studied. The swine cornea preparations contained a total of 4 components, 2 of which sedimented at rates different from components in the other preparations. The above sedimentation values for the components in the mixtures cannot be taken as sedimentation coefficients of the respective components because these values were not corrected to conditions which would obtain in a theoretical medium having the density and viscosity of water at 20°C at zero protein concentration (standard conditions).

*Conclusions.* These studies show that there are fundamental differences in number of protein fractions and particle size distribution in extracts of water soluble corneal proteins from 3 different mammalian species. A marked difference was also noted in number of fractions and particle size distribution in preparations from New Zealand White rabbits and mongrel rabbits.

*Summary.* 1. Methods of concentrating soluble proteins are reported for aqueous extracts of cornea. These methods apparently do not denature the proteins. 2. These methods were applied to increase concentration of protein from aqueous extracts of bovine, swine, New Zealand White rabbit and mongrel rabbit corneas to a level which would permit ultracentrifugal analysis of these extracts. 3. Fundamental differences in number of protein fractions and particle size distribution were noted in these extracts. This suggests a species difference in soluble corneal proteins. 4. Differences were noted in sedimentation diagrams of extracts from New Zealand White rabbits and mongrel rabbits, suggesting differences in soluble corneal protein content of different strains of the same species.

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## Comparative Fat and Fatty Acid Intestinal Absorption Test Utilizing Radioiodine Labeling—Results in Normal Subjects.* (22515)

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Disorders of intestinal absorption may be divided arbitrarily into two groups: (a) those with deficiency of digestive enzymes; and (b) a group with normal enzyme pattern but impaired absorption due to intestinal dysfunction. The most direct way to distinguish between (a) and (b) is by analysis of duodenal contents for enzymes(1,2). Another method, admittedly indirect, involves measurement of products of digestion and absorption in the blood. In theory, a person with a normal enzyme pattern and intestinal absorption would show a comparable response to ingestion of both unhydrolyzed and hydrolyzed substances; a person lacking enzymes but possessing normal absorptive ability should show a normal response only to the hydrolyzed form. This hypothesis has been thoroughly tested in starch-sugar(3) and in protein-amino acid(4,5) tolerance tests. We have devised a similar test for fats utilizing $I^{131}$-labeled fats and fatty acids. This report describes the technique and results of its use in normal human subjects.

*Methods.* The whole fat selected was commercial olive oil since it is not unpalatable, has a high iodine number, 79-88, and an absorption coefficient of 98. It consists of approxi-

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mately 75% glycerine trioleate and its major fatty acid, oleic acid, can easily be obtained and iodinated. By a modification of previous methods(6), we have carried out the iodination as follows: one ml of 1% sodium iodide containing 500 $\mu$c of $I^{131}$ is added to approximately 50 ml of acidified chloroform. This purple-colored solution is extracted with additional chloroform and the iodine is then halogenated by a stream of chlorine gas forming iodine monochloride (Wijs' method). The end point of halogenation is the disappearance of the purple color. Eighty grams of olive oil or oleic acid in an equal volume of chloroform are then added and the solution is allowed to stand for 24 hrs after which it is washed 5 times with 60 ml amounts of 10% sodium carbonate in water removing any free iodine and precipitating any free fatty acid. Oleic acid is washed with 10% sodium thiosulfate instead of carbonate. The solution is dried over anhydrous sodium sulfate and filtered until clear. The chloroform is then evaporated under vacuum while bubbling nitrogen through the solution. The resultant iodinated oil or fatty acid is clear and is odorless with respect to chloroform. Eighty % of the initial radioiodine can be expected to become bound to fat or fatty acid with the remainder lost in the washings. Twenty-seven patients without evidence of gastrointestinal disease served as subjects. Lugol's Solution (10 drops b.i.d.) was administered for 2 days prior to the test to saturate the thyroid. $Iodine^{131}$ tagged olive oil was used as the test material in 7 subjects and oleic acid in 20. Following an overnight fast the test material (5-10 ml containing approximately 50 $\mu$c of radioactivity) was administered by mouth, the precise volume ingested being determined by difference in weight of the vessel. One hour later breakfast was given and the subjects were allowed to be ambulatory. Blood, urine, and stool were collected at frequent intervals up to 72 hours (Table I). Serums were separated and stools prepared by homogenization. Samples were pipetted into counting tubes and counted to an accuracy ( $\pm$ 1.S.D.) of 2% or better in a well type scintillation counter, proper decay factors and coincidence corrections being applied when necessary. Dilution of the standards for counting was carried out in ether. All results were expressed in percentage of total dose given. Figures for serum samples represent percentage of the total dose which is in the entire blood stream at the time of sampling and are based on an assumed serum volume of 4.5% of body weight. Urine and stool data are each expressed in cumulative figures calculated into percent of dose administered.

