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NOMENCLATURE OF FATTY ACIDS.

	NAME	FORMULA	ABBREVIATION
1.	Palmitic	$C_{16}H_{32}O_2$	16:0
2.	Palmitoleic	$C_{16}H_{30}O_2$	16:1
3.	Stearic	$C_{18}H_{36}O_2$	18:0
4.	Oleic	$C_{18}H_{34}O_2$	18:1
5.	Linoleic	$C_{18}H_{32}O_2$	18:2
6.	Linolenic	$C_{18}H_{30}O_2$	18:3
7.	Gadoleic	$C_{20}H_{38}O_2$	20:1
8.	Arachidonic	$C_{20}H_{32}O_2$	20:4
9.	Erucic	$C_{22}H_{42}O_2$	22:1

ABSTRACT

This project compares the compositional changes in fatty acids in several tissues after ingestion of a diet high in erucic acid and a diet rich in linoleic acid.

Two groups of rats were fed at weaning a semisynthetic diet containing either 20% mustard seed oil (25% of its total fatty acids consisted of erucic acid) or 20% corn oil (62% of its total fatty acids consisted of linoleic acid). In some experiments, a third group of rats was used. These consumed a mustard seed oil diet with a moderate erucic acid content (not exceeding 13.23% of the diet total fatty acids). The experimental period was one and three weeks of feeding. Half of all the rats were fasted overnight prior to the end of the experiment. Animals were killed and lipids extracted from the different tissues. Separation of fatty acids derived from glycerides and free fatty acids was achieved by gas-liquid chromatography (GLC). This analysis revealed similar percentages of erucic acid in plasma and in the diet. However, after an overnight

period of fasting, the proportion of erucic acid in plasma decreased strikingly, suggesting that adipose tissue and liver did not store erucic acid. This last observation was in agreement with the GLC results. In the heart, erucic acid percentage was higher than that in the mustard seed oil with high or moderate erucic acid content. A possible explanation for this finding is a decreased ability of the heart to oxidize erucic acid. Furthermore, the concentration of triglycerides in hearts was determined after one, three and six weeks on the diet. Cardiac tissue from rats fed a high erucic acid diet for one week accumulated triglycerides to levels several fold more than tissue from rats under corn oil diet. However, after feeding the diet for six weeks, the cardiac lipid accumulation clearly diminished. The data are interpreted to suggest some adaptation to the high erucic acid diet.

Gas-liquid chromatographic analysis revealed that skeletal muscle tissue from the hind limb did not appear to retain a high concentration of erucic acid when it was in the diet. Further experiments were carried out with

this tissue. After one, three and six weeks on the experimental diets (corn oil or mustard oil - diet), the ability of skeletal muscle tissue to oxidize a labelled fatty acid (FA) was determined. Tissue was incubated with 1-^{14}C-octanoic acid. The results showed a similar rate of FA oxidation from rats fed either diet for only one week. However, after three and six weeks of feeding, skeletal muscles from rats fed corn oil diet oxidized labelled octanoic acid at a greater rate while the rate of oxidation did not seem to change when mustard oil diet was fed for a longer period. The data could suggest a certain metabolic adaptation to the high fat corn oil diet when ingested for three weeks.

INTRODUCTION

A. HISTORICAL REVIEW

In the diet of man, erucic acid has become the best known long chain fatty acid with 22 carbons. It is 13-docosenoic acid and is formed by the elongation of oleic acid carbon chain.

Rich sources of erucic acid are to be found in seed oils from members of the CRUCIFERAE family, such as rapeseed, Brassica napus or B. campestris and mustard, B. juncea or B. nigra.

The early use of rapeseed oil was recorded by ancient civilizations in Asia and along the Mediterranean for illumination purposes and later it was used in foods and as a cooking oil. Although the crop was grown in Europe in the 13th century, its use was not extensive until the development of steam power when it was found that rapeseed oil would cling to water and steam-washed metal surfaces better than any other lubricant. In

fact, it was the need for this oil by ships of the Allied Navies which brought rapeseed cultivation to Canada in 1942.

The agronomical success of rapeseed was demonstrated by its extensive cultivation in northern Europe and Canada, as well as in western and central Europe, Australia and Asia. In terms of vegetable oils and oilseeds traded in world markets, rapeseed ranked fourth, exceeded only by soybean, peanut and sunflower.

As the production of rapeseed grew, increasing amounts of docosenoic acids appeared in the Canadian diet during the 1960s and up to 1972. The change arose from a greater use of rapeseed oil, both in the liquid form as a salad and cooking oil and in the partially hydrogenated form as margarine and shortening. Such hardened products also contained other docosenoic acids from partially hydrogenated marine oils. This dietary trend towards more and more docosenoic acids was of nutritional concern because of their physiological and pathological effects (p. 13-20).

Although the average consumption of rapeseed oil was estimated to be 7g/day, the intake among those concerned with the economy of food costs would probably be higher than that. Furthermore, a practical and palatable diet for humans was designed in which rapeseed oil constituted 58% of the daily fat intake. When a high erucic rapeseed oil was provided in such a diet, erucic acid accounted for 20% of the fat or 7.6 Cal %.

The development of very low erucic rapeseed was achieved by plant breeding. To select appropriate stock, the fatty acid composition was determined on individual seeds. As a result of the success in changing the fatty acid composition of rapeseed, the supply of erucic acid diminished in the diet.

On the 1st of December, 1973, the Canadian rapeseed industry voluntarily established as a guideline that erucic acid would not constitute more than 5% of the total fatty acids in margarines, shortenings, mayonnaise, salad oils and dressings, and cooking oils. Rapeseed oil with less than 5%

erucic acid has been given the name Canbra oil to distinguish it from rapeseed oil previously used.

As for mustard crops, commercial Canadian production was not undertaken until 1936 but since that time the demand has been growing. Mustard seed is used in salad dressing and pickles but the main market is for the prepared and powdered mustard for table use. Since mustard seed oil contains 15.25% of erucic acid in its fatty acid composition and since this oil is not used for daily cooking (except in India) the supply of erucic acid in the diet from mustard seed is minimal.

B. GENERAL BIOCHEMISTRY AND PHYSIOLOGY OF FATS

1. Chemical nature of dietary fat:

All over the world, human beings consume a variety of fats with different compositions. For example, in the normal North American diet, approximately 40 to 45 percent of the calories

are derived from fats which is about equal to the calories derived from carbohydrates. By far the most common fats of the diet are the neutral fats, also known as triglycerides, each molecule of which is composed of a glycerol nucleus and three fatty acids. Neutral fat is found in food of both animal origin and plant origin.

The major differences between the fatty acids are:

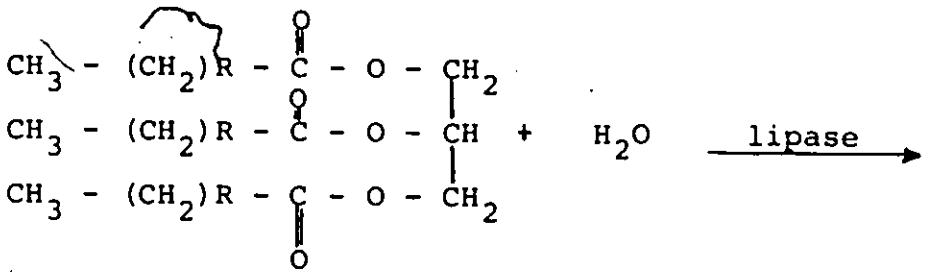
(a) the length of the carbonaceous chain
(b) the degree of unsaturation of the chain

Fatty acids in the body have two important functions: They constitute an energy reserve of storage fat, and, as a component of phospholipids, they take part in the architecture of the cell membrane. In addition to triglycerides, fat in the diet usually contains small quantities of phospholipids, cholesterol, and cholesterol esters.

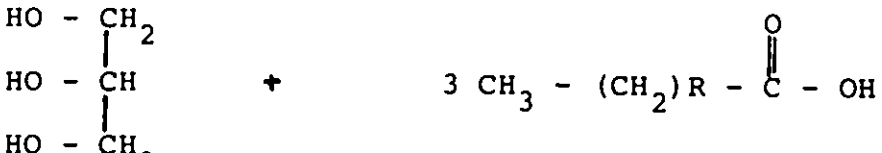
B. 2. <u>The digestion and absorption of fat</u>:

Fat digestion begins in the duodenum, pancreatic lipase being the most important enzyme involved; this enzyme acts on fats that have been emulsified mainly by the detergent action of bile salts. Most of the triglycerides of the diet are finally split into monoglycerides and free fatty acids and glycerol.

<u>Hydrolysis of neutral fat catalyzed by lipase</u>.

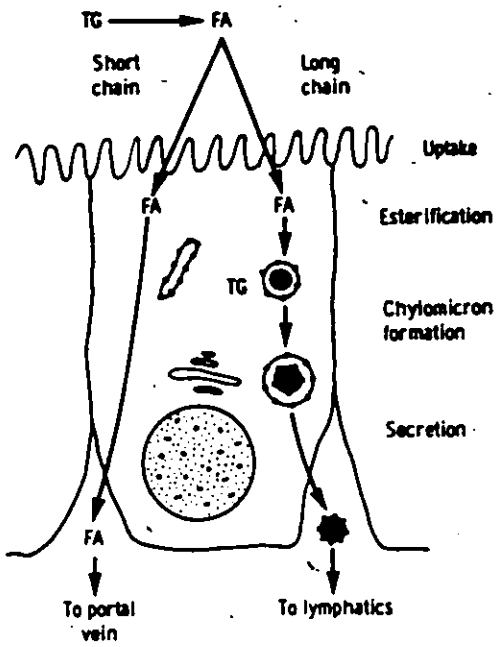


triglyceride



glycerol fatty acid

Monoglycerides and fatty acids combine with bile salts to form micelles. The lipids are thus brought into close contact with the mucosal cells permitting the monoglycerides and fatty acids to enter the cells and leaving the bile salts to pass on to the ileum, where they are reabsorbed. The subsequent fate of the fatty acids depends on their size. Fatty acids containing less than 10 or 12 carbon atoms pass from the mucosal cells directly into the portal blood, where they are transported as free (unesterified) fatty acids. The fatty acids containing more than 10-12 carbon atoms are reesterified to triglycerides in the mucosal cells. They are then coated with a layer of lipoprotein, cholesterol and phospholipid to form chylomicrons, having sizes between 0.03 and 0.5 microns, which leave the cell and enter the lymphatic system.



Absorption of fat by intestinal mucosal cells

The chylomicrons are then transported up the thoracic duct and emptied into the venous blood at the junction of the jugular and subclavian veins.

The chylomicron concentration in the plasma may rise to as high as 1 to 2 percent immediately after a meal that contains large quantities of fat; and because of the large sizes of the chylomicrons, the plasma appears turbid and sometimes yellow. However, the chylomicrons (with a half-life of less than an hour) are

removed within a few hours and the plasma becomes clear once again.

Most of the chylomicrons are removed from the circulating blood as they pass through the capillaries of adipose tissue and the liver, but to a lesser extent other tissues as well. Both the adipose tissue and the liver contain large quantities of an enzyme called lipoprotein lipase. In the capillary endothelium this enzyme hydrolyzes the triglycerides of chylomicrons that stick to the endothelial wall, releasing fatty acids and glycerol. The fatty acids, being highly miscible with the membranes of the cells, immediately diffuse into the fat and liver cells. Once within these cells, the fatty acids are resynthesized into triglycerides, new glycerol being supplied by the metabolic process of the cells.

Thus the major function of adipose tissue is storage of triglycerides until they are needed to provide energy elsewhere in the body. During starvation or in any other condition in which

fat is being utilized rapidly for energy, large quantities of triglycerides appear in the liver. In these conditions, the triglycerides are mobilized from the adipose tissue, transported as free fatty acids in the blood, and then redeposited as triglycerides in the liver, where the initial stages of much of the fat degradation begin. Thus, under normal physiological conditions, the total amount of triglycerides in the liver is controlled to a great extent by the overall rate at which lipids are being utilized for energy.

C. REVIEW OF THE LITERATURE

In the literature, the first report on rapeseed oil appeared in 1918 when Holmes fed 82 grams of rapeseed oil to each of four humans and obtained an average coefficient of digestion of 98.8%. But it was not until 1940 that reports in the literature started revealing that feeding of large amounts of rapeseed oil to laboratory animals causes some growth retardation and changes in some tissues of the body.

The physiological and pathological effects of rapeseed oil have been studied.

1. Longevity

The expectation that rapeseed might be associated with increased longevity was aroused by a report that rats fed 50 Cal% of high erucic rapeseed oil lived longer than those fed an equivalent level of butterfat (Thomasson, 1955). However, the subsequent experimentation showed no relationship in the rat between the level or type of these dietary fats and longevity (Vles, 1975). From the evidence so far accumulated on experimental animals kept under normal conditions, rapeseed oil appeared not to affect longevity.

2. Body Weight Gain

In the early 1940's, Boer found lower body weights in rats fed rapeseed oil than in those fed butterfat. Thomasson and Boldingh, in

1955, concluded that poor growth was related to the presence of erucic acid. The rate of body weight gain was initially depressed in rats consuming rapeseed oil and this was mainly attributed to a lowered food intake caused by a depressed appetite (Thomasson 1955, Beare 1959, Kramer 1973). Then there appeared to be an adaptation to the rapeseed oil evidenced by similar weight gains with both the rapeseed and standard oils (Beare 1959).

