THE NUTRITIVE VALUE OF PURE FATTY ACID ESTERS*

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There is an extensive literature dealing with the utilization by the animal body of various complex natural triglycerides, but up to the present time little attention has been paid to the separation and study of the individual fatty components for their ability to supply energy and support growth. There is no apparent reason to suppose that the individual components of a fat should be any less efficient from a nutritional standpoint than is the original mixture. Our views concerning the metabolism of fats recognize no essential difference in the method of combustion and transport of different fatty acids. The question has taken on an added interest with the recent discovery by Burr and Burr (1) of a particular fatty acid essential for well being. The writer undertook to fractionate a natural fat (coconut oil) and to study its various components by feeding experiments upon rats of the Wisconsin inbred strain.

Certain fatty glycerides have previously been investigated. Davis (2) fed 15 per cent of tributyrin to chickens and reported that the material was distinctly toxic. Eckstein (3) fed 15 per cent of tricaproin to rats and found good growth, although no caproic acid could be detected in the body fat. Powell (4) investigated tricaprylin and trilaurin by feeding these substances to adult, previously fasted rats at a 25 per cent level. She found traces of caprylic acid in the body fat of rats fed tricaprylin, and the body

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The expenses of this investigation were defrayed in part by a grant from Mead Johnson and Company for the study of fat metabolism.
fat of rats receiving trilaurin contained some 25 per cent of laurie acid. Rats fed tricaprin deposited 15 per cent of capric acid in the body depots (5). Ozaki (6) has studied the growth-promoting effect of various fats, purified esters, and synthetic compounds. He restricted the food intake of young rats to 9 gm. a day, and when they came to constant weight allowed them, ad libitum, the same diet with varying amounts of fats. He expressed the relative Nährwert of different compounds as gm. of weight gained above the constant weight level. It is evident that 9 gm. of food per day will allow a young rat to grow to almost an adult weight, and that weight changes after a long preparatory period, followed by ad libitum feeding, are subject to more factors than can be attributed to the type of fat employed in the diet. Eckstein (7) fed 17.6 per cent of free myristic acid to rats, and observed good growth.

Balance experiments with stearic esters and palmitic esters were initiated by Arnschink (8) in 1890, and Frank (9) in 1898. Arnschink found that two dogs absorbed 9 and 13.8 per cent of tristearin, respectively, and Frank recorded the absorption of ethyl stearate by dogs as 12.7 per cent, and of ethyl palmitate as 86.3 per cent. More recently Lyman (10) reported the absorption of ethyl stearate and ethyl palmitate in dogs to be 10 and 50 per cent, respectively, and of the corresponding glycerides as 12 and 95 per cent. The unabsorbed fat was chiefly in ester form. Müller and Murschauser (11) found these esters to be better absorbed by dogs than have other investigators. Respiratory experiments showed that the absorbed material was utilized.

It is evident that previous investigators have used different species of experimental animals receiving diets of varying composition, and that the purified fat constituent was fed at relatively low levels. With the exception of the toxicity of ethyl butyrate to chickens no marked deleterious effect from other esters has been reported, although the absorption of the compounds seemed fairly good until the longer chain acids were employed.

**EXPERIMENTAL**

Coconut oil was saponified with alcoholic potassium hydroxide, acidified with sulfuric acid, and the fatty acid layer washed several times with water. 95 per cent alcohol (4 moles of alcohol per mole of fatty acid) containing 3 per cent HCl and anhydrous CaCl₂ were
added, and the mixture refluxed overnight. The mixed ethyl esters after washing contained less than 2 per cent of free acid. This was removed by shaking with dilute ammonia in 50 per cent alcohol. The mixed esters were fractionated in a 6 foot laboratory fractionating column.

