each series were also injected with 1% and 0.1% suspensions of the same virus material. One series of 12-day eggs was injected with 0.2 cc doses of a 10% suspension of glycerolated BK virus cord pool. Two series, 9- and 12-day eggs were injected with 0.3 cc of a 10% suspension of glycerolated St virus cord pool. Three or more eggs were injected in each series and after 3 to 4 days' incubation the embryonic nervous tissue and the yolk sacs were recovered separately from each egg, pooled, ground and injected into monkeys to determine infectivity. All results were negative in the 22 monkeys employed. Control animals given the virus suspensions used in the egg injections showed typical infection.

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A Spectrophotometric Method for the Study of Fat Transport and Phosphorylation*

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The study of fat transport has been facilitated by the use of labelled molecules whose fate in the animal body could be followed. The tracers thus applied to fat transport studies have been: iodized fatty acids,[1] elaidic acid,[2] deuterium,[3] and the radioactive isotope of phosphorus.[4] Miller and Burr[5] have followed the transport and distribution of tung oil in rats by utilizing the characteristic absorption band of eleostearic acid which makes up more than 90% of tung oil. The characteristic absorption spectra of eleostearic acid, due to its 3 conjugated double bonds, makes possible the distinction of this acid from normal body fats by spectroscopic analysis. However, tung oil offers two disadvantages. First, it is poorly tolerated by animals. And second, one double bond may be selectively destroyed

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1 Artom, C., *Arch. intern. physiol.*, 1933, **36**, 101.

2 Sinclair, R. G., *J. Biol. Chem.*, 1935, **111**, 515.

3 Schoenheimer, R., and Rittenberg, D., *J. Biol. Chem.*, 1935, **111**, 163.

4 Hevesy, G., *Nature*, 1936, **136**, 754.

5 Miller, E. S., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 726.

(by hydrogenation or some other method) giving rise to a new band at 2350 Å which may slightly affect the calculations. An activated corn oil has been found to overcome both difficulties.

Moore,[6] and later Kass, Miller, and Burr,[7] found that if fatty acids more unsaturated than oleic were subjected to prolonged saponification there was a marked increase in ultraviolet absorption due to a conjugation of the double bonds, and that the position of spectral absorption was dependent on the degree of unsaturation. Corn oil (Mazola), which contains a large amount of linoleic acid. but no acid more unsaturated than linoleic, gives rise to a single band at 2350 Å with an absorption coefficient, $E^{1\%}_{1cm}$ of about 500 when subjected to a modification of Moore's saponification treatment.[8] Normal body fat has an $E^{1\%}_{1cm}$ at 2350 Å from about 5 to 15, depending on the tissue or organ from which it is extracted. After feeding this conjugated fat, any absorption at 2350 Å over the basal level for the particular tissue and fat fraction being studied will be due to tagged fat incorporated in the body fat. By accurate spectroscopic measurement at this wave length the exact amount of this fat present may be determined. Because of the great tendency for unsaturated fatty acids with conjugated double bonds to oxidize, certain modifications of the conventional methods of extraction and spectrophotometric analysis of tissue fats have been made.

Method. The tagged fat is fed by stomach tube in the form of the methyl or glycerol esters of the conjugated fatty acids of corn oil. After a predetermined interval of time the tissues to be examined are removed from the animal and immediately placed in a boiling water bath for 5 minutes. It has been found[9] that enzymatic transfer of phospholipid fatty acids proceeds rapidly in certain excised tissues, and heating apparently stops this action. The tissue is then minced and frozen with solid carbon dioxide. The frozen tissues are then dried *in vacuo* at a pressure less than 1×10^{-4} mm Hg, ground fine, weighed and extracted with Bloor's mixture (3 parts alcohol and 1 part ether). This extraction and all subsequent evaporations must be carried out under nitrogen. A convenient way to accomplish this is to extract under a reflux condenser in a flask with a side arm. A constant stream of nitrogen is allowed to run in the side arm while the extraction is proceeding. After ex-

[6] Moore, T., *Biochem. J.*, 1937, **31**, 138.

[7] Kass, J. P., Miller, E. S., and Burr, G. O., *J. Biol. Chem.*, 1938, **123**, LXVI.

[8] Kass, J. P., Miller, E. S., and Burr, G. O., unpublished data.

[9] Barnes, R. H., Miller, E. S., and Burr, G. O., PROC. SOC. EXP. BIOL. AND MED., in press.

tracting 1.5 to 2.0 hours the alcohol-ether mixture is evaporated under partial vacuum, a slow stream of nitrogen still running in the flask, and the residue extracted by washing several times with low boiling petroleum ether. The petroleum ether is filtered into a volumetric flask and evaporated *in vacuo*. The extract is then taken up in aldehyde-free ethyl ether and made to volume. An aliquot portion of the ether solution is taken for the determination of tagged fat in the "total fat extract." The remainder is evaporated almost to dryness and the phospholipids precipitated with acetone. For convenience and protection against exposure to atmospheric oxygen for longer than necessary, the precipitation is best carried out in a centrifuge tube and centrifuging rather than filtration used in separating the phospholipids. The acetone-soluble layer is poured off, washed, then evaporated and made up to volume in aldehyde-free ether. If the tissues are placed in a boiling water bath immediately after excision, and caution employed in preventing oxidation, the phospholipids will readily dissolve in ether and may then be made up to volume.