3. Digestion and Absorption

In human subjects, it had been first reported (Holmes 1918) and then confirmed (Deuel et al. 1949) that the coefficent of digestion was 99% when rapeseed oil was ingested. The findings of Vaisey et al. (1973), also indicated that humans possess a high capacity to absorb the fatty acids from traditional rapeseed oil and more than 99% of erucic acid from this oil was absorbed.

In rats, some discrepancies appeared in the

literature concerning coefficient of digestibility (values between 58% and 95% have been obtained). The main reason was the different strains of rats used by different researchers (Carroll 1957, Murray 1958, Deuel 1948, Beare 1960).

All of the above observations did not lead to any actions as far as human consumption was concerned until it was found more recently that rapeseed oil could produce pathological effects in some tissues.

4. Myocardial Changes

Roine et al. (1960) and later, Rocquelin and Cluzon (1968) found that rats fed rapeseed oil for two to six months showed foci of histiocyte infiltration in the myocardium. Abdellatif and Vles (1970) subsequently showed that histiocyte infiltration represented only one stage in the cardiac effects of rapeseed oil. In rats, an intracellular cardiac lipidosis was observed

as early as 3 hours after the ingestion of rapeseed oil (Ziemlansky et al. 1973). Lipidosis reached peak intensity after three to six days (the heart then looked cream-coloured to the naked eye) and decreased afterwards (Abdellatif 1972). The stage of histiocyte cell infiltration described by Roine et al. (1960) and by Rocquelin and Cluzan (1968) was replaced after 32 weeks of ingesting the rapeseed oil by interstitial fibrosis of the myocardium. The accumulation of heart lipids has been demonstrated in most species tested, including monkeys, but there are species differences (Abdellatif 1972, Beare-Rogers 1976, Bremer 1982). In rats, both males and females were susceptible to heart lipidosis although Engfeldt and Brunius (1975) found that it was less pronounced in the female rat where its regression was also more rapid.

Direct evidence that cardiac function is impaired by erucic acid has been established by Ten Hoor (1973) and Christophersen et al. (1972).

The accumulated heart lipids are almost exclusively triglycerides (TG) (Pointillart et al. 1975;

Joffrain et al. 1974). No increase in phospholipids or cholesterol esters has been found (Saurer et al. 1980; Dow-Walsh et al. 1975). The acute TG accumulation has been mainly attributed to an inhibition of fatty acid oxidation (Beare-Rogers 1976, Kako and Vasdev 1979, Bremer and Norum 1982).

5. Other tissue changes

Studies of the effects of rapeseed oil on other tissues in the body were pursued but did not show any striking changes induced by this oil (Beare-Rogers 1977).

No fat infiltration or lesions have been observed in the liver (Christiansen et al. 1977; Beare-Rogers 1977).

Some enlargement of the adrenal cortical cells in Wistar rats was noticed (Abdellatif 1970) but Beckett and Boyd (1975) detected no

structural alteration as revealed by electron microscopy.

Cheniti et al.(1967) found no specific defect in testicular development or spermatogenesis in the rat fed rapeseed oil and Coniglio et al. (1974) observed no histological change in the rat testes. Walker et al. (1972) indicated an increase in cholesterol in the ovaries of rats fed rapeseed oil. Offsprings of rats fed this oil for four generations were fewer and smaller than the controls (Beare-Rogers et al. 1961).

6. Causative agent for rapeseed oil effects

Most of the characteristics of dietary rapeseed oil have been attributed to its content of erucic acid. More and more evidence pointed to erucic acid being responsible for the pathogenicity of rapeseed oil (Abdellatif and Vles, 1970; Mattson, 1973; Levin, 1973).

Erucic acid is not normally found in animal tissue lipids. Rats fed rapeseed oil diets show less erucic acid and more oleic acid in their tissue lipids (with the exception of the cardiac tissue) than in the diet (Vasdev 1978, Bremer 1982). Metabolic conversion of long chain fatty acids from dietary rapeseed oil to oleic acid is now experimentally proven (Carreau et al. 1968; Lapous et al. 1970; Pinson and Padieu 1974).

In short, at this stage of our knowledge, there are still many questions to be answered about the pathological effects of erucic acid in human beings. Since it would seem that it can produce cardiac lipidosis, precautions for man might best be those of moderation and avoidance for an over-dependency on one vegetable oil or oils having similar characteristics.

AIMS OF THE PROJECT

Certain vegetable oils contain large amounts of erucic acid, a long chain unsaturated fatty acid that is not normally found in animal tissues. Abnormalities in lipid metabolism and pathological changes in cardiac tissue have been found following diets containing a high percentage of these oils.

In this study, we attempt to identify the eartly steps in the chain of events which follow the intake of diets containing a high erucic acid level. To do this, we compare the effects of diets containing vegetable oils with high, moderate and zero erucic acid levels on Sprague-Dawley rats. The lipids of different tissues are analysed to determine to what extent erucic acid is incorporated after feeding these diets for different periods of time.

In addition, the total triglyceride content of some of these tissues is determined to obtain a picture of the general effects of erucic acid on tissue lipid metabolism.

Finally, the capacity of tissues to oxidize fatty acids is evaluated following the different dietary regimes. This is done using a model system of skeletal muscle with octanoate as substrate. This tissue was chosen since it represents the largest mass of tissue which utilizes lipids and probably accounts for the greater part of the oxidation of ingested lipids.

MATERIALS AND METHODS

Two studies were carried out; Study I analysed the fatty acid composition of different tissues. These tissues were removed from rats under specific test diets. Study II dealt with skeletal muscle tissue incubated with a labelled fatty acid. Rate of fatty acid oxidation was determined.

Animals and diets

For Study I, male albino rats (40-60g) of the Sprague-Dawley strain at weaning (3 weeks old) were purchased from Canadian Breeding Farm and Laboratories Limited, Montreal, Quebec. Rats were studied at their weaning age, facilitating comparison with earlier studies by other authors (Abdellatif and Vles 1970, Beare-Rogers et al. 1972, Vasdev and Kako 1979).

Rats were divided into two groups, each consisting of 12 rats. They were randomly assigned three per cage and were fed the different diets. The diets were semisynthetic containing 20% casein, 20% sucrose, 30%

corn starch, 2% vitamin mixture, 4% salt mixture, 4% brewers yeast and 20% test oil. The test oil was either corn oil or mustard seed oil from commercial sources. Mustard seed oil high in erucic acid was used in the experiments but some additional experiments with mustard seed oil containing moderate amount of erucic acid, were run too.

Rats of each group were provided with respective food and water ad libitum for a period of one week or three weeks. Half of each group of rats had been fasted overnight prior to the end of the experimental period to determine the effects of fasting on erucic acid storage in the liver and adipose tissue.

The rats were sacrificed by decapitation and their blood, heart, aorta, liver, adipose tissue and skeletal muscles were immediately removed for lipid analysis.

The second part of the project (Study II) consisted of incubation of skeletal muscles. The rats were purchased at three different ages, so that at the end of the experimental period (one, three and six weeks) they would all weigh between 250 and 280 grams.

Rats of the same weight were divided into two groups, six rats each. They were housed three per cage and they were placed respectively on one of the diets described earlier (corn oil diet or high erucic-mustard seed oil diet). They were fed ad libitum and had free access to water. They were killed by decapitation and skeletal muscles tissue from their legs was excised.

The purpose of using larger rats in Study II was to provide the amount of skeletal muscle tissue needed in these experiments. It has been shown (Kako and Vasdev 1978) that similar tissue changes occur in both weanling and adult rats after feeding the mustard seed oil diet.

Experimental procedure

Study I: Accumulation of tissue lipids after ingestion of mustard seed and corn oil diet.

(a) Fatty acid composition of tissues.

(i) Extraction of lipids.

Rats were decapitated. Their blood was collected and immediately centrifuged (Lourdes Instrument Corporation) at a

speed of 1000 G for 10 minutes. The plasma was transferred carefully to another container. Several tissues (heart, aorta, skeletal muscle from the legs, liver, adipose tissue) were removed for study. Only the ventricles were used from the heart, and the adipose tissue was retroperitoneal (Vasdev and Kako 1978).

Lipids were extracted by the method of Folch et al. (1957) as follows: The different organs were crushed using a pestle and a mortar and homogenized with 20 volumes of 2:1 chloroform-methanol mixture (v/v). Then the extract was filtered through a fat-free filter paper into a glass-stoppered cylinder. The crude extract was mixed thoroughly with 0.2 volume of a salt solution (0.74% KCl) and the mixture was allowed to separate into two phases by standing. As much of the upper phase as possible was removed by aspiration and the interphase was washed three times with 2 ml pure solvents upper phase ($CHCl_3$:MethOH:H_2O - 3:48:47) without disturbing the lower phase.

Finally, the lower phase was cleared of water droplets by the addition of methanol. The extract was dried under nitrogen gas at 30°C. When samples were stored, they were dissolved in chloroform and kept in a freezer.

(ii) Separation of neutral lipids from phospholipids. Samples were dissolved in 20ml chloroform and filtered through a column of activated silicic acid (silicic acid was activated by heating for two hours at 100°C and could be kept in this state for one week in a closed bottle). The column retained the phospholipids and the eluate was collected and dried under nitrogen gas at 40°C. Phospholipid composition was not studied because it was reported that erucic acid does not significantly incorporate into this lipid fraction (Kako et al. 1978).

(iii) Separation of neutral lipids from cholesterol esters.

The dry sample was dissolved in 30ml petroleum ether: benzene (8:2) and passed through a new column of activated silicic acid. Cholesterol ester was eluted and discarded. Twenty ml chloroform were added to the column

and this last filtrate contained the neutral lipids (glyceride and free fatty acid).

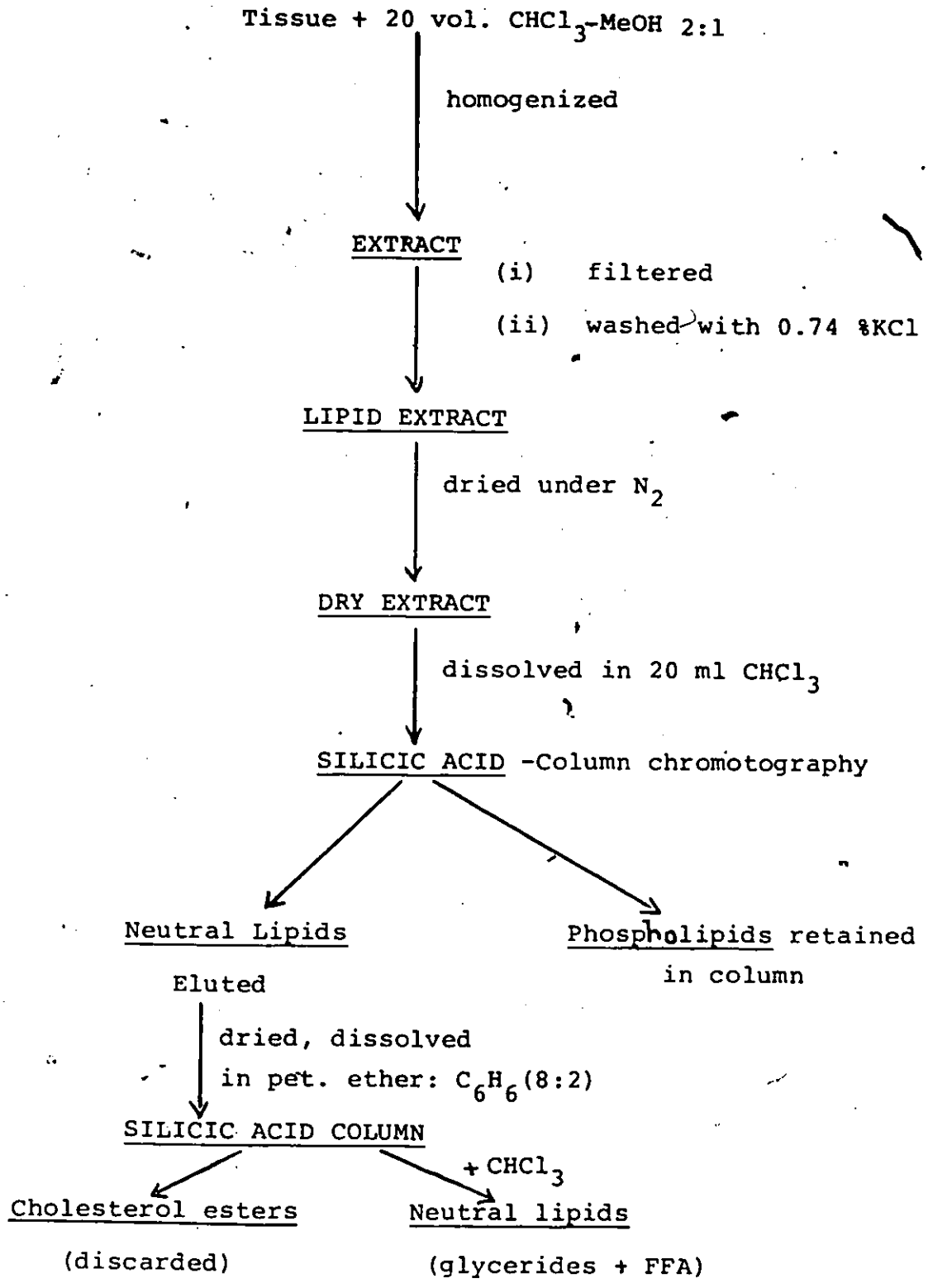
(iv) Esterification

(Method of Metcalfe et al., 1961).

The esterification method described below has been used for the routine preparation of methyl esters of fatty acids for gas-liquid chromatography.

