sections taken at the nape, center, and tail of the fish. Greatest differences (Table III) were found in oil content, although protein and moisture varied slightly. The nape contained the highest oil and the lowest protein and moisture contents. The tail contained the lowest oil values, often less than one half that of the nape, but the amount of protein was similar to that in the center steak section.

The average oil content for edible flesh in each lot (Table II) was similar to the average values for the center steak sections. In the lots with higher oil values, the oil content in all three sections increased, indicating that the fish had increased in general fatness.

Composition of Lipid Deposits. Both the nutritionist and the processor are interested in the composition of lipid deposits and in whether these deposits should be removed. Removal of lipid deposits will significantly decrease total oil content and increase storage life of frozen fish. Three areas of silver salmon that contained high amounts of oil and low amounts of protein were the belly flap, the dark meat along the side, and the dorsal fatty layer along the back of the fish (Table IV). These sections were analyzed in the last lot of fish obtained each year, except that the lot in the third year contained so little meat in the dorsal fatty layer along the back that it could not be analyzed. In the other two lots, the dorsal meat had by far the greatest concentration of oil in the fish. The belly flap from lot 9, however, contained more oil (61.3%) than any other section in these three lots. The average values for oil content in these three sections are not significant because of the wide variation, but they do show that these areas definitely have concentrations of oil that may decrease storage life of frozen fish. Even when the fish had a low amount of oil—(e.g., Table II, lot 3 at 3.10%)—the belly flap, dark meat, and dorsal layer had high concentrations of oil (Table IV, 12.71, 17.05, and 24.92%, respectively).

Acknowledgment

The authors express their appreciation to Mabel A. Edwards and Dave H. Wieg for their assistance in the analysis of samples.

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Received for review May 8, 1963. Accepted September 12, 1963.

CELLULOSE DIGESTIBILITY

Birefringence of Plant Fibrous Cellulose and Microcrystalline Cellulose in Human Stools Freezer-Stored Immediately after Evacuation

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With pure crystalline cellulose (high alpha cellulose and microcrystalline cellulose, at least), celluloses which do not contain more than relatively small amounts of very low molecular weight hemicellulosic fractions, xylans, mannans, or mixed sugar polymers, chemical and/or physical degradation normally does not occur to any significant extent within the human digestive tract. Microscopic evidence of the retention of definitive birefringence of cellulose substantially intact in human feces, provided the stools are freezer-stored immediately after evacuation from the body, is presented.

The digestibility of cellulose in animals varies considerably, depending on the species of the animal, the source of cellulose, and the composition of the so-called crude fiber (*14*). The evidence (*6, 8, 9, 14*) for the utilization of cellulose by ruminant animals is not only extensive, but also quite convincing. On the other hand, the literature on the digestibility of cellulose in nonruminant animals and man appears to be not only confusing, but also contradictory (*2, 3, 5, 7, 11, 12, 14*).

One of the underlying variables that appears to have received insufficient attention is the fact that the cellulose source and the degree of its purity can influence digestibility data markedly. For example, Williams and Olmsted (*15*) and Hoppert and Clark (*6*) demonstrated that cellulose in fruits and some vegetables seems more digestible than the cellulose in, for example, cereal grain. Fung *et al.* (*3*) claim that even hemicelluloses are completely unmetabolized by the rat.

Various types of natural plant celluloses, however, are known to have wide variations in noncellulosic carbohydrate components. These noncellulosic carbohydrate materials have a fine structure distinctly different from pure cellulose. Some of them are amorphous, three-dimensional sugar polymers and do not exhibit a definitive x-ray diffraction pattern or sharp birefringence under crossed Nicols in the microscope. These latter noncellulosic carbohydrate components may be present in relatively large amounts in natural cellulose sources and in themselves may indeed show partial digestibility. Normally they are classed together with the material called crude fiber, which is then equated to pure cellulose by many of the available analytical procedures used to determine crude fiber in foods.

