vestigation enables some additional observations to be made. After storage at 21°C. the extracted low-temperature meal did not support as rapid growth as did the extracted meals dried at higher temperature. When the meals were stored at −25°C., the extracted low-temperature meals again did not promote as rapid growth as did the extracted meals that had been dried at higher temperatures. Of the unextracted meals the overheated commercial meal gave the fastest rate of growth. It was also observed (11) that controlled heating of herring meals dried at a low temperature did not lower the nutritive value of the meals and that in many instances chicks grew better with the heated meals. From the results of these various experiments it appears that certain changes in herring meal occur at a faster rate or take a different course when the processing and storage temperatures are low. The absence, in the present experiment, of significant differences in the biological responses obtained to the meals subjected to different temperatures is probably accounted for by the fact that, in contrast to the previous experiments, none of the meals was processed at low temperature.

Antioxidant treatment of the meal prevented a decrease in ether-extractability of the fat and to a large extent prevented a decrease in iodine value of the ether-extractable fat. The increase noted with some of the meals in the HCl-acetone extract during the first six weeks of storage appears to represent the formation of lipid complexes with protein which are split by HCl treatment to yield an ether-soluble lipid component. BHT apparently prevented the formation of these complexes. By inhibiting oxidation of the unsaturated lipid in the meal, BHT may have retarded the various types of polymerization that have been postulated for systems containing fat and protein and subjected to oxidative conditions (12,13, 14). It may be noted that postponing the BHT treatment of the meals until one week after manufacture permitted oxidative reactions in the fat that were, in this short time, apparently accompanied by polymerization with protein. Consequently the *in vitro* digestibility of the meal treated immediately with BHT was better throughout the entire storage period than that of the meal treated one week after manufacture. It is interesting that the advantage obtained with BHT treatment was constant at each sampling date. Although the amount of ether-extractable lipid remained similar for the two antioxidant-treated meals during storage, the iodine value of the meal stored without BHT for the first week dropped in the course of that week but did not change appreciably thereafter. Immediate addition of BHT to the hot meal, on the other hand, inhibited oxidation so that after one week the iodine value was higher than that of the normal meal on the day after manufacture.

Insofar as the biological tests are concerned, it should be noted that the diets fed were formulated so that in no instance was the available energy content of the fish meal a factor in the response of the chicks. The extent to which the fat content of the meals was utilized by the chick did not therefore affect the response of the chicks. With the purified diets however, in which a relatively high level of fish meal was fed, it is possible that the depression in growth noted with meal D could have resulted from destruction of vitamins in the diet by the oxidizing fat in the meal. Although all of the vitamins were present in considerable excess, they are especially labile in this type of diet. A separate study is being made of the nutritive value of the fat content of fish meal prepared and stored under different experimental conditions.

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Metabolic Studies of Glyceride Esters of Adipic Acid[1,2,3]

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Data on the digestibility, absorption, and *in vivo* oxidation of two types of adipic acid esters of glycerides, a diglyceride adipate and a polymer of fatty acids, adipic acid, and glycerol, have been presented. Findings indicate that these compounds have high digestibility coefficients and that the stearic acid moiety is well absorbed. However, although the stearic acid moieties are oxidized slowly in both cases, which is compatible with previous findings that a slow rate of *in vivo* oxidation of the stearic acid moiety of glycerides obtains (6), the rate of oxidation of the stearic acid is greater when fed as the diglyceride adipate than as the polyester.

THE POSSIBLE EDIBLE USE of polyesters of short-chain dibasic acids and glycerides has recently been suggested (1,2). The acylation of mono- and diglycerides of fat-forming acids with adipic acid produces a series of viscous compounds with a number of potentially useful properties. Thus polymers

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[2] Contribution No. 470 from the Department of Biochemistry and Nutrition, School of Medicine, University of Southern California, Los Angeles, Calif.
[3] This is a partial report of work done under contract with the U.S. Department of Agriculture and authorized by the Research and Marketing Act. This contract was supervised by the Southern Utilization Research and Development Division of the Agricultural Research Service.
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of saturated fatty acids, adipic acid, and glycerol tend to be relatively low-melting and possess a high resistance to oxidation.

The nutritional safety of adipic acid has been reported by Horn and co-workers (3). Although there are indications that the glyceride esters of adipic acid are edible, in order to ascertain the innocuousness of a foodstuff it is common to make a toxicological investigation including acute, subacute, and chronic toxicity studies. Frequently however biochemical studies such as absorption, distribution, excretion, and organ-function tests are used. It has been pointed out that, "in cases in which a biological and metabolic approach is possible, the proof of harmlessness of a food additive can be established more quickly, more cheaply, and on a more sound scientific basis than is possible with the present toxicological approach" (4).

Therefore the digestibility as well as the absorption and the *in vivo* oxidation of the stearic acid moiety of adipic acid polyesters were studied in accordance with the metabolic approach to obtain at least some data toward determining the nutritional safety of these materials.