After evaporating the sample, it was mixed with 2 ml boron trifluoride-methanol and 5 ml benzene and heated in a boiling water bath for 40 minutes. Then, 15 ml petroleum ether and 20 ml distilled water were added in a separatory funnel, mixed thoroughly and allowed to stand. The aqueous phase was discarded and the petroleum ether phase was passed through a fat-free filter paper to remove water and evaporated. The dry sample was finally dissolved in a small volume of hexane in readiness for gas-liquid chromatographic analysis.

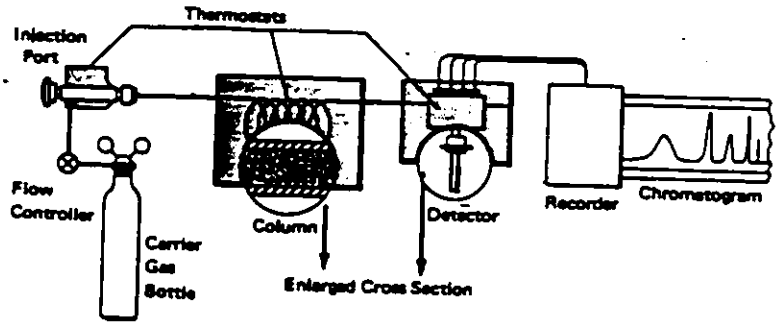
FLOWSHEET OF LIPID EXTRACTION AND ANALYSIS



The reason for fatty acids methyl esterification is that it decreases their boiling point and thus, a less elevated temperature in the chromatograph is needed. Furthermore, methyl esters are less strongly adsorbed on the column than the free acids which facilitates their elution.

(v) Chromatographic separation of fatty acids.

In gas-liquid chromatography (GLC) the components to be separated are carried through the column by an inert gas (Carrier Gas). The sample mixture is partitioned between the carrier gas and a non volatile solvent (Stationary Phase) supported on an inert size-graded solid (Solid Support). The solvent selectively retards the sample components, according to their distribution coefficient, until they form separate bands in the carrier gas. These component bands leave the column in the gas stream and are detected by a flame ionization detector and recorded on a recorder.



Schematic drawing of a gas chromatographic system

In this project, chromatography was utilized to achieve the most satisfactory separation of fatty acids at elevated temperatures. GLC was performed with an Aerograph instrument, equipped with a 6 feet x 1/8 inch stainless steel column packed with 10% SP-2300 on Chromosorb W (Supelco Inc. Belafonte, Pa.) - Chromosorb is Johns Manville registered trademark for support material for G.C.-. Nitrogen was used as a carrier gas. The injection-port temperature was set at 250°C, the column

temperature at 180°C, and the hydrogen flame detector temperature at 300°C. The sample (1 to 3 µl depending on the sample concentration) was introduced instantaneously into the column. The sample was injected with a hypodermic syringe needle. After 25 to 30 seconds, peaks for fatty acids began displaying on a strip chart recorder (the chromatogram), where the abscissa indicated the time and the ordinate represented the output from the hydrogen flame detector. The retention time of standard methyl esters of fatty acids were used to identify the molecular species of fatty acids in the samples. Concentration of fatty acids was calculated using retention time and peak height as described by Carroll (1962). The values were expressed as means of assays carried out in duplicate.

(vi) Test of methodology.

The efficiency of both the Folch method and the Metcalfe method was tested. A known

amount of 1-^{14}C palmitic acid (New England Nuclear Corporation, Boston, Mass.) was added to the tissue at the beginning of Folch method. Prior to and after methylation, 0.5 ml (x2) of the sample was transferred to a glass scintillation vial. Ten ml of Econofluor (New England Nuclear Corporation) was added to each vial, and the radioactivity was determined in a liquid scintillation counter (Beckman scintillation spectrometer LS-150). The recovery before esterification was approximately 90% and after esterification 87%.

As for gas-liquid chromatography, a standard containing five different fatty acids in equal quantity (20% each) was run twice in the chromatograph. The results were as follows:

Palmitic acid	(16:0)	18.8	20.2
Stearic acid	(18:0)	18.7	18.7
Oleic acid	(18:1)	21.5	21.7
Linoleic acid	(18:2)	20.7	19.2
Linolenic acid	(18:3)	20.3	20.2

The variance calculated from the difference between replicates is 0.426 which gives a S.D. of 0.653. This gives a measure of reproducibility of the method for single estimates.

(b) Determination of tissue triglycerides

The determination of tissue triglycerides was based on the quantitative measurement of the glycerol moiety of the molecules, according to a modification of the procedure of Van Handel and Zilversmit (1957).
This procedure consists essentially of four steps: the selective quantitative extraction of triglycerides from tissues, the complete saponification of triglycerides, the periodate oxidation of glycerol to formaldehyde and formic acid, and the colorimetric estimation of formaldehyde.

(i) Extraction of lipids (Folch et al. 1957)

Half gram of tissue (heart, liver, or skeletal

muscle) was minced using a mortar and pestle, then well homogenized with 2:1 choroform-methanol mixture (v/v) to a final dilution 20-fold the volume of the sample. Then, it was transferred to a 25 ml cylinder and shaken well for 3 to 5 minutes.

If standard was to be added to check loss of lipids, it was done at this point. Then the homogenate was filtered through fat-free filter paper into another cylinder and was mixed thoroughly with 0.2 volume of 0.74% KCl.

The mixture was allowed to form two phases by standing.

The upper phase was removed by siphoning and washed three times, each time with 2 ml of pure solvents upper phase without disturbing the lower phase. Finally, methanol was added to the lower phase to clear it and all of the extract was dried under N_2 at 30°C.

(ii) Removal of phospholipids

Two ml of chloroform were mixed with the dry lipids. Two grams of activated silicic acid were placed in a glass-stoppered tube and the solution of lipids was transferred to the tube. 18 ml more of chloroform was added.

The tube was stoppered and shaken vigorously for about 10 minutes. Then, all of the contents of the tube were filtered through a fat-free filter paper. The phospholipids were absorbed by silicic acid.

(iii) Estimation of triglycerides

Glass-stoppered test tubes were used. From each sample, two different amounts of extract (each in duplicate) were taken to verify the final concentration. The tubes were dried under N_2 at 40°C. At the same time, 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml of working standard (tripalmitin), each in duplicate, were

dried too. Then, one of each duplicate (from the sample tubes and the standard tubes) were saponified with 0.5 ml of working alcoholic KOH (o.4%) and the second duplicate was unsaponified, being mixed with 0.5 ml of pure ethanol.

All of the tubes were stoppered and incubated at 60^{o}-70^{o}C for 30 minutes. Then, 0.5 ml of 0.2N H_2SO_4 was added to each tube, mixed well and the tubes were placed in a gently boiling water bath for about 15 minutes to evaporate the alcohol (it was advisable to keep the water level of the bath only slightly above the surface of the reaction mixture in order to avoid evaporation of the water from the tubes).

Then, in the absence of excessive light, the following steps were carried out:
0.1 ml of fresh soldium metaperiodate was added to all of the tubes and mixed well. After 10 minutes, further oxidation was prevented

by the addition of 0.2 ml of fresh sodium bisulfite. After mixing well and waiting for 5 to 10 minutes, 5 ml of fresh chromotropic acid reagent was added and mixed. The tubes were heated in a boiling water bath for 30 minutes. After cooling to room temperature, (from this point, exposure to light presented no problem), 0.5 ml of 5% thiourea was added to all tubes and mixed very well; the optical density was determined at 570 nm. The colour remains stable for several hours.

(iv) Test of methodology

The accuracy of the triglycerides determination was checked by measuring triglyceride after adding a known amount of standard material to tissue samples.

The recovery was expressed as a percentage, as follows:

$$\% \text{ Recovery} = \frac{\text{mg TG in tissue + STD} - \text{mg TG tissue alone}}{\text{mg TG in STD}} \times 100$$

The recovery in this method was 94.06% $\pm$ 1.35 (N = 15, mean $\pm$ SD)

Study II: Effect of the diets on the oxidation of octanoic acid by skeletal muscle tissue in vitro.

In these experiments, a known amount of labelled short chain fatty acid was added with skeletal muscle tissue.

The tissue was incubated at 37°C; $^{14}CO_2$ was collected after incubation in a vial for liquid scintillation counting.

(i) Incubation procedure

Rats weighing 250-280g were decapitated after being fed either a corn oil diet or a mustard seed oil diet for one, three and six weeks.

All skeletal muscles from the legs were excised and three grams of muscles were taken randomly for each incubation. They were minced manually with scissors in 4 ml

Krebs-Ringer solution to which octanoic acid (2mMole/l) and NaOH (2mMole/l) had been added. Modified Erlenmeyer flasks (20ml) with attached center well and two side arms were used in these experiments. Before tissue addition, Erlenmeyer flasks containing 1-^{14}C-octanoic acid (sodium salt; 25.1mCi/mmol; New England Nuclear, Boston, Mass.) in ethanol (50µCi/1ml EtOH) were flushed with a mixture of 95% oxygen and 5% CO_2 for approximately 5 minutes to remove any labelled CO_2. According to some authors (Kikuchi et al. 1970), without this aeration a high blank value of $^{14}CO_2$ was invariably observed. The tissue homogenate in Krebs-Ringer solution was added to the main chamber, and tiny pieces of filter paper were put into the center well. Incubation at 37^{o}C was started by placing the flasks in a Dubnoff metabolic shaking incubator. After three hours, the reaction was stopped by injecting 0.5 ml 3N HCl and 0.3 ml 8% TCA through a rubber stopper to the tissue. At the same time, 0.1 ml of 10% KOH was added to the center well which contained the filter paper, facilitating

CO_2 collection.

The carbon dioxide, liberated by shaking the flask at 37°C for 30 minutes, was absorbed by the KOH solution in the center well.

Carbon dioxide production as a function of time, was found to be constant between 2½ hours and 4 hours.

(ii) Determination of oxidation by $^{14}CO_2$ measurement.

The trapped $^{14}CO_2$ was eluted from the filter paper by soaking in 10 ml Aquasol (New England Nuclear, Boston, Mass.) in a scintillation vial.

Each experiment was run in 6 flasks. Three flasks were incubated for 3 hours and the three others had the reaction stopped without incubation. While absolute rates of oxidation may be underestimated due to metabolic exchange of CO_2, the relative amount of oxidation was derived from the difference in radioactivity between the two sets of flasks (experimental -

control); and the radioactivity (cpm CO_2) was converted to picomole CO_2 produced. The results were expressed as picomole per hour per gram of skeletal muscle tissue.

Results in this present work were expressed as mean $\pm$ S.D. and on a wet weight basis, since there was no significant difference in water content between the experimental groups.

The significance of difference was calculated with the Student's t-test.

(iii) Test of methodology

The efficiency of the method was tested by adding a known amount of $NaH^{14}CO_3$ in a simulated experiment. Incubation of tissue was stopped by acidifying the sample. After completion of the entire procedure, an average of 97.54% $\pm$ 0.97 (N=3) of the label was recovered.

RESULTS

FATTY ACID COMPOSITION OF THE TEST DIETS

The fatty acid composition of fat in the diets are shown in Figure 1.

In corn oil, linoleic acid (18:2) accounted for 62.1% of the total fatty acids and oleic acid (18:1) for 25.9%. There is no erucic acid in this oil.

However, in mustard seed oil, erucic acid (22:1) amounted to 26.52%, oleic acid (18:1) to 23.25% and gadoleic acid (20:1) to 25.54% of the total fatty acids. Thus, three quarters of the fatty acids in mustard seed oil consisted of monoenoic acids (sum of 18:1, 20:1 and 22:1).

When mustard seed oil with moderate erucic acid content was used in the diet, 13.23% of its total fatty acids consisted of erucic acid, 40.22% of oleic acid and 19.73% of gadoleic acid.

In this work, when the term "mustard seed oil diet" is used as such, it refers to the diet with 26.52% erucic acid.

Legend Fig. 1

Fatty acid composition (%) of test oils.