Commercial grades of alpha cellulose, which are so widely used in the preparation of control and test diets in animal feeding research programs, as well as in human research studies, also contain varying amounts of potentially digestible but not necessarily metabolizable noncellulosic carbohydrate fractions. This is true even though alpha cellulose is the end product of severe chemical purification treatments of raw material from which plant celluloses are extracted. For example, commercial fibrous alpha celluloses may contain as much as 4 to 12% of hemicellulose components. Because of the empirical nature of hemicellulose classification, the "hemicelluloses" in alpha cellulose normally contain xylan, mannan, and glucomannan fractions, and some of the

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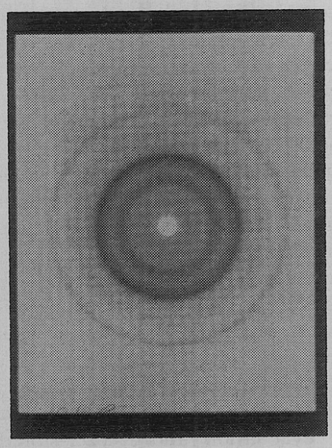

Figure 1. X-ray diffraction powder pattern of fibrous alpha cellulose

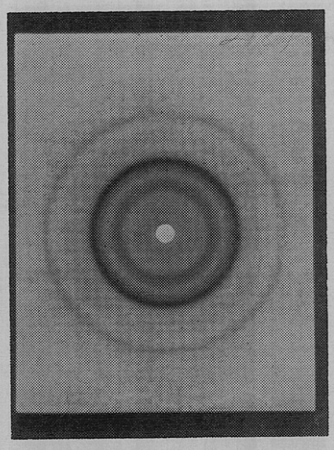

Figure 2. X-ray diffraction powder pattern of microcrystalline cellulose in flour form

Figure 3. Deterioration of the birefringence of microcrystalline cellulose in flour form in the stool of patient A

Sample was not freezer-stored immediately upon evacuation

Figure 4. Deterioration of the birefringence of microcrystalline cellulose in gel form in the stool of patient B

Sample was not freezer-stored immediately upon evacuation

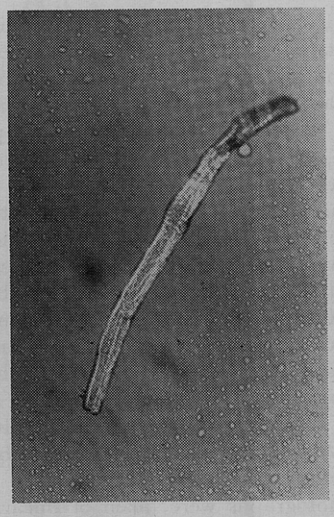

Figure 5. Particle of fibrous plant cellulose in stool of patient A, freezer-stored immediately upon evacuation

Figure 6. Microcrystalline cellulose in flour form in stool of patient A immediately after evacuation

Figure 7. Microcrystalline cellulose in gel form in stool of patient B immediately after evacuation

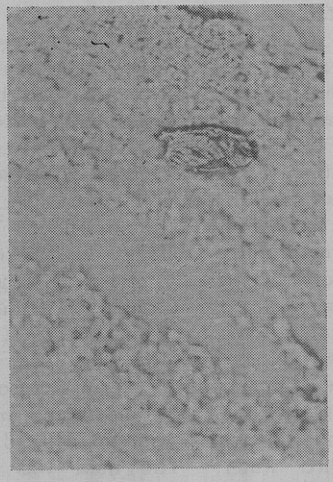

Figure 8. Thin smear of stool from patient A 2 weeks after concluding the microcrystalline cellulose diet supplementation, showing complete absence of any birefringent particles of microcrystalline cellulose

Figure 9. Photograph of particles of fibrous alpha cellulose (control)

Figure 10. Photograph of microcrystalline cellulose flour (control)

Figure 11. Photograph of microcrystalline cellulose in gel form (control)

latter materials conceivably are potentially digestible. Therefore, even a raw material such as alpha cellulose, which is substantially purer than the so-called crude fiber present in normally edible plant cellulose sources, may still not be completely free from small amounts of potentially digestible noncellulosic carbohydrates and/or metabolizable fractions. Furthermore, great care must be exercised in not equating digestibility of crude fiber as measured by indirect analytical procedures and true physiological assimilation via metabolic processes. Schiff (*13*) and Johnson *et al.* (*10*) have pointed out special situations which can affect the complex problems relating to the digestion of food which depends on alteration of bacterial microorganisms due to such variables as illness or the intake of antibiotics which can influence the role of intestinal flora.