Materials. Radioactive diglyceride adipate was prepared by the random interaction of stearoyl-1-C^{14} chloride (0.0066 mole), oleoyl chloride (0.0198 mole), and bis(2,3-dihydroxypropyl) adipate (0.0060 mole) in a chloroform solution and in the presence of pyridine (0.0290 mole). After two days at room temperature the reaction mixture was purified by washing successively with dilute acid, dilute alkali, and water. The structural formula for the reaction product is given in Figure 1.

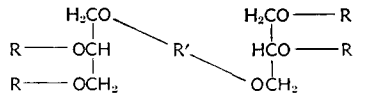

FIG. 1. Structural formula of the radioactive diglyceride adipate. *R* represents oleoyl and stearoyl groups (present in the ratio of 3:1); some stearoyl groups were labelled in the carboxyl position with carbon-14. *R'* represents the adipic acid group.

In the preparation of the radioactive polymeric fat, adipyl chloride (0.0126 mole), 1-monostearin, labelled in the carboxyl carbon of the stearic acid (0.0084 mole), 1-mono-olein (0.0084 mole), and pyridine (0.0462 mole) in chloroform solution were allowed to interact at room temperature for two days. Then a chloroform solution of unlabelled stearoyl chloride (0.0042 mole) and oleoyl chloride (0.0042 mole) were added, and the reaction was allowed to proceed for an additional two days. The reaction product was purified by washing successively with dilute acid, dilute alkali, and water. Figure 2 represents the structure of a typical molecule present in the fat product.

The unlabelled diglyceride adipate was obtained by converting peanut oil fatty acids to 1,3-diglycerides (diglyceride content, 98%) and then treating these diglycerides with adipyl chloride in the presence of quinoline; the reaction was carried out in chloroform solution. The reaction product, which was purified in the usual manner, contained 2.02% free fatty acids and had a saponification value of 245.4.

The unlabelled polymeric fat was obtained by the random esterification of six moles of peanut oil fatty

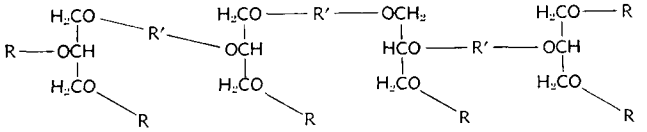

FIG. 2. Structural formula of a typical molecule of the radioactive polymeric fat. *R* represents oleoyl and stearoyl groups (present in equal proportions); some stearoyl groups were labelled in the carboxyl position with carbon-14. *R'* represents the adipic acid group.

acids, three moles of adipic acid, and four moles of glycerol. Stannous chloride dihydrate (0.001 mole/100 g. of total acids) was used to catalyze the reaction, which was carried out over the temperature range of 140 to 200°C. and at a pressure of 100 mm. of mercury. The reaction product was washed with dilute acid, bleached with activated clay and carbon, and deodorized by steam distillation at a low pressure. Analyses: combined glycerol, 15.7%; saponification value, 281.5; hydroxyl value, 25.7; number average molecular weight, 1633; and free fatty acids, 1.8%, calculated as peanut oil fatty acids.

Digestibility Studies. In these studies digestibility coefficients of unlabelled diglyceride adipate and unlabelled polymeric fat were determined as follows.

Male and female rats of the University of Southern California strain, which had been maintained on a commercial pellet diet (Rockland Rat Diet) for 15 weeks of post-weaning were pre-fed these two fats at a level of 15% of a synthetic diet for a three-day orientation period. The composition of the diets is listed in Table I. The animals were continued on

TABLE I
Composition of Diet

Component	Percentage
Sucrose	52.55
Casein (comm.)	24.00
Fat	15.00
Wesson salt mixture (5)	4.00
Celluflour	4.00
Choline	0.24
Vitamins [a]	0.212

[a] Fat and water-soluble vitamins. Used in adequate amounts.

these diets for nine days subsequently in the case of animals fed the polymeric fat from peanut oil fatty acids and for seven days in the case of animals fed the diglyceride adipate. During these periods feces were collected from five animals of each sex. At the conclusion of the experimental period the individual samples of feces were dried, weighed, and ground to a fine powder. Aliquots were acidified, then extracted with chloroform:methanol (2:1) to determine the unabsorbed fat.

Absorption and Oxidation Studies. Fasted adult male rats weighing between 200 and 220 g. were fed a known amount, approximating 0.5 g., of the radioactive glyceride ester of adipic acid in a 30% fat diet. One animal was used in each experiment. The animals were then placed in an all-glass metabolism [7] apparatus for 8 hrs. Hourly collections were made of expired carbon dioxide, which was trapped in 2*N* NaOH. Total carbon dioxide expired during each hour was determined titrimetrically, and the C^{14} content was determined after precipitation as barium carbonate.

[7] Delmar Scientific Laboratories, Chicago, Ill.

