ingesta (*9*) suggests protozoal proliferation.

Alternatives to our interpretation that the lipolysis is followed by hydrogenation are: (i) that hydrogenation of intact glycerolipids renders them selectively susceptible to lipolysis; or (ii) that stearic acid is synthesized by other relatively important pathways than hydrogenation of C_{18} unsaturated acids from feed. Evidence to support either of these is lacking. Study of the mechanism of rumen microbial hydrogenation of unsaturated fatty acids indicates that a free carboxyl group is required (*10*). This, too, implies that lipolysis of glycerolipids precedes hydrogenation of fatty acids in the rumen.

Possibly, practical measures may be developed for bypassing rumen hydrogenation of unsaturated fatty acids. For example, infusion of unsaturated fat emulsions into the cow's bloodstream significantly increases unsaturation in her milk fat (*11*), and the feeding of certain purified diets to sheep produces a tallow that is much more unsaturated than normal (*12*). Manipulation of the rumen microflora to favor organisms with little or no ability to hydrogenate is also conceivable. However, even if practical feeding procedures and feed forms can be developed to bypass or suppress rumen hydrogenation of feed lipids, it is important to consider whether economy and efficiency of meat and milk production could be maintained and at what point of polyunsaturation, if any, the health of the animals would become impaired.

Table 2. Fatty acid composition (percentage by weight) of lipid fractions from feed and rumen ingesta, as determined by analysis of peak areas from gas chromatograms.

Fatty acid*	Neutral lipids		Free fatty acids		Polar lipids	
	Feed	Rumen	Feed	Rumen	Feed	Rumen
12:0	0.4	0.7	0.7	0.2	0.5	1.2
14:0	0.6	1.4	1.1	0.5	0.7	3.1
16:0	13.8	21.4	29.2	12.9	26.5	55.0
18:0	2.4	6.6	13.5	70.0	2.2	7.1
18:1	26.0	24.7	13.1	13.4	13.1	17.7
18:2	49.8	41.6	26.6	2.3	36.4	14.6
18:3	7.1	3.6	15.9	0.0	20.5	1.3
Saturation	17.2	30.1	44.5	84.5	29.9	66.4

* Numbers of carbons in the fatty acid are to the left of the colon, and numbers of double bonds (unsaturation) are to the right. Saturation is the sum of the acids with no double bonds.

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