The diet was composed of 20 per cent casein (acid-washed), 55 per cent fat, 13 per cent maize starch, 6 per cent salts (12), 5 per cent dried brewers' yeast, and 1 per cent cod liver oil. 77.0 per cent of the calories was furnished by the variable fat. To keep the very liquid esters from separating from the other diet constituents, a starch paste of suitable consistency was prepared and the fat whipped in with an electric mixer. The remaining ingredients were then added and the mixture stirred until a smooth paste was obtained. After rapid cooling the diets were stored in the ice box. Little or no separation of the esters took place. The diets were freshly made not less often than every 2 weeks. Four rats, two males and two females, weighing approximately 50 gm. at the age of weaning, were placed in wire bottom screen cages, and allowed to eat of the diet ad libitum. The food cups were weighed, cleaned, and refilled daily, and record kept of the food consumed.

Results

There is but little information in the literature on the relative ease of intestinal lipolysis of glycerides as compared with ethyl esters of fatty acids. However, there is no reason to suppose that the more fluid esters are less readily split than the higher melting glycerides. Considerable simplification was effected in the present work by feeding the ethyl esters of the acids. This position is substantiated by the weight curves of control rats fed lard and coconut oil (as the fat constituent of the diet) when compared with the weight curves of rats receiving ethyl esters of lard and of coconut oil (Fig. 1). It is evident that there is no significant difference between the slopes of any of the first four control curves or of that of the fifth curve—obtained by feeding all of the esters of coconut oil which were distillable at 15 mm. Lard was used as the control fat, inasmuch as good growth had been obtained by Smith and Carey (13) after feeding it at a level even higher than that employed here.
Fig. 1. Composite weight curves of rats, two males and two females on each diet, fed various fats at a level of 55 per cent of the diet (77 per cent of the calories). Fraction A was composed of the ethyl esters of coconut oil which distilled below ethyl laurate. Fraction B was ethyl laurate. Fraction C contained all esters of coconut oil boiling higher than ethyl laurate which were distillable at 15 mm. pressure.
In a preliminary study the coconut oil esters were separated into three fractions: compounds boiling at a temperature lower than ethyl laurate were distilled under 100 mm. pressure, and incorporated in the above diet as Fraction A; ethyl laurate, comprising some 50 per cent of coconut oil, was fed as Fraction B; and all esters distillable at 15 mm., above ethyl laurate, were collectively termed Fraction C. The composite growth curves are given in Fig. 1. Fraction A was markedly inferior in growth-permitting ability to the control fats; the animals survived and maintained their weights, but growth was subnormal. The behavior of rats on Fraction B, ethyl laurate, was most surprising. Almost without exception the animals receiving this fat died suddenly before the 14th day of the experiment. Very good growth was obtained with Fraction C.

Following the unexpected results after feeding the only pure compound used, ethyl laurate, about 80 liters of coconut oil esters were fractionally distilled in order to obtain the single esters which constituted the previous fractions. This allowed the preparation of ethyl caproate, ethyl caprylate, ethyl laurate, and ethyl myristate in sufficient quantities to permit separate investigation. The series was completed by adding triacetin and ethyl butyrate, purchased from the Eastman Kodak Company, and ethyl palmitate and ethyl stearate obtained by fractional distillation of Procter and Gamble’s commercial stearic acid.

The average weight curves obtained when these substances were incorporated in the basal diet at a level of 55 per cent, are presented in Fig. 2. Over a 60 day period only two esters allowed fair growth, ethyl myristate and glycercyl acetate; one, ethyl caprylate, allowed survival of the animals but no growth; ethyl butyrate, ethyl laurate, ethyl palmitate, and ethyl stearate resulted in the death of all animals within 14 days. Three of the four animals on ethyl caproate died, but the one which survived gained weight fairly well. Two of the four receiving ethyl caprate died; the survivors exhibited mediocre growth.