The aliquot portions of the acetone-soluble and insoluble fractions thus prepared are taken for spectrophotometric analysis. The remainder of the ether solutions is evaporated *in vacuo* and weighed. Spectral absorption measurements cannot be made on the weighed material because of the difficulty in evaporating to absolute dryness

TABLE I.

Fraction description	Results in percentages			
	Absorption time 5 min.		15 min.	
	Control	Iodoacetic acid	Control	Iodoacetic acid
1. Acetone-soluble extract in dry mucosa	17.2	13.9	18.7	24.1
2. Acetone-insoluble extract in dry mucosa	11.0	10.5	11.0	11.7
3. Total fat in dry mucosa (1 + 2)	28.2	24.4	29.7	35.8
4. Tagged fat in acetone-soluble extract	33.7	33.9	37.4	45.0
5. Acetone-insoluble fatty acids as tagged fat	0.72	0.90	1.4	1.3
6. Total fatty acids as tagged fat	26.7	25.4	30.7	34.6
7. Acetone-soluble tagged fat in dry mucosa	5.8	4.7	7.0	10.8
8. Acetone-insoluble tagged fat in dry mucosa	0.08	0.09	0.16	0.16
9. Total tagged fat in dry mucosa	7.5	6.2	9.1	12.4
10. Recovery (7 + 8) × 100 ÷ 9	78.5	77.4	79.2	89.0

without some oxidation. The weights obtained after removal of the aliquot portions are used in the calculation of absorption coefficients and percentages of the extracts in the dried tissue by applying the proper dilution factor. Blank determinations must be made on the tissues of animals not receiving tagged fat, and these values subtracted from the experimental values.

Table I gives the results of a typical experiment. In this case the incorporation of tagged fat in the acetone-soluble and acetone-insoluble fat fractions of the mucosal tissue from the small intestine of rats was studied. An emulsion of the methyl esters of conjugated corn oil, bile salts, lipase (Pancreatin), and water was injected directly into the duodenum of fasted, male, albino rats. Some of them had iodoacetic acid included in the emulsion in a concentration of 1:10,000. Five and 15 minutes after the introduction of the fat emulsion the animals were etherized and their intestines washed out by the method of Barnes, *et al.*[10] The washed intestines were carefully removed from the animals, placed on a glass plate, and the outside gently scraped with the edge of a microscope slide. In this way the intestinal mucosa was expelled from the intestine without contamination with intestinal muscle or mesenteric fat. The mucosa was immediately placed in a boiling water bath and the extraction and analysis carried out as has been described.

Calculations: The $E^{1\%}_{1cm}$ at 2350 Å of the tagged fat fed was 493 in this experiment. The blank $E^{1\%}_{1cm}$ for intestinal mucosa was: Total fat extract, 7; acetone-soluble, 6; and acetone-insoluble, 8. Then $[E^{1\%}_{1cm}\ (^{\text{acetone}}_{\text{soluble}}) - 6] \times 100 \div 493 =$ % tagged fat in acetone-soluble fraction (Row 4). If both fatty acids of lecithin were tagged fat with an $E^{1\%}_{1cm}$ 493, then lecithin would have an $E^{1\%}_{1cm}$ 356. The percent of acetone-insoluble fatty acids, as tagged fat calculated on this basis, is $[E^{1\%}_{1cm}\ (^{\text{acetone}}_{\text{insoluble}}) - 8] \times 100 \div 356$ (Row 5). In calculating the percent tagged fat in the total fat extract the denominator factor must be calculated for each extract and depends on the proportion of the total fat which is acetone-soluble and acetone-insoluble (Row 6). The percent tagged fat of the various fat fractions in the dry mucosa (Rows 7, 8, and 9) are calculated by multiplying the percent tagged fat in the different fat fractions by the percent of the respective fractions in the dry mucosa and dividing by 100. The percent acetone-soluble tagged fat (Row 7) plus the percent acetone-insoluble tagged fat (Row 8) should equal the percent total tagged fat in the dry mucosa (Row 9). As can be seen

[10] Barnes, R. H., Wick, A. N., Miller, E. S., and MacKay, E. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 651.

in Row 10 these are approximately 20% low. This discrepancy is probably due in some part to errors in transfer, weighing, and spectrophotometric determinations, but can undoubtedly be accounted for in large measure by unavoidable oxidation. The magnitude and direction of the error has been found fairly constant, so the interpretation of data is not seriously affected.

The conjugated acids of corn oil used in this experiment had an $E^{1\%}_{1cm}$ of 493 at 2350 Å. Pure 10-12 linoleic acid has an $E^{1\%}_{1cm}$ 1200. Therefore approximately 54% of the fatty acids fed were no spectroscopically active. The calculations given above are based on the supposition that the spectroscopically active and inactive acids reacted alike in becoming incorporated with already existing body fat. This is purely an assumption and in order to express the data on the basis of spectroscopically active acids, 46% of the tagged fat values should be used.

Changes undoubtedly take place in the conjugated linoleic acid after remaining in the body for long periods of time. These changes might cause a loss of the configuration necessary for spectroscopic measurement, but still leave the intact carbon chain present in the body fat. For this reason all experiments using this tagged fat have been arbitrarily restricted to data collected not more than 6 hours after a single feeding of the fat.

Summary. By conjugating the double bonds of the linoleic acid of corn oil, a fat may be prepared which can be spectroscopically distinguished and quantitatively measured in body fat. A method for the extraction and determination of this tagged fat in animal tissues has been described.