Mustard seed oil with high erucic acid content

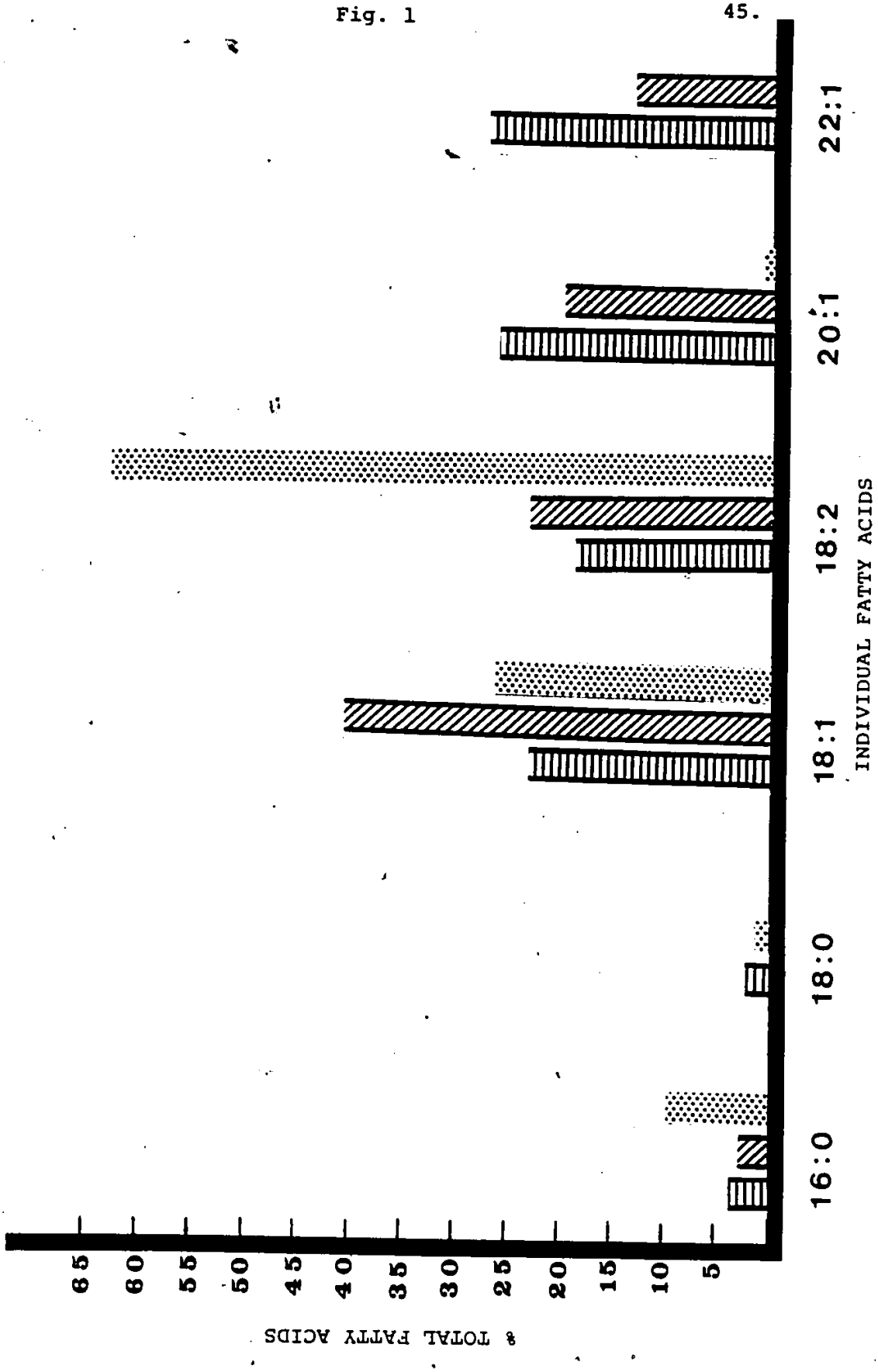
Mustard seed oil with moderate erucic acid content

Corn oil

The fatty acid composition of the test oils is expressed as percentage of the tabulated fatty acids.

Fatty acids are designated by their chain length:number of double bonds; 16:0 (palmitic), 18:0 (stearic), 18:1 (oleic) 18:2 (linoleic), 20:1 (gadoleic), 22:1 (erucic).

Fig. 1


% TOTAL FATTY ACIDS
85
80
55
50
45
40
35
30
25
20
15
10
5
16:0
18:0
18:1
18:2
20:1
22:1
INDIVIDUAL FATTY ACIDS

TISSUE FATTY ACID COMPOSITION OF RATS FED THE TEST DIETS

Although some experiments on tissue fatty acid composition were run only on one batch of rats, this was not allowed to happen before having been assured that variability between different batches is acceptable.

The following examples are paired determinations from two different batches of rats given the same treatment.

Plasma from rats fed corn oil diet for one week and fasted overnight.

FA composition	Batch (1) (%)	Batch (2) (%)
16:0	17.3	19.2
18:0	5.3	4.8
18:1	19.3	21.8
18:2	43.8	41.3
20:4	14.3	12.9

(Variance between batches equals 1.832 and the S.D. equals 1.353).

Heart from rats fed mustard seed oil diet for one week.

FA composition	Batch (1) (%)	Batch (2) (%)
16:0	3.1	2.6
18:0	3.1	1.5
18:1	22.8	23.4
18:2	12.6	13.7
18:3	4.3	5.0
20:1	14.6	12.8
22:1	39.5	41.0

(Variance between batches equals 0.74 and the S.D. equals 0.86).

Differences in FA composition between batches greater than two S.Ds likely indicate a significant effect of the treatment.

The fatty acid composition of glycerides and free fatty acids (FFA) of various tissues of rats fed the corn oil diet differed greatly from that of the respective tissues from rats fed the mustard seed oil diet. This difference appears to be due to the different ratios of dietary fatty acids.

The most remarkable differences between tissues from rats on a corn oil diet and those on a mustard seed oil diet were first, that erucic acid was only found in tissues from rats fed a diet containing the material (i.e. mustard seed oil diet) (Figure 3). Second, arachidonic acid (20:4), although not present in the diets, appeared in all tissues taken from rats fed the corn oil diet (Figure 5) and only in liver and plasma of rats on a mustard seed oil diet (Figure 3). The above observations were noted regardless of duration of treatment.

When a diet rich in mustard seed oil was fed to rats for one week, the fatty acid composition of the glycerides and FFA in plasma (Figure 2) resembled the composition of the test oil - with the exception of gadoleic acid percentage.

The duration of the experimental feeding period did not affect the plasma fatty acid composition when the mustard seed oil diet was fed. After three weeks on the diet (Figure 3) the plasma fatty acid composition was essentially similar to that of rats fed for one week.

As regards the compositional changes of fatty acids, aortic tissue resembled plasma when erucic acid was in the diet (Figure 2). Aorta fatty acid composition was also not influenced by the duration of the feeding (Figure 3).

Adipose tissue, liver and skeletal muscle contained less than 12% of erucic acid after one week on a mustard seed oil diet (Figure 2). This percentage decreased even more after three weeks on the diet (Figure 3). On the other hand, all of these three tissues had a high percentage of oleic acid compared to its percentage in the diet and this value increased significantly after the third week of feeding.

As for the heart tissue, after one week of mustard seed oil diet (Figure 2), erucic acid in the cardiac

Legend Fig. 2

Weanling rats were fed the mustard seed oil diet for one week.
Tissues were removed for lipid analysis.
The abscissa indicates the individual tissue fatty acids, derived from glycerides and FFA. The ordinate indicates the percentage of each of these fatty acids.
The values are means of two experiments and each chromatographic assay in duplicate.

Fig. 2

TISSUE FATTY ACID COMPOSITION

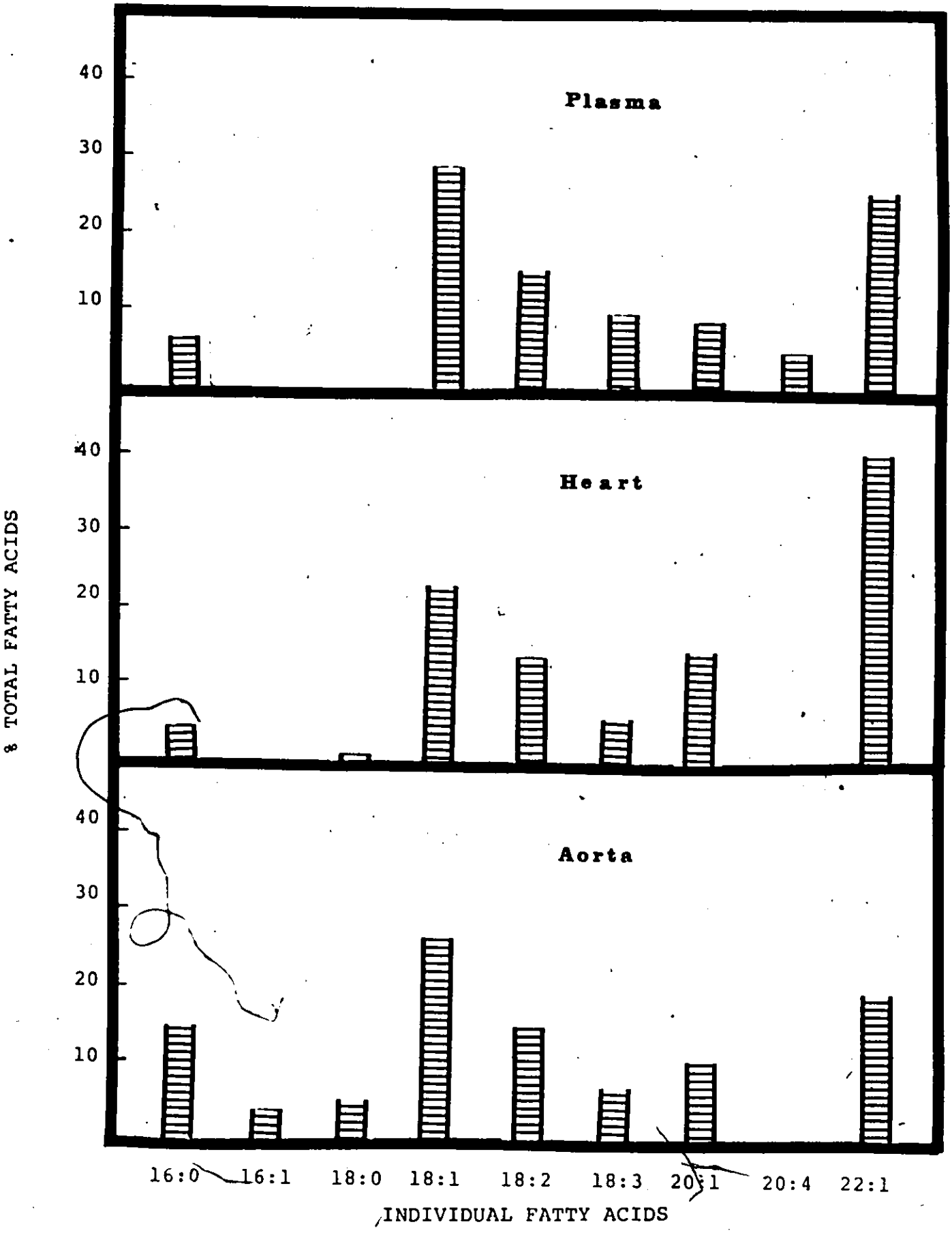
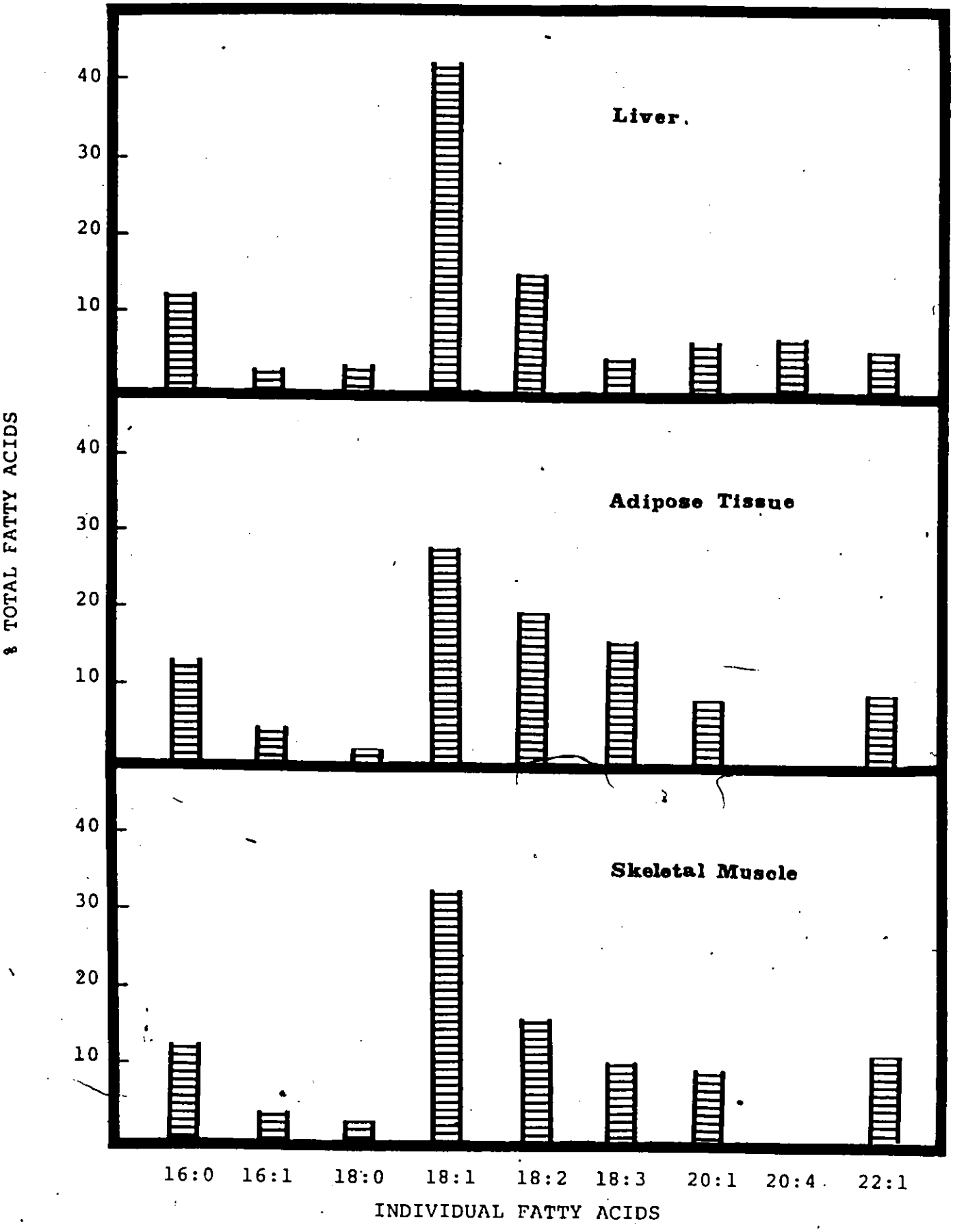


Fig. 2

TISSUE FATTY ACID COMPOSITION



Legend Fig.3

Weanling rats on a mustard seed oil diet for one week.

Weanling rats on a mustard seed oil diet for three weeks.

Tissues were removed for lipid analysis.

The abscissa indicates the individual tissue fatty acids, derived from glycerides and FFA. The ordinate indicates the percentage of each of these fatty acids.
The values are means of two experiments and each chromatographic assay in duplicate.

Fig. 3

TISSUE FATTY ACID COMPOSITION

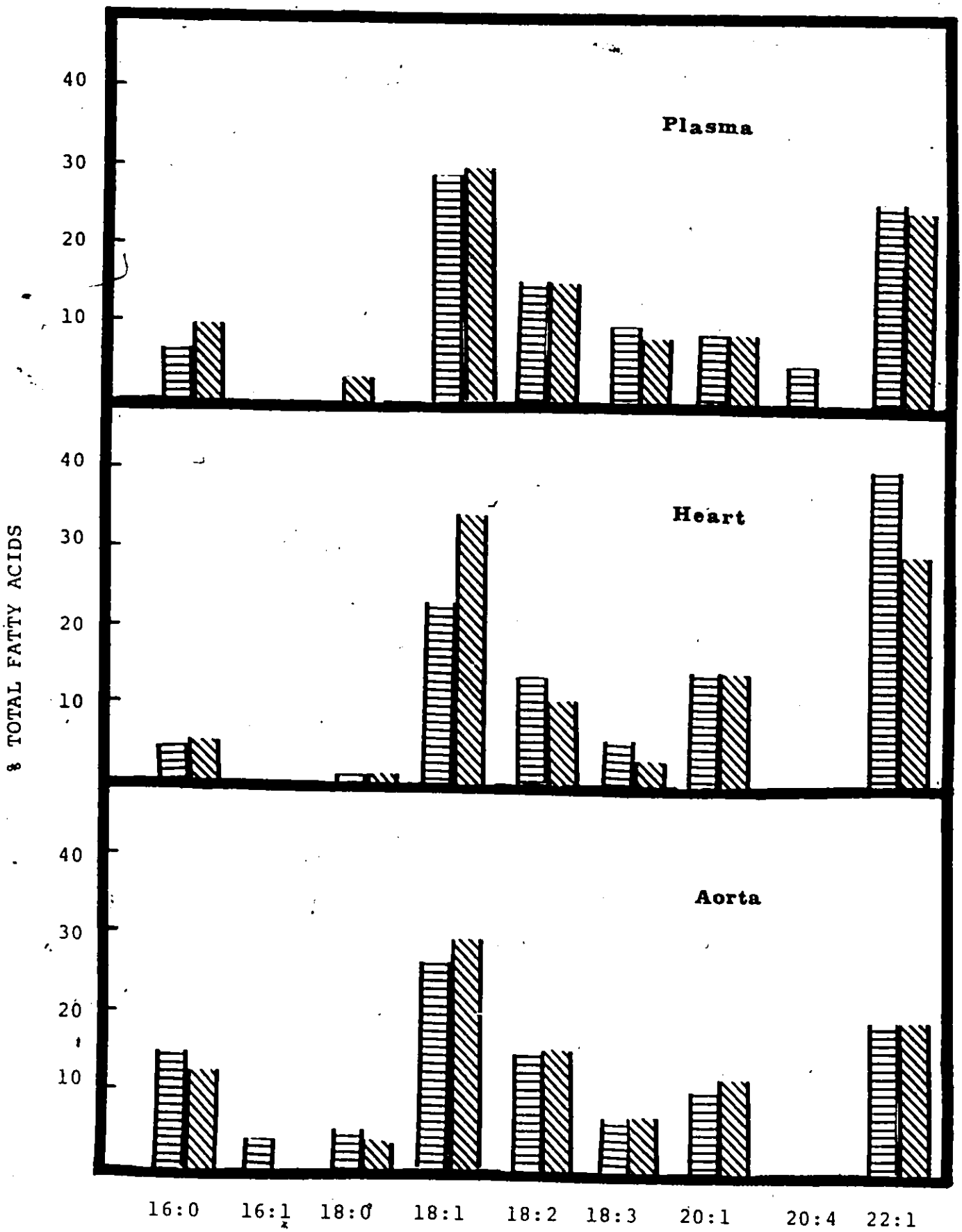
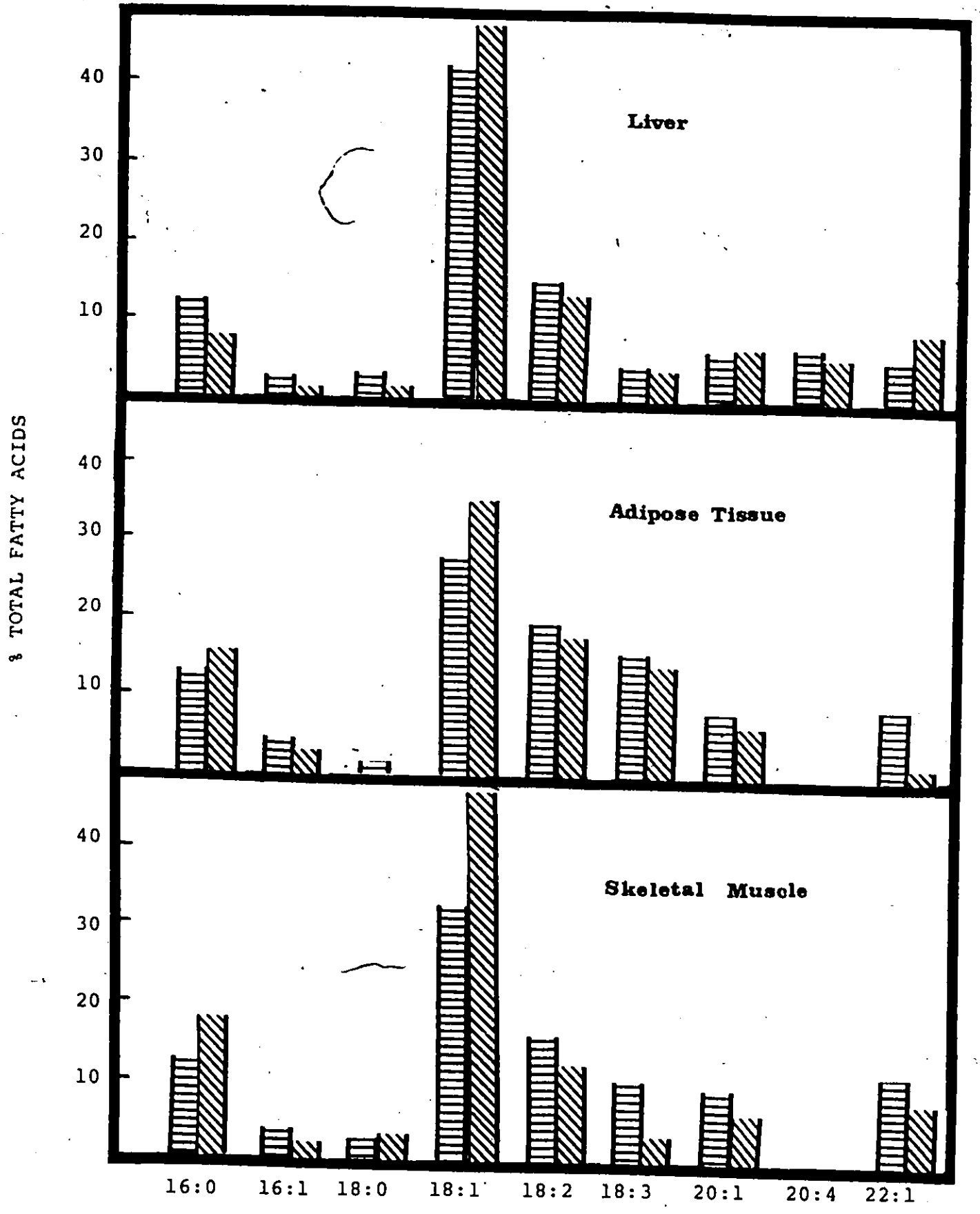


Fig. 3

TISSUE FATTY ACID COMPOSITION



Legend Fig. 4

Weanling rats were fed the corn oil diet for one week.
Tissues were removed for lipid analysis.
The abscissa indicates the individual fatty acids in tissues, derived from glycerides and FFA. The ordinate indicates the percentage of each of these fatty acids.
The values are means of two experiments and each chromatographic assay in duplicate.

Fig. 4

TISSUE FATTY ACID COMPOSITION

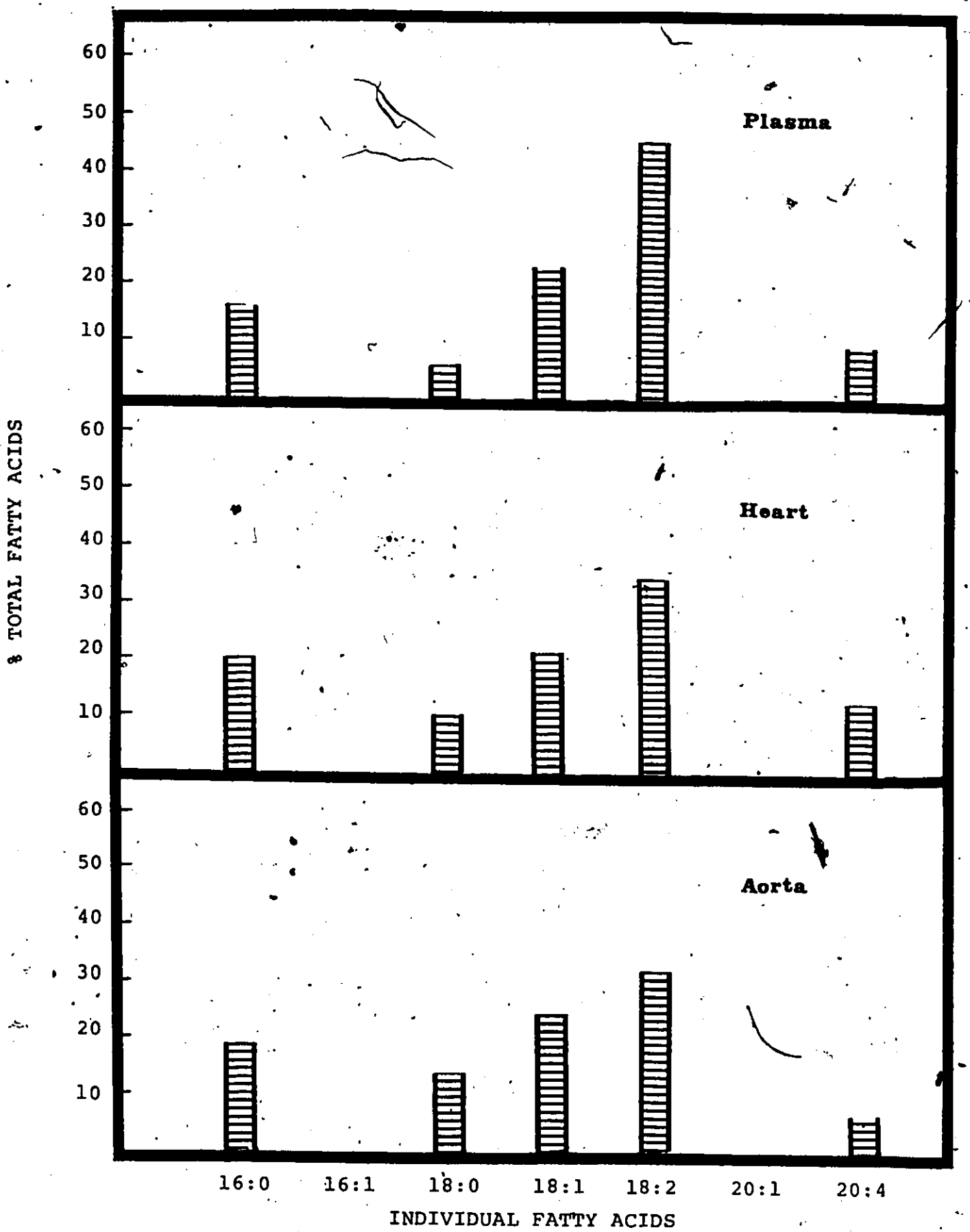
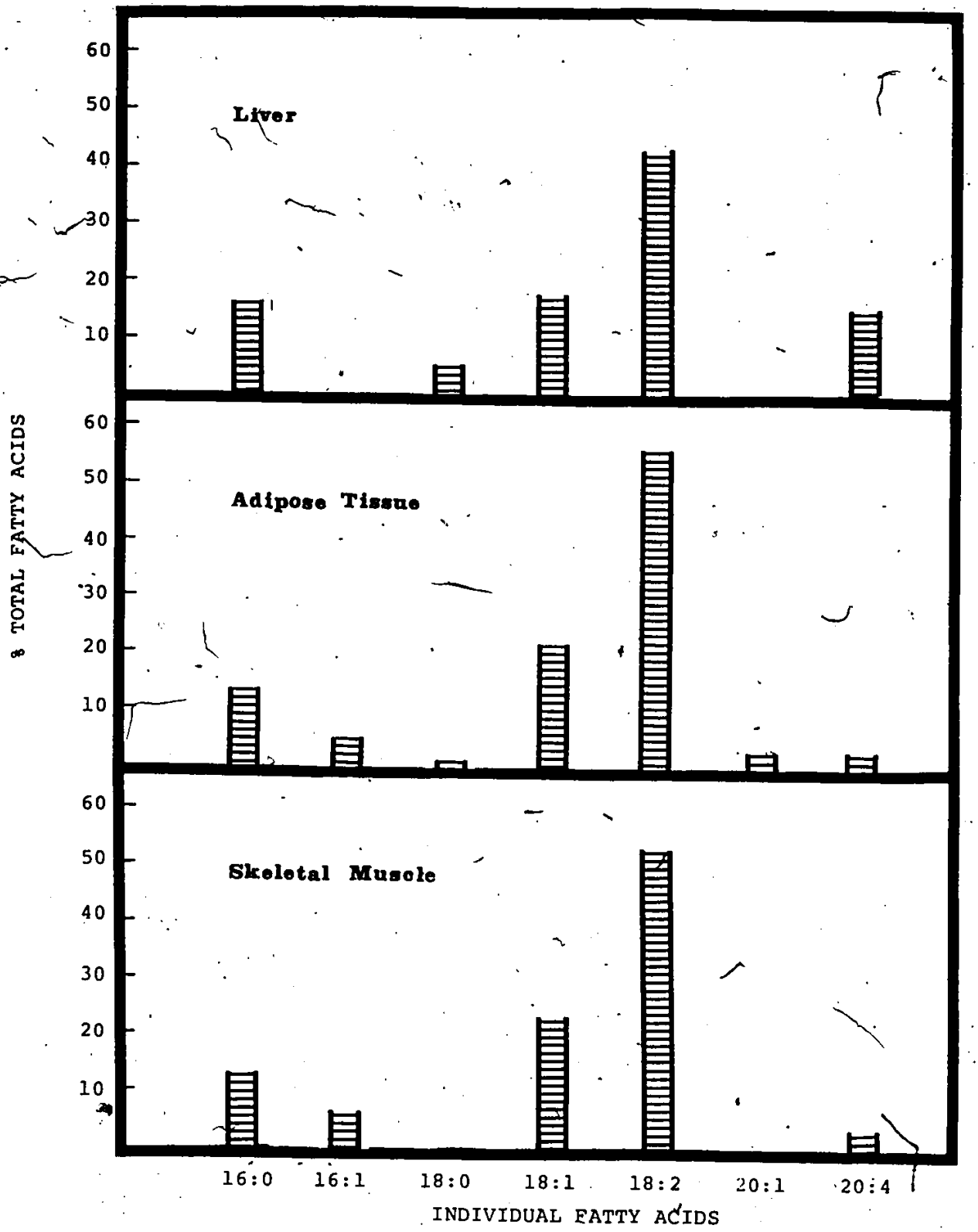


Fig. 4

TISSUE FATTY ACID COMPOSITION



Legend Table 1.

Concentration of TGs in heart, liver and skeletal muscle is estimated by a modification of Van Handel and Zilversmit method

The number of rats is shown in parentheses.
Values are means ± standard deviation in mg/g of wet tissue.
* Indicates a significant change ($P < 0.05$) compared to the value obtained from corn oil-fed rats.
# Indicates a significant change ($P < 0.05$) compared to the value obtained from rats fed the same diet for one week

Table 1

CONCENTRATION OF TRIGLYCERIDES IN TISSUES

HEART

	Corn oil diet		Mustard seed oil diet	
1 week	1.18 ± 0.5	(N =10)	9.2 ± 3.1*	(N =9)
3 weeks	1.40 ± 0.4	(N =5)	8.04 ± 1.7*	(N =5)
6 weeks	1.20 ± 0.2	(N =5)	4.66 ± 1.3*#	(N =5)

LIVER

1 week	11.5 ± 2.6	(N =4)	9.13 ± 1.0	(N =4)
3 weeks	15.0 ± 3.5	(N =3)	6.13 ± 2.4*	(N =3)
6 weeks	16.2 ± 1.8#	(N =3)	11.0 ± 3.4	(N =3)

SKELETAL MUSCLE

1 week	3.8 ± 2.0	(N =3)	3.38 ± 1.2	(N =4)
6 weeks	4.69± 3.0	(N =5)	3.28 ± 1.5	(N =4)

tissue reached a very high percentage compared to its percentage in other tissues studied or in the diet (40% erucic acid for the heart vs. 26% for the diet). When mustard seed oil diet was administered for 3 weeks, percentage of erucic acid - although a little decreased compared to one week figure - was still very elevated (Figure 3).

A relative increase in erucic acid always accompanied a relative decrease in oleic acid.

A very significant accumulation of cardiac triglycerides was observed concurrently in rats on a mustard seed oil diet compared to that in cardiac tissue from animals on a corn oil diet (9.2 mg/g vs. 1.18 mg/g after one week) (Table 1).

Weanling rats fed corn oil diet for one week (Figure 4) had a percentage of linoleic acid in their tissues less elevated than that of the diet. On the contrary, percentage of palmitic acid was higher in tissues than in the diet.

This could be explained by the process of shortening a long chain fatty acid in the body. The continuous ingestion of the diet for three weeks led to a further increase in linoleic acid in tissues (Figure 5), and in adipose tissue it reached a percentage close to that in the diet.

In contrast to myocardial tissue that accumulated triglycerides after one week when rats were on a mustard seed oil diet, rats on a corn oil diet did not accumulate myocardial triglycerides. Moreover, the percentage of linoleic acid in hearts was little more than half its percentage in the diet (34% vs. 62%) (Figure 4).
Note: The precise percentage of fatty acids in tissues as determined by chromatographic analysis are tabulated in the appendix.

FATTY ACID COMPOSITION IN RATS TISSUE AFTER A MUSTARD SEED OIL DIET WITH MODERATE ERUCIC ACID CONTENT.

After one week of feeding weanling rats the mustard seed oil diet with moderate erucic acid content, plasma analysis showed once more the similarity between fatty acid composition of plasma glycerides and FFA, and the fatty acid

composition of the diet; moreover, the erucic acid plasma again reflected very closely its relative amount in the diet (Figure 6).

Also, fatty acid composition of aorta resembled that of the plasma with a similar percentage of erucic acid in the two tissues (13.17% erucic acid in aorta vs. 12.68% in plasma).

As for the heart, the same trend that was observed in cardiac tissue after a high erucic acid diet, appeared once more when the diet contained a moderate amount of erucic acid: percentage of erucic acid was very high in comparison with its percentage in the diet (28.59% erucic acid in heart vs. 13.23% in the diet).

FATTY ACID COMPOSITION OF RATS TISSUE AFTER OVERNIGHT FASTING

Before an overnight of fast, rats were either on a corn oil diet or on a mustard seed oil diet (high in erucic acid) for one and three weeks.

When on a corn oil diet, fatty acid composition of tissues from fasted rats is shown in Figure 7 and 8.

Legend Fig. 5

Weanling rats on a corn oil diet for one week.

Weanling rats on a corn oil diet for three weeks.

Tissues were removed for lipid analysis.

The abscissa indicates the individual tissue fatty acids, derived from glycerides and FFA. The ordinate indicates the percentage of each of these fatty acuds.
The values are means of two chromatographic assays.

Fig. 5

TISSUE FATTY ACID COMPOSITION

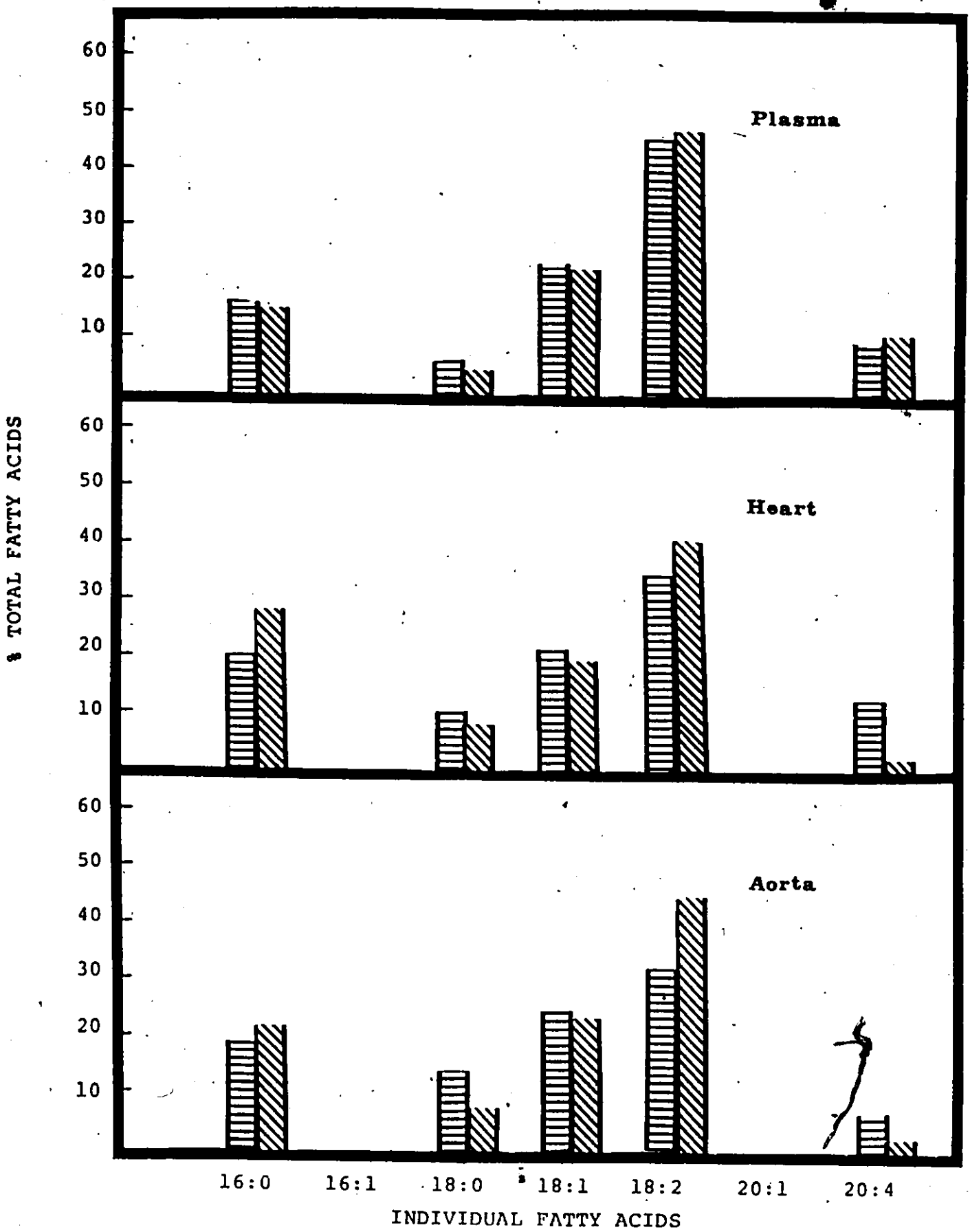
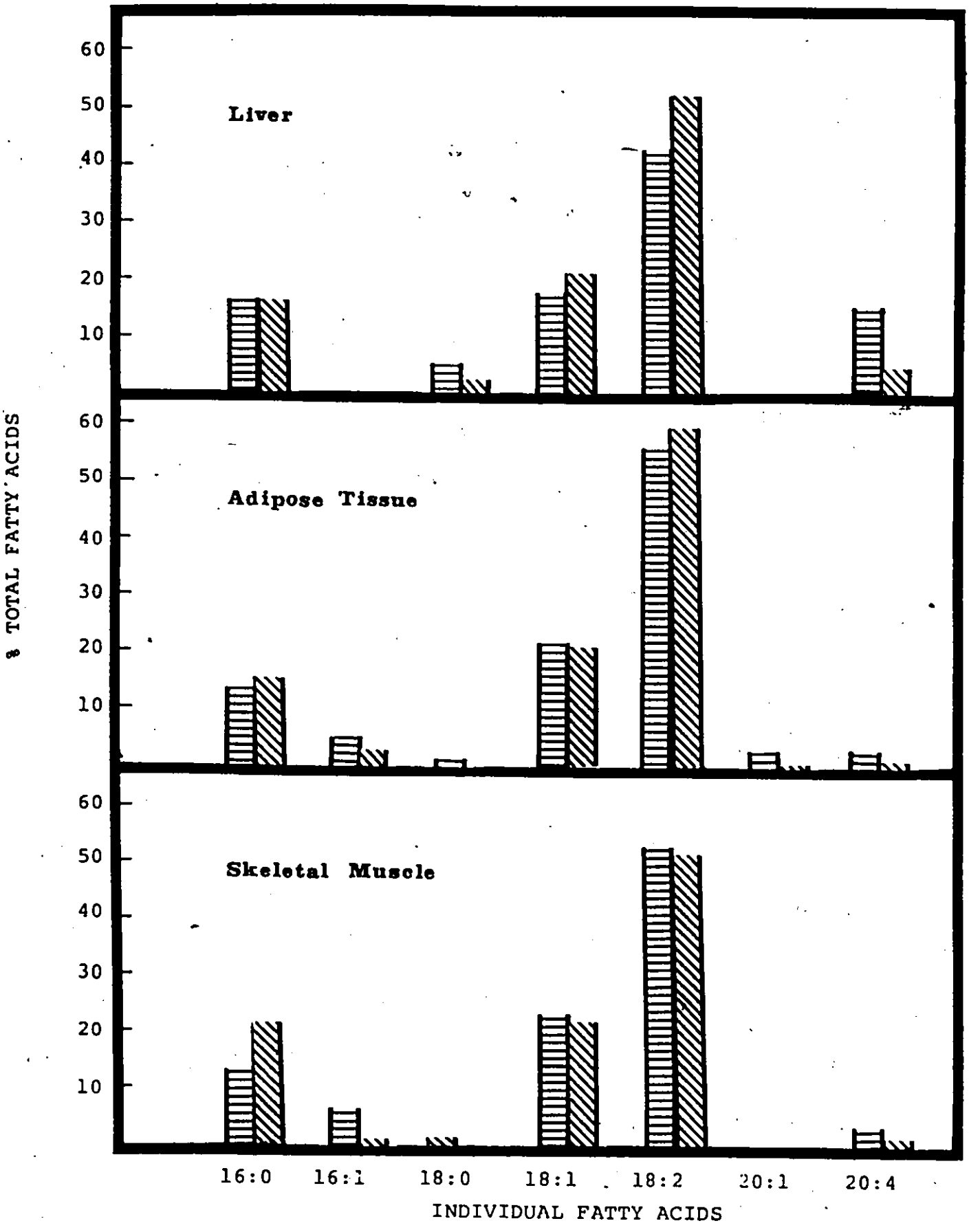


Fig. 5

TISSUE FATTY ACID COMPOSITION



Legend Fig. 6

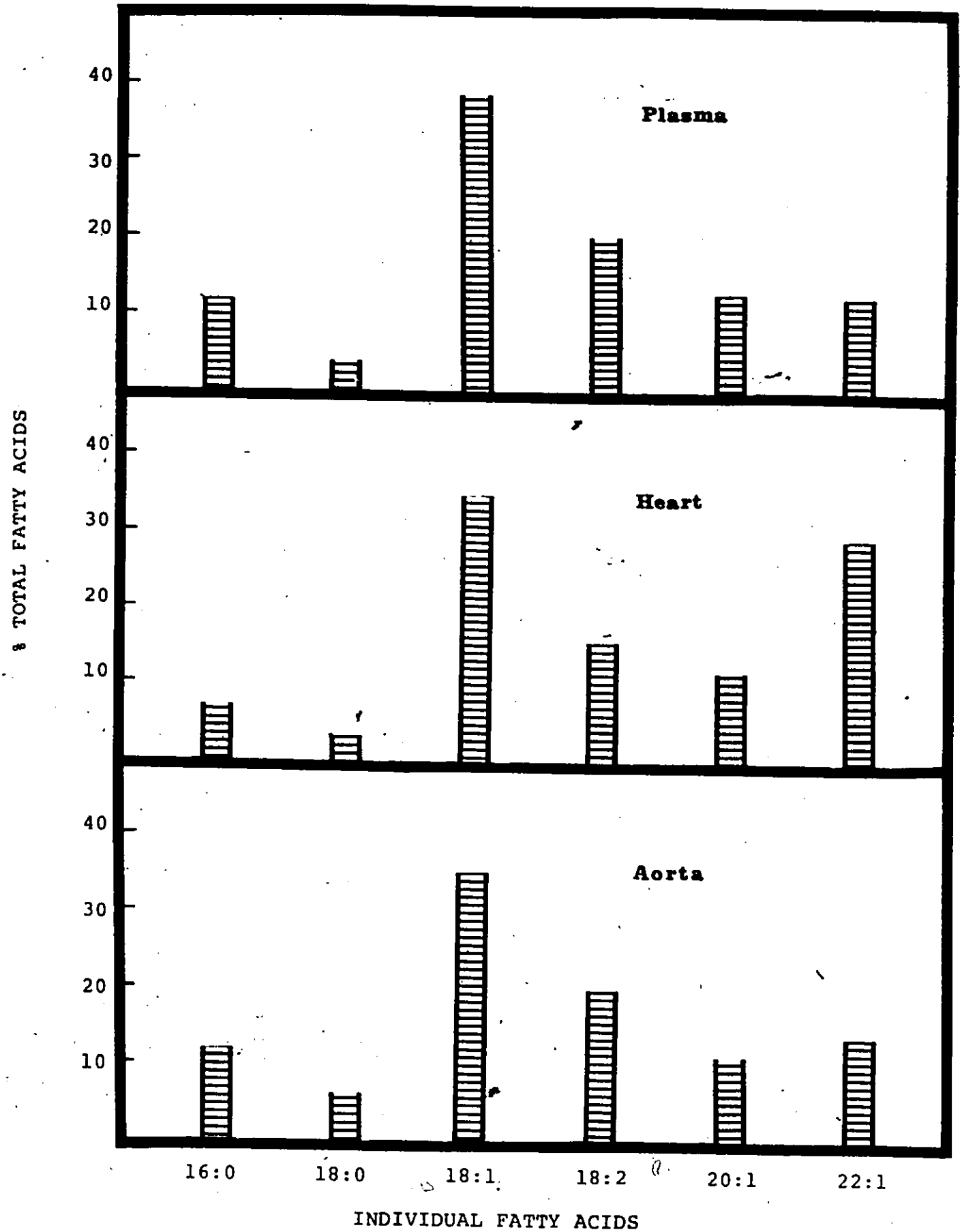
Weanling rats were fed the mustard seed oil diet low in erucic acid for one week.

Plasma, heart and aorta were removed for lipid analysis. The abscissa indicates the individual tissue fatty acids, derived from glycerides and FFA. The ordinate indicates the percentage of each of these fatty acids.

The values are means of two chromatographic assays.

Fig. 6

TISSUE FATTY ACID COMPOSITION



Similarities in fatty acid composition between fasted and non-fasted animals on a corn oil diet was noticed.

These similarities are probably due to the fact that first, in postabsorptive states, FFAs are supplied by the adipose tissue; and second, the plasma TG is synthesized by the liver, which utilizes plasma FFA as the source of the acyl moiety of TG. Thus, because rats under the corn oil regimen retained a relatively large amount of linoleic acid in their tissues, the stored fatty acid could be used for synthesizing new TG in the plasma when animals were fasting.

From comparing the fatty acid composition of plasma in fasted and non-fasted rats on a mustard seed oil diet, it was evident that erucic acid was effectively eliminated from the plasma during the withdrawal of food. This last observation was true regardless of the duration of feeding - one or three weeks - (Figure 9 and 10). A sharp decrease in erucic acid in rats plasma after an overnight of fast was noticed (a drop from 26.15% to 9.45% after one week on the diet, or from 25.02% to 8.54% after 3 weeks). This decrease was accompanied by an

Legend Fig. 7

Weanling rats fed the corn oil diet for one week.

Weanling rats fed the corn oil diet for 6 days then fasted overnight.

Tissues were removed for lipid analysis.

The abscissa indicates the individual tissue fatty acids, derived from glycerides and FFA. The ordinate indicates the percentage of each of these fatty acids.
The values are means of chromatographic assay run in duplicate.

Fig. 7

TISSUE FATTY ACID COMPOSITION

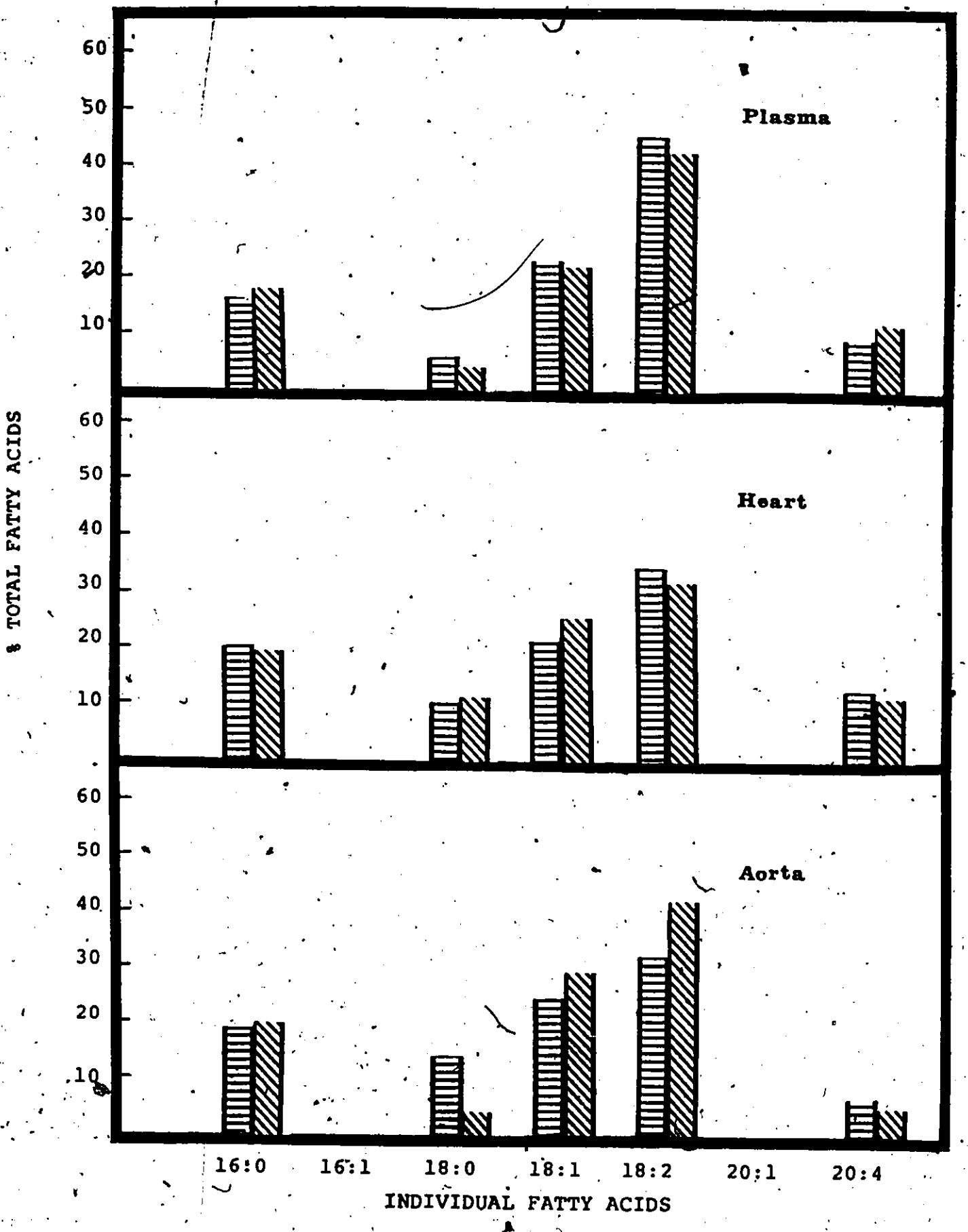
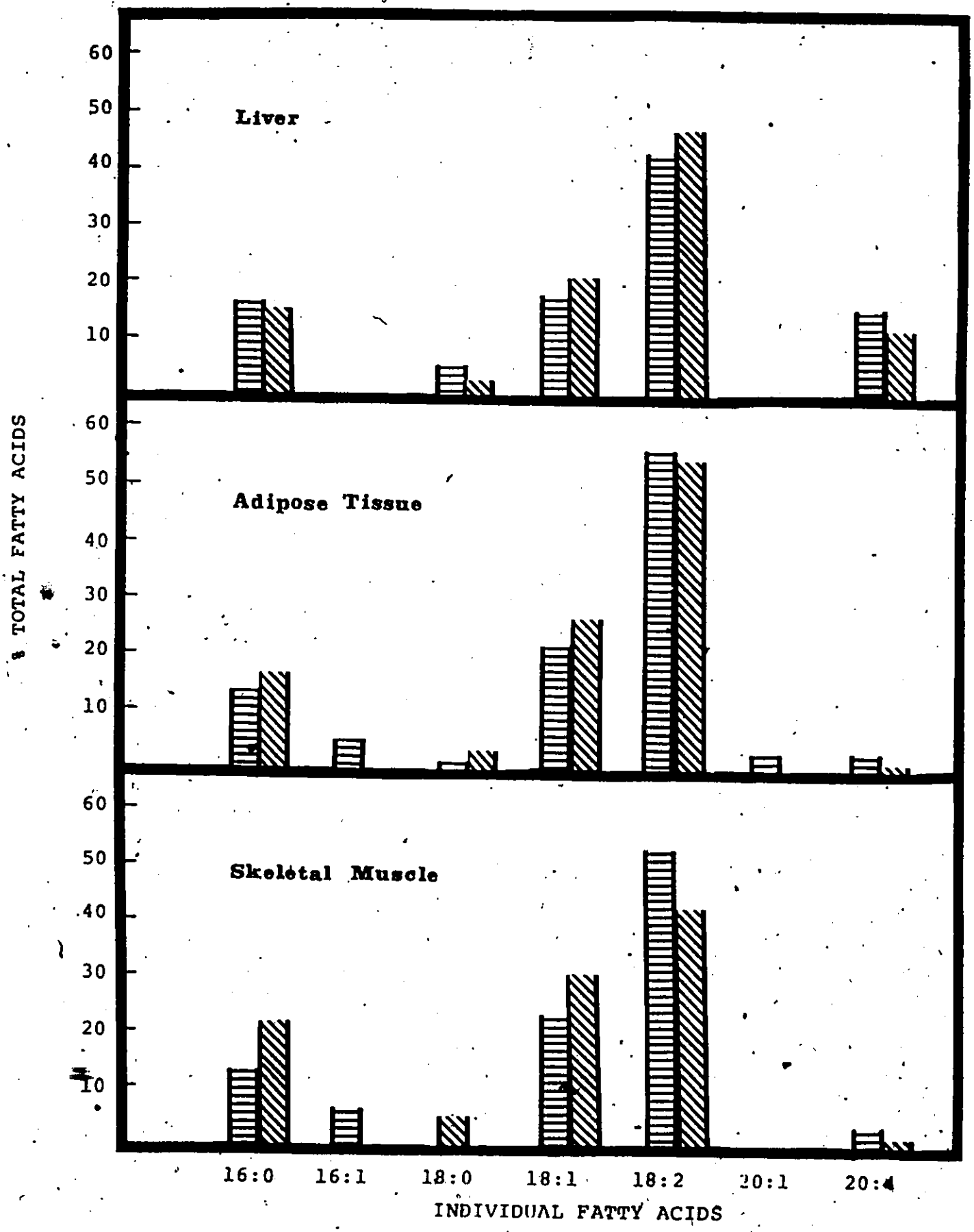


Fig. 7

TISSUE FATTY ACID COMPOSITION



Legend Fig. 8

Weanling rats were fed the corn oil diet for 20 days,
then fasted overnight.
Tissues were removed for lipid analysis.
The abscissa indicates the individual tissue fatty acids,
derived from glycerides and FFA. The ordinate indicates
the percentage of each of these fatty acids.
The values are means of chromatographic assay run in duplicate.

Fig. 8

TISSUE FATTY ACID COMPOSITION

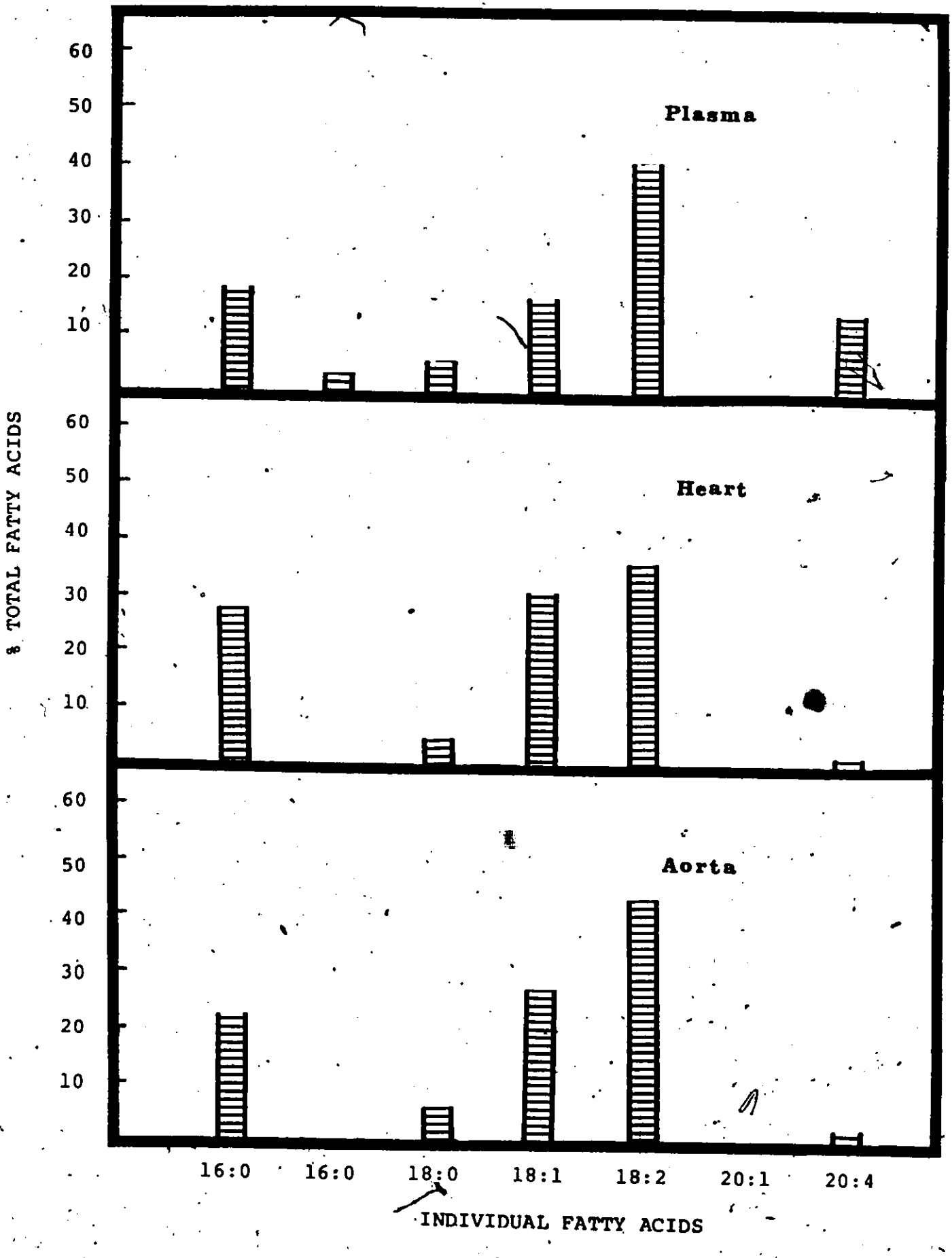
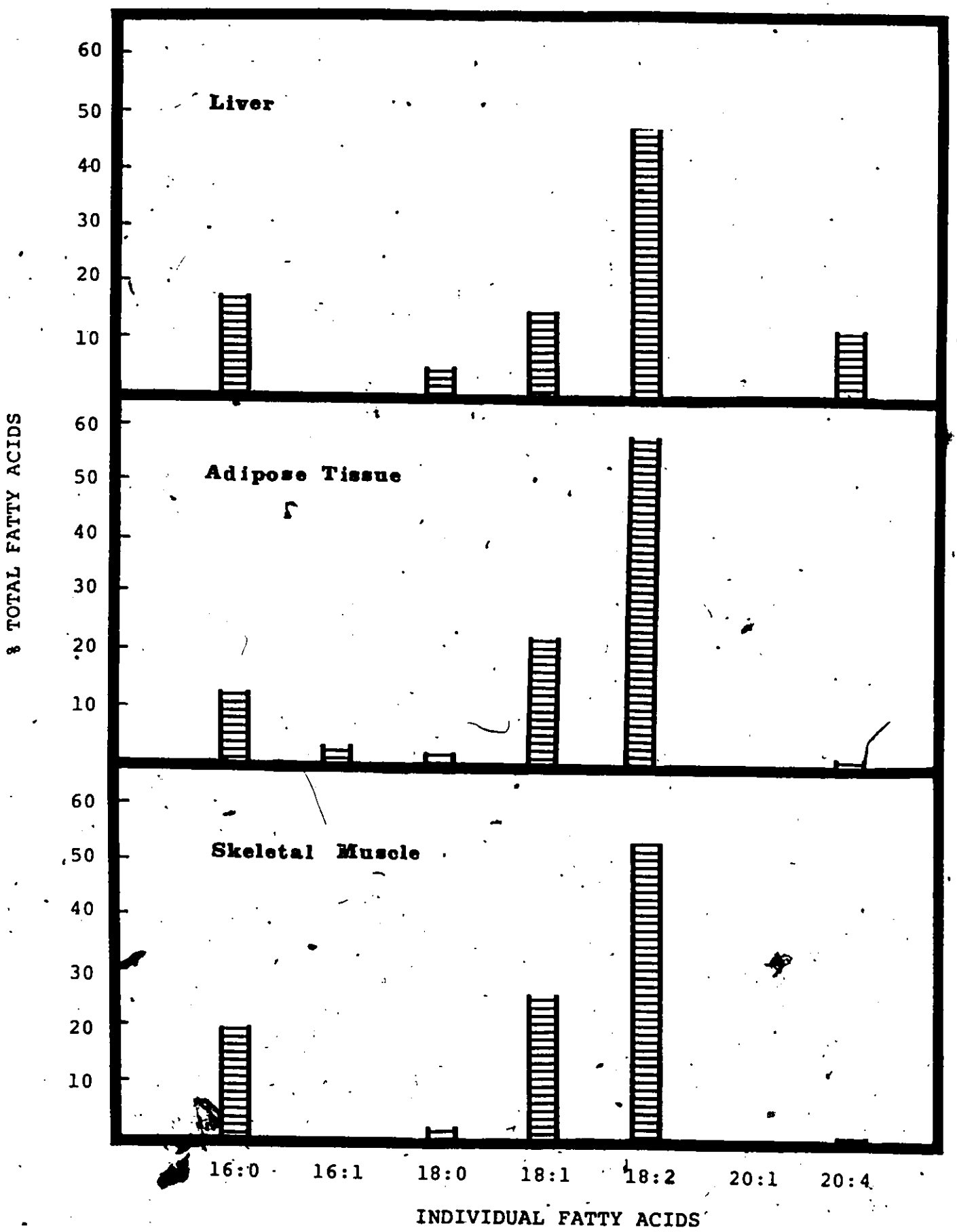


Fig. 8

TISSUE FATTY ACID COMPOSITION



increase in shorter chain fatty acids, and mainly palmitic acid.

Moreover, after three weeks on the diet and an overnight of fast, the high relative amount of erucic acid in cardiac tissue dropped markedly (Figure 10). Along with it, the percentage of palmitic and oleic acid increased in the same tissue.

Finally, aorta which resembled plasma in its fatty acid composition, also showed a significant decrease in its erucic acid percentage when diet was withdrawn and that regardless of the feeding duration.

ESTIMATION OF TRIGLYCERIDES IN TISSUES

Myocardial triglyceride concentration in rats fed the corn oil diet was similar in all rats irrespective of duration of treatment (Table 1).

In rats fed the mustard seed oil diet for one week, myocardial triglyceride concentration was several fold greater than that of rats fed the corn oil diet (9.2 mg/g vs. 1.18 mg/g of wet tissue). Prolonging the dietary

Legend Fig. 9

Weanling rats fed the mustard seed oil diet for one week.

Weanling rats fed the mustard seed oil diet for 6 days and fasted overnight.

Tissues were removed for lipid analysis.

The abscissa indicates the individual tissue fatty acids, derived from glycerides and FFA. The ordinate indicates the percentage of each of these fatty acids.
The values are means of chromatographic assay run in duplicate.

Fig. 9

TISSUE FATTY ACID COMPOSITION

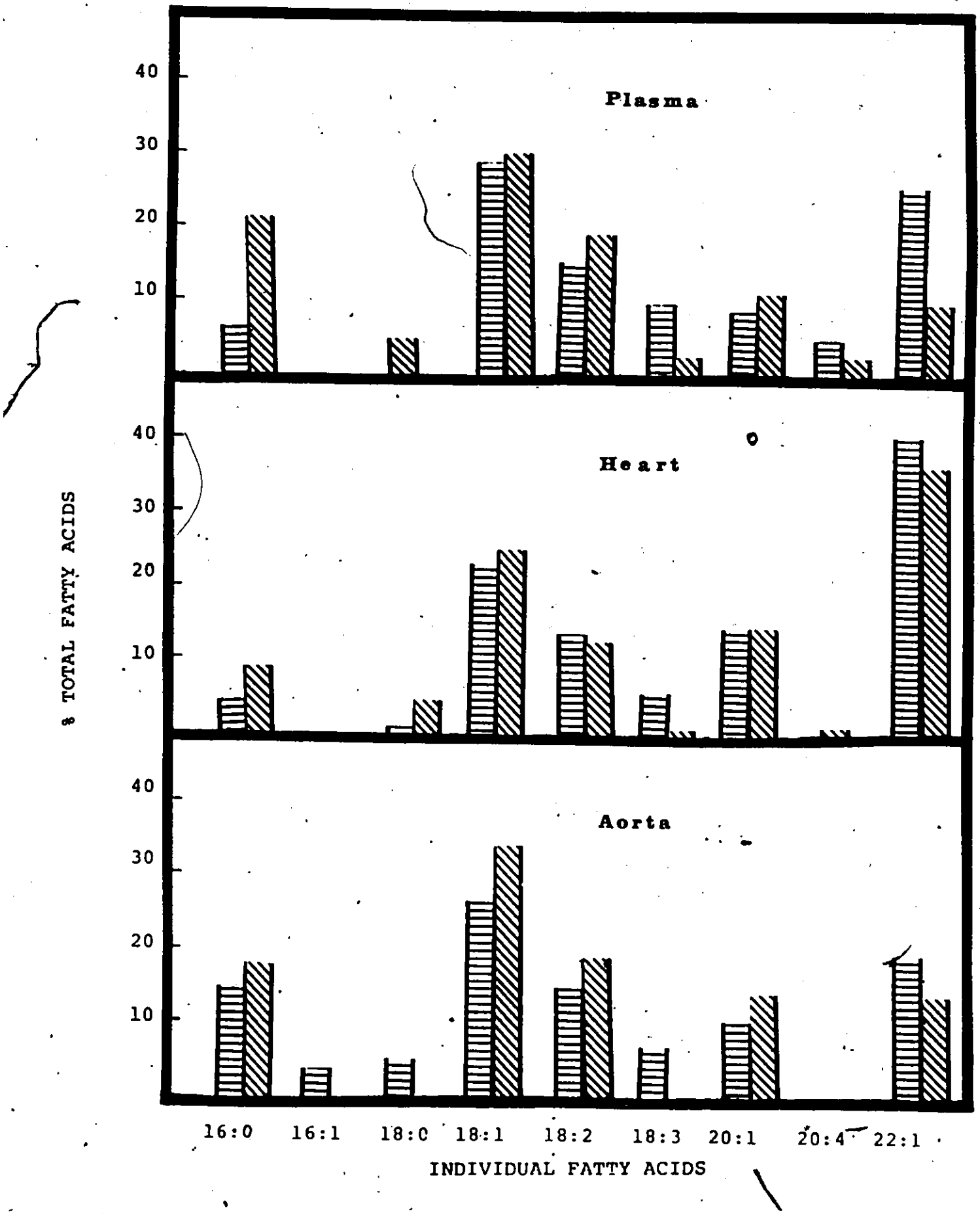