Although the relatively small number of rats in each group prevents any broad generalization, the following interpretation seems in accord with the data. There are three distinct portions of the series in which the animals do poorly, separated by others in which good or fair results are obtained. The groups of esters
giving very poor results consist of ethyl butyrate and ethyl caproate (C₄ and C₆), ethyl caprate and ethyl laurate (C₁₀ and C₁₂), and ethyl palmitate and ethyl stearate (C₁₆ and C₁₈). These

![Composite growth curves of four rats, two males and two females on each diet, fed pure ethyl esters of the fatty acid series at a level of 55 per cent of the diet (77 per cent of the calories). Triacetin was the only glyceride employed. The numerical designation refers to the number of carbon atoms.](image)

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groups are separated by triacetin (C₃), which permits the best growth of any member of the series, ethyl caprylate (C₁₀), and ethyl myristate (C₁₄). Ethyl caprylate supported life but not growth; weight was almost at a standstill, the rats lost all their hair, and
stored a negligible amount of body fat—behaving in these respects similarly to those rats receiving Fraction A. Ethyl myristate supported fair growth, although not equal to that of rats receiving the mixed esters. There was a marked flattening of the weight curve before a normal adult weight was reached.

The cause of death in the three fatal regions does not appear to be identical. Food consumption records explain the death of the animals receiving ethyl butyrate; they died since they would not partake of the odorous ester. The same explanation probably holds true for the three animals which died within 10 days after being offered the ethyl caproate diet. Food records indicate sufficient caloric intake to allow one animal to survive; one did survive and grew fairly well.

Ethyl Palmitate and Ethyl Stearate—The animals receiving diets containing these esters likewise died, but for a different reason. Although both compounds are liquid at the body temperature of the rat, after saponification the free acids melt considerably above that point. Consequently the free acids or soaps were unavailable for metabolic purposes and were excreted. This was grossly evident from the physical appearance of the fecal pellets; they were numerous, very large, white, hard masses. Fig. 3 shows the percentage retention of ethyl stearate, and the form in which the fat was excreted in the feces. It is worthy of note that the majority of the excreted lipids was free acid and soap, while there was but little relative increase in the excretion of unsplit ester. This would seem to answer the question of the relative lipolysis of ethyl esters and glycerides. This is in contradistinction to the findings of Frank (9) and Lyman (10) on dogs, as most of the lipid excreted by these animals, after saturated ethyl esters were fed, was unsplit. The figures for retention of ethyl stearate are in close agreement in both species.

An attempt to accustom rats to ethyl stearate by feeding decreasing percentages of lard mixed with the stearate was unsuccessful. The rats died when transferred to a pure ethyl stearate diet.

Ethyl Laurate—After the death of the first four rats receiving ethyl laurate, various other groups were employed, in all twenty-five animals. Uniformly, death ensued within 2 weeks. Glyceryl

1 I am indebted to Miss Dorothea M. Cross for these analyses.
laurate caused similar results. Evans and Lepkovsky (14) report that 30 per cent of their animals receiving glyceryl laurate (at a level of 60 per cent) died very suddenly before the 50th day of life. They were unable to explain this phenomenon, and no physical treatment which might have purified the material seemed to lessen the toxicity. Strach and coworkers (15) have recently reported that methyl laurate introduced directly into the small intestine of dogs is toxic.

![Figure 3](http://www.jbc.org/)

**Fig. 3.** The percentage retention of ethyl stearate when fed at a level of 55 per cent of the diet (77 per cent of the calories), and the distribution of the excreted fat.

The animals eat the diet containing this fat as well as they do when other fats are used, and during the few days of life they gain weight, and appear well—until just before death. Metabolism experiments show the material to be almost completely absorbed.

Dr. G. Lyman Duff of the Department of Pathology, the Johns Hopkins Medical School, very kindly made a complete histological examination of these rats. Ten animals were placed in two cages, and when the first three had died, all were killed. Stomach, heart, kidney, brain, liver, bone marrow, and spleen were examined. No pathological condition was found. Cultures of peritoneal contents taken with aseptic precautions were negative. Blood chemistry studies were likewise negative.
The rats used in these experiments weighed between 40 and 60 gm. when placed on the test. It was thought worth while to put larger animals on the diet, to see if death would similarly occur. Six rats weighing between 60 and 100 gm. were first tried; half of these died within the allotted time, the remaining half lived and grew subnormally. Of four adult rats, weighing more than 200 gm., none died and after losing some 10 per cent of their body weight, they regained and maintained their original weights for a period of 3 months.