The C^{14} activity in the intestinal contents was also determined. In this instance aliquots of the intestinal washes were plated directly.

Radioactivity was determined on an auto scaler (Nuclear) and gas-flow counter. All counts were corrected for background and self absorption.

Results and Discussion

The digestibility coefficients, presented in Table II, reveal that in female rats these fat products are absorbed as readily as are ordinary fats. The digestibility coefficients of approximately 93% compare well with the digestibility coefficients of approximately 94% reported for cottonseed oil and other edible oils in female rats. The digestibility of the adipic acid polyesters or glycerides in male rats is not as complete, which is a common observation for other fats as well.

TABLE II
Digestibility Coefficients of the Unlabelled Adipic Acid Esters

Type of ester	Digestibility coefficients[a] %	
	Males[b]	Females[b]
Polymer	84 ± 1	93 ± 1
Diglyceride adipate[c]	89 ± 2	93 ± 1
Cottonseed oil[d]	92 ± 1	94 ± 1

[a] (Fat intake [total fecal fat-endogenous fecal fat]) 100/fat intake.
[b] Includes standard error of the mean.
[c] Endogenous fecal fat, as determined in animals simultaneously fed fat-free diets, was 0.065 g./g. feces for males and 0.0436 g./g. feces for females.
[d] Endogenous fecal fat, as determined in animals simultaneously fed fat-free diets, was 0.0181 g./g. feces for males and 0.0104 g./g. feces in females.

The digestibility coefficients also correlate well with the degree of absorption of the C^{14} stearic acid moiety of both diglyceride adipate and the polymeric fat during an 8-hr. experimental period, which is approximately 81% for both fats (Table III).

TABLE III
Absorption and Oxidation of Adipic Acid Esters Labelled with Stearic-1-C^{14} Acid[a]

Category	Diglyceride adipate			Polymeric fat			
	I	II	Av.	I	II	III	Av.
C^{14} absorbed in 8 hrs., %	73	88	81	91	71	82	81
Absorbed C^{14} expired, %	43	15	29	11	10	....	11

$$C^{14}\text{ absorbed} = \frac{C^{14}\text{ ingested} - C^{14}\text{ intestinal contents}}{C^{14}\text{ ingested}}(100)$$

However the percentage of the absorbed C^{14} material expired during the 8-hr. experimental period differs markedly for the two fats (Table III). In one experiment four times as much of the radioactivity from stearic acid appears in the expired carbon dioxide when the diglyceride adipate is fed than when the polymeric fat is fed. However, in a second experiment, there was no significant difference in the percentage of absorbed C^{14} expired between the animals fed diglyceride adipate and those fed the polymeric fat. These results indicate that perhaps the rate of hydrolysis of the stearic acid from the diglyceride adipate is greater than that from the polymer.

In Figure 3 the total C^{14} expired after the administration of diglyceride adipate and polymeric fat is presented graphically. Although there is some variation between experiments in the animals fed the diglyceride adipate, results indicate that the stearic acid moiety of this compound is oxidized, and possibly absorbed, more rapidly in comparison with the polymeric fat.

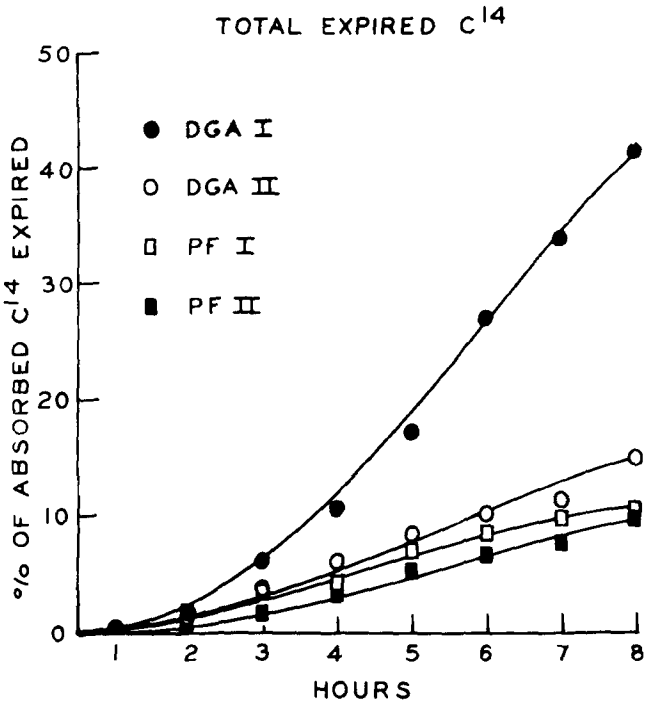

FIG. 3. Cumulative $C^{14}O_2$ expired after the administration of C^{14}-stearic acid-containing glyceride esters of adipic acid. ○, Diglyceride adipate, Experiment I; ●, diglyceride adipate, Experiment II; □, polymeric fat, Experiment I; ■, polymeric fat, Experiment II.

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