Recently (*1*) a purer form of commercial cellulose, known as microcrystalline cellulose, has become available in which the noncellulose carbohydrate fractions and the inorganic contaminants are reduced to a minimum. More importantly, the macromolecular fine structure of microcrystalline cellulose comprises only the crystalline fraction of natural cellulose (Figures 1 and 2). The impurities in natural cellulose, both organic and inorganic, necessarily are all concentrated in the noncrystalline or so-called amorphous regions of plant cellulose, and these are substantially removed during the manufacture of microcrystalline cellulose, which is the crystalline residue of natural plant cellulose.

The unique crystallinity of cellulose (it is perhaps the most perfect natural isotactic polymer) makes it possible to use the microscope in conjunction with crossed Nicols to study bacterial or enzymatic degradation of plant cellulose—including crude fiber—and more particularly of highly crystalline microcrystalline cellulose. The definitiveness of the birefringence deteriorates rapidly as bacterial attack proceeds. Therefore, 75° Nicols should be used for this purpose, as was done in this study, to permit clearer delineation of background materials which are noncellulosic in nature.

In human clinical studies with microcrystalline cellulose, the birefringence of cellulose was utilized to help establish whether or not this purified form of cellulose passed through the human digestive processes with or without physical breakdown. This study was part of a broader clinical investigation (*1*, *4*) and did not include a metabolic materials balance.

Photographs were taken of smears of stools from two different patients cooperating in the study. One patient (male, patient A) had been on a regular free-choice diet supplemented by 30 grams of dry microcrystalline cellulose (commercially available as Avicel microcrystalline cellulose) flour per day for $5^1/_2$ weeks at the time of sampling for this study. The other patient (female, patient B) was a person who had been on a similar dietary schedule, except that her diet was supplemented by the addition of microcrystalline cellulose in gel form at the rate of 30 grams per day (dry basis) in lieu of the dry flour. The microcrystalline cellulose in colloidal gel form has particles of a much smaller average dimension than the microcrystalline cellulose in flour form (*1*).

The initial samples were from the supernatant fluid of fresh, unfrozen stools which were homogenized in a Waring Blendor in 500 to 700 ml. of distilled water. The fluid was obtained by decantation and stored in a refrigerator for approximately 3 days prior to making a smear and taking a photograph of the smear under crossed Nicols. Examination of aliquots of these fluid specimens showed that the microcrystalline cellulose, as well as various fibrous natural cellulose components in the feces, had become severely degraded as evidenced by a substantial degradation of the birefringence (Figures 3 and 4). If one concluded the experiments at this point, one would be forced to interpret the results as indicating that a substantial amount of digestion and/or physical breakdown of the cellulose had occurred as it passed through the human digestive tract, or during storage of the samples during the postevacuation period.

When samples of stools from the two patients were recovered immediately after evacuation and freezer-stored at −9° C., smears were made of the undiluted specimens and photographed. The results in Figures 5, 6, and 7 were obtained. The natural fibrous cellulose, the microcrystalline cellulose in dry flour form, and the microcrystalline cellulose in the colloidal gel form have completely normal and definitive birefringence patterns indicative of no significant chemical or physical breakdown within the body. However, when these specimens were allowed to remain in the air at room temperature, degradation was gradually initiated, and the same type of destruction of the birefringence of the cellulose as was found in the feces samples shown so clearly in Figures 3 and 4, respectively, was observed. The deterioration of the birefringence of cellulose in the stools was followed with time at room temperature under the microscope. As the cellulose was destroyed, presumably enzymatically, the larger particles became smaller and smaller until finally only pinpoints of birefringence could be observed at around 0.5 micron, and finally these also disappeared and no further birefringence could be observed in the sample. No doubt this process is used by nature to complete the cycle of photosynthesis whereby the cellulose is reconverted back into the carbon dioxide and water out of which it was originally made. A smear of a freezer-stored stool from patient A, 2 weeks after the conclusion of the supplementation of the diet with Avicel dry flour, is shown in Figure 8. No evidence whatsoever of any birefringent particles of microcrystalline cellulose was found in this specimen, as shown.

Control photographs were prepared using water slurries of dry microcrystalline cellulose flour (Figure 10) and microcrystalline cellulose in colloidal gel form (Figure 11). Figures 6 and 7 may be compared directly with Figures 10 and 11, respectively. The birefringence of the microcrystalline cellulose control samples is identical with that observed, after these respective forms of cellulose had passed through the human body, in the stools freezer-stored immediately after evacuation.

Figure 5 and Figure 9 are not directly comparable, on the other hand. Figure 5 shows the birefringence of a particle of plant fibrous cellulose from food which had passed through the human body and was recovered in the stool which was freezer-stored upon evacuation. Figure 9, conversely, is a particle of commercial alpha cellulose which had not been eaten, but which is included to show the characteristic birefringence of fibrous cellulose per se.

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Received for review July 10, 1963. Accepted November 13, 1963.

NUTRIENTS IN ALFALFA

Lipids of Dehydrated Alfalfa (*Medicago sativa*)

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Lipids of dehydrated alfalfa (6.4%) were fractionated by silicic acid chromatography. The first fraction, accounting for 30% of the total lipids, contained 30% low molecular fatty acids, mainly C_8. The main glyceride lipids were mono- and digalactosyldiglycerides. The galactolipids contained approximately 80% linolenic acid. Considerable amounts of unsaponifiables were present in all fractions.

Loss of carotene during storage of dehydrated alfalfa is of sufficient magnitude to be responsible for an appreciable decrease in the economic value of the alfalfa. Noncarotene lipid constituents probably play an important role in this loss, and since details of their composition have so far not been completely elucidated, such studies have been initiated in this laboratory. Hilditch (*12*) summarized information on the nature of green leaf lipids. Recent developments are those of Carter *et al.* (*7–9*), who isolated a mono- and a digalactosyldiglyceride from flour lipids; Benson *et al.* (*3*), who showed that the major chloroplast lipids are galactolipids; Lepage *et al.* (*17*), who described a sulfolipid in alfalfa and identified it as a 6-sulfo-6-deoxy-*O*-α-D-glucopyranosyldiglyceride; Weenink (*22*), who demonstrated that the major lipids of the acetone-solubles of red clover are mono- and digalactolipids; and Sastry and Kates (*19*), who isolated and characterized mono- and digalactosyldiglycerides from runner-bean leaves. The purpose of this paper is to report some observations on the nature of the lipids in dehydrated alfalfa meal.

The dehydration of alfalfa is usually accomplished at initial air temperatures above 550° C., although the temperature of individual pieces may vary considerably (*16*). Analysis of the lipids of dehydrated alfalfa might be expected to be complicated by the products of oxidation, degradation, or rearrangement. A comparison of the results obtained in this study with those obtained from fresh alfalfa is in progress.

Material and Methods

Alfalfa meal was obtained immediately after dehydration from the Dixon Dryer Co., Dixon, Calif., transferred to 1-gallon, crimped-top cans, and stored at −18° C. The lipids were isolated from the alfalfa meal by continuous extraction with 95% ethanol for 48 hours in a modified Soxhlet apparatus. The ethanolic solution was dried in vacuo at 45° C. with a flash evaporator (Laboratory Glass and Instrument Co.). The residue was taken up in chloroform; the deep green chloroform solution was washed twice with water and evaporated as before. The residue was a green, viscous material amounting to 6.4% by weight of the original alfalfa. It was again dissolved in chloroform and stored under nitrogen at −18° C., protected from light until used. Efforts to separate phospholipids from the main lipid with cold (0° C.) acetone were unsuccessful.

The lipids were separated into fractions by silicic acid (Mallinckrodt, 100 mesh, analytical grade) chromatography. Chloroform with increasing amounts of methanol was the eluent (*21*). The various fractions were saponified by the method described by James (*15*). The saponified acids were methylated with 2,3-dimethoxypropane (courtesy of The Dow Chemical Co.) (*18*), and the resulting methyl esters were analyzed by gas chromatography. Diethylglycol succinate was used as the liquid substrate, and siliconized (*5*) firebrick (30 to 60 mesh), as the stationary phase.