On the supposition that possibly an auxiliary fat was necessary for complete utilization of ethyl laurate, a small amount (5 per cent) of ethyl palmitate was substituted for an equivalent amount of ethyl laurate. To our surprise the animals survived and grew during the crucial 2 week period, and when two of the four eventually succumbed it was apparently due to causes unrelated to the toxicity reported. Similarly, when ethyl oleate, prepared from olive oil, was substituted for 5 per cent of the ethyl laurate, the animals survived and grew fairly well.

In order to determine whether the ethyl laurate was available as a source of energy or was merely stored as inert material, the following experiment was devised. A series of diets was prepared which provided quantities of non-fatty ingredients (plus cod liver oil) identical to those eaten by normal rats of the same weight receiving the control diet. The quantity of food was restricted so that this amount of non-fatty material should not be exceeded. Thus about 10 calories per day of non-fatty materials were fed—this being quite insufficient for growth. One lot of rats was fed this basal ration alone, while others were given additional quantities of ethyl laurate, enough to provide in one instance 2 additional calories per day, and in others 5 and 10 additional calories per day. This last diet provided 50 per cent of the calories as ethyl laurate. Other diets were prepared providing 60 and 70 per cent of the calories as ethyl laurate, in contrast with the original diet in which 77 per cent of the calories had been supplied as this ester. The weight curves of this series of experiments are presented in Fig. 4. It is apparent that growth is limited by the caloric intake; the animals receiving no laurate or only small amounts failed to gain, whereas those receiving more laurate did correspondingly better. After 20 days one group of animals was allowed to eat the diet ad libitum and then gained weight rapidly. This diet, and the one
providing 60 per cent of the calories as ethyl laurate are of particular interest. The animals grew well and did not succumb as did those previously given 77 per cent of their calories as this ester. Of four rats receiving 70 per cent of their calories in the form of ethyl laurate two survived and two died.

![Graph](image)

**Fig. 4.** Average growth curves of rats receiving: Curve 1, 10 calories daily of the non-fatty constituents (including cod liver oil) of the standard diet; Curve 2, the same, plus 2 calories daily of ethyl laurate; Curve 3, the same, plus 5 calories daily of ethyl laurate; Curve 4, the same, plus 10 calories daily of ethyl laurate (at the point marked by the arrow the animals were allowed to eat of the diet *ad libitum*); Curve 5, *ad libitum*, a diet furnishing 60 per cent of the calories (35.7 per cent of the diet) as ethyl laurate.

It is apparent that the lethal effects encountered with ethyl laurate cannot be attributed to failure of utilization. No specific reason for death can be advanced. The weight of the rat and the quantity of ester appear to be significant factors. It is possible that the survival of animals in which a small amount of palmitate or oleate was substituted for laurate was due to the lowered intake
of laurate; however, it is worthy of comment that these animals, which did fairly well, actually received slightly more laurate than did the four given 70 per cent of their calories as laurate, two of which died. This point cannot be stressed because of the small series employed, but it seems possible that the addition of the longer chain esters may have lessened the toxic effect of the laurate. Further observations will be required to settle this point.