TABLE I. Percentage of Ingested Material in Blood Stream, Urine, and Stool at Various Time Intervals.

| | Oleic acid | | | Olive oil | | |
|---|---|---|---|---|---|---|
| | Subjects | Mean | S.D. | Subjects | Mean | S.D. |
| Serum | | | | | | |
| 1 hr | 15 | 1.8 | .7 | 6 | 3.8 | 1.6 |
| 2 | 18 | 3.5 | 1.7 | 6 | 9.0 | 4.0 |
| 3 | 16 | 5.2 | 1.9 | 7 | 13.1 | 3.6 |
| 4 | 20 | 6.7 | 1.9 | 7 | 14.7 | 4.0 |
| 6 | 20 | 8.0 | 2.2 | 7 | 11.2 | 3.6 |
| 8 | 7 | 7.1 | 2.3 | — | — | — |
| 24 | 20 | 2.9 | 1.1 | 7 | 3.3 | .7 |
| 48 | 4 | 1.0 | .4 | — | — | — |
| Urine* | | | | | | |
| 12 hr | 13 | 30.3 | 10.0 | 5 | 47.9 | 12.6 |
| 24 | 13 | 54.2 | 8.9 | 5 | 70.3 | 21.6 |
| 48 | 9 | 72.3 | 10.0 | — | — | — |
| 72 | 7 | 79.2 | 9.1 | — | — | — |
| Stool | | | | | | |
| 72 hr | 7 | 14.5 | 6.2 | — | — | — |

* Urine figures are cumulative.

*Results.* The data for our normal subjects are presented in the accompanying table and figure. Of particular interest is the fact that radioactivity appears in the serum at a higher concentration following the ingestion of tagged fat than fatty acid. This is true of all sampling times individually including the peak concentration which reaches 14.7 $\pm$ 4.0 (S.D.) % of dose in the case of fat but only 8.0 $\pm$ 2.2 (S.D.) % for fatty acid. Peak serum concentration is reached in 3½ to 4 hours with fat but not until 5-6 hours with fatty acid. In both cases only 2 to 4% of the dose remains in the blood stream at 24 hours. Urinary excretion is likewise more rapid in the case of fat with some 70% of the radioactivity recovered in the urine within 24 hours but only 55% with fatty acid. More prolonged collection leads to nearly complete

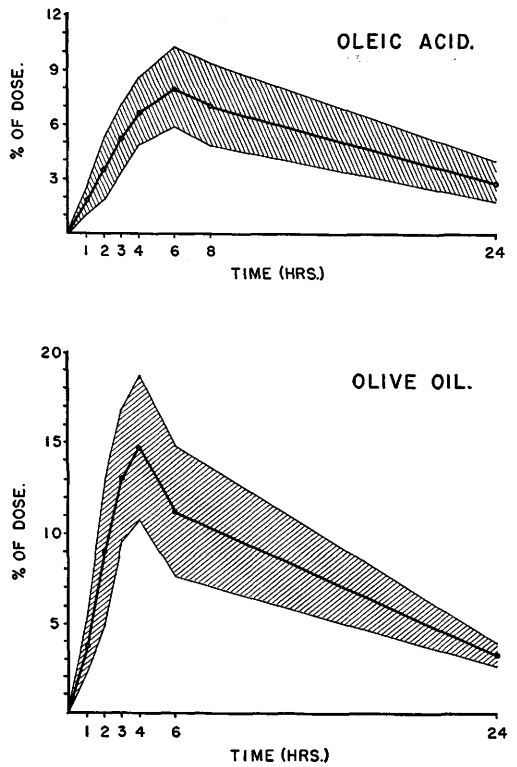


FIG. 1. Percentage of ingested $I^{131}$ dose in circulation at various time intervals. Based on serum volume of 4.5% of body wt. Zero time is time of ingestion. Shaded areas represent plus or minus one standard deviation.

recovery when urine and stool figures are added. Our data for the fat studies agree closely with those of others(6,7). Fatty acid figures have not been previously available in humans to our knowledge.