Observations on Fat Storage—It has been well known since the pioneer work of Anderson and Mendel (16) that long chain fatty acids, when fed, are regularly found in the depot fat. From the literature cited above it would appear that this also holds true for acids containing as few as 10 carbon atoms, but that only traces of 8-carbon fats have been so recognized and no shorter chains. In view of the very few observations on these short chain esters it seemed desirable to determine some of the constants of the depot fat in our rats. The fat was extracted from the carcass after removal of the abdominal and thoracic organs and the head. The carcasses were minced in a meat chopper and extracted once by shaking with cold 95 per cent alcohol, and then extracted in a continuous type Soxhlet apparatus with 95 per cent alcohol for about 2 hours. The alcohol was removed and the final extraction with ether allowed to proceed for 8 hours. The first two alcohol extractions removed some water, most of the phospholipids and coloring matter, but very little fat. The ether extract was usually a white solid or liquid uncontaminated by coloring matter. Duplicate determinations of the saponification number, and the iodine number (Wijs) of this extract were made. These values are presented graphically in Fig. 5, after conversion of the saponification value into the mean molecular weight.

The parallelism between the curves of these values is at once apparent. The fat obtained from rats receiving acids containing 2 to 6 carbon atoms differs markedly from the fat deposited when the control fat, coconut oil, was fed. Both the iodine value and mean molecular weights are higher, indicating a conversion of the short chain saturated acids into longer chain, unsaturated acids. However, with the 8-carbon acid there is the beginning of a change in metabolism: the acids from this point to the C₁₈ acid are deposited to a greater or less degree in the depot fat without change by the organism. This change is indicated by the change in the
slope of the curves occurring between C₈ and C₁₄. The iodine number of the depot fat decreases rapidly—apparently reaching a minimum iodine number of about 28. Both the C₁₂ and C₁₄ acids gave this value and although no determinations were possible after feeding C₁₆ and C₁₈ acids (because of death), it is presumed that this value is very close to the value which would have been found after feeding these acids. This minimum iodine value may be due either to (1) admixture with fat of high iodine number stored before the beginning of the experiment and carefully conserved; (2) a storage of unsaturated acids from the 1 per cent of dietary cod liver oil; or (3) extraction of the élément constant of Terroine and Belin (17) and Mayer and Schaeffer (18), characteristic of tissue fat, and, due to the method of extraction, mixed with depot fat.

The change in slope of the iodine number curve is paralleled by the curve of mean molecular weights. As fats of longer chains
are stored in increasing amounts, the molecular weight drops, reaching a minimum with lauric acid, and then rises, corresponding to the fact that the molecular weight of trimyristin, if stored pure, would be higher than that of trilaurin. The calculated mean molecular weights of the three (C₁₄, C₁₆, and C₁₈) pure triglycerides are indicated in Fig. 5 by a straight line. It is thus apparent that the depot fat of the C₁₆-fed rats approaches fairly closely the mean molecular weight of pure trimyristin. An extension of the curve indicates that if pure tristearin could be successfully metabolized the depot fat would be practically pure tristearin. These observations on the storage of depot fat confirm the findings of Eckstein and of Powell.

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SUMMARY

1. Feeding experiments have been carried out on white rats with esters fractionated from coconut oil, and the series of saturated even carbon fatty acids between 2 and 18 carbon atoms has been studied.

2. It is shown that in the rat mixed ethyl esters permit practically as good growth as do mixed triglycerides. The fat is well split, in contrast to the findings of others on dogs.

3. When individual saturated fatty acid esters supply 77 per cent of the caloric intake, nutrition, as measured by growth, is in no case equal to that obtained with mixtures of esters.

4. Three portions of the saturated series were encountered with which fatal results ensued. Death occurred when ethyl butyrate and ethyl caproate were included in the diet because the animals usually refused to eat them. Ethyl palmitate and ethyl stearate did not support life because of inadequate absorption. With ethyl caprate, but more regularly with ethyl laurate, the rats died suddenly within 2 weeks. The toxicity is a function of the weight of the animal and of the level at which the fat is fed. No characteristic chemical, bacteriological, or pathological changes were demonstrable.

5. It is shown that saturated fatty acids with shorter chains than 10 carbon atoms when fed to rats fail to appear to any conspicuous
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extent in the body fat of the animal, in contrast to the longer chain acids which appear regularly.

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