*Discussion.* The validity of radioactive-tagged fat technics in the study of intestinal absorption rests on the assumption that the $I^{131}$-fat complex remains intact during passage through the intestinal membrane. This is probably true since the iodine is incorporated chemically and much higher levels are achieved when carrier-free iodine is given(8). Furthermore, the major part of the iodine is in the lipid fraction of blood in the early stages of absorption(6). If the label remains intact during passage through the intestinal wall then the presence of radioactivity in the blood stream indicates absorption regardless of the durability of the tag after absorption. Unless there is inconsistent extravascular sequestering, it is probable that the serum levels achieved during the early hours of sampling are closely proportional to the actual amount of fat or fatty acid absorbed since disposal is slow at first. It is our current opinion from preliminary analysis of serum curves in about 200 studies in patients with a variety of disorders(8) that serum curves are more informative than cumulative stool or urinary excretion figures. Even without serum figures, however, considerable information can be gained from simple 24 hour urinary excretion figures when the principle of comparative fat and fatty acid absorption is employed. The explanation for differences in serum radioactivity following ingestion of fatty acid and fat in normal subjects is not apparent. Some studies(9-11) have suggested that fatty acids are absorbed by way of the portal system thus presenting them to the liver in high concentration while fats are absorbed by way of the lymphatics. Others (12-15), however, have failed to find any difference in routes of absorption. Possibly gastric emptying time or transit time to level of predominant absorption is different for the two substances. The presence of iodine in the molecule may alter transport across intestinal cells with the more profound effect in the case of the smaller molecule. We do not feel that the 25% portion of olive oil which is made up of molecules other than trioleate is responsible for the differences observed since our results with olive oil are almost identical with those reported for trioleate(7). It is quite possible that the oleic acid is absorbed equally or more rapidly than the olive oil but that differences exist in rate of disposal from the blood stream. Since urinary loss is actually slower following oleic acid, the difference would have to be in more widespread distribution within the body or selective concentration in certain tissues. There is no appreciable uptake by the thyroid in properly prepared patients(8). That the difference may be one of absorption is suggested by the higher fecal recovery following fatty acid administration.

It should be emphasized that the differences between the oleic acid and fat tests in normal

subjects do not lead to confusion when the test is applied clinically. In patients with enzyme deficiencies oleic acid gives higher serum concentrations of radioactivity than does fat(8) which is the reverse of the case with normals. Furthermore, the serum levels observed in abnormals are usually so much lower than those in normals that interpretation is not difficult.

*Summary.* A test has been proposed for evaluation of intestinal malabsorption states. It involves administration of isotopically tagged whole fat on one day and fatty acid on a separate occasion. By comparison of resultant radioactivity in blood and urine one can judge whether an abnormality found is due primarily to lack of proper digestive enzymes or to intrinsic difficulty with the absorption process. Normal figures with standard deviations are given for serum levels of radioactivity and for urinary and fecal loss. It was found that in normal subjects serum concentrations of radioactivity were lower following ingestion of tagged fatty acid than whole fat and the possible significance of this difference is discussed.

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## Effects of Orally Inoculated Bacteria on Gastrointestinal Flora of Newborn Mice. (22516)

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This study was undertaken to ascertain if overwhelming feedings of both normal and abnormal microorganisms at birth would produce any obvious changes in the ecology of the intestinal bacterial flora and in the health and development of white mice. Since part of the normal flora is already established by the fifth day after birth(1), the organisms were fed to newborn mice, in order to eliminate the possibility that the preexisting flora would prevent the establishment of these organisms. Because of the recent interest in *Pseudomonas aeruginosa* and *Proteus vulgaris* as agents of infantile diarrhea, they were chosen as 2 of the test organisms.

Most of the attempts to produce experimental shigellosis have been with young or adult laboratory animals. Since the antagonistic action of the gastrointestinal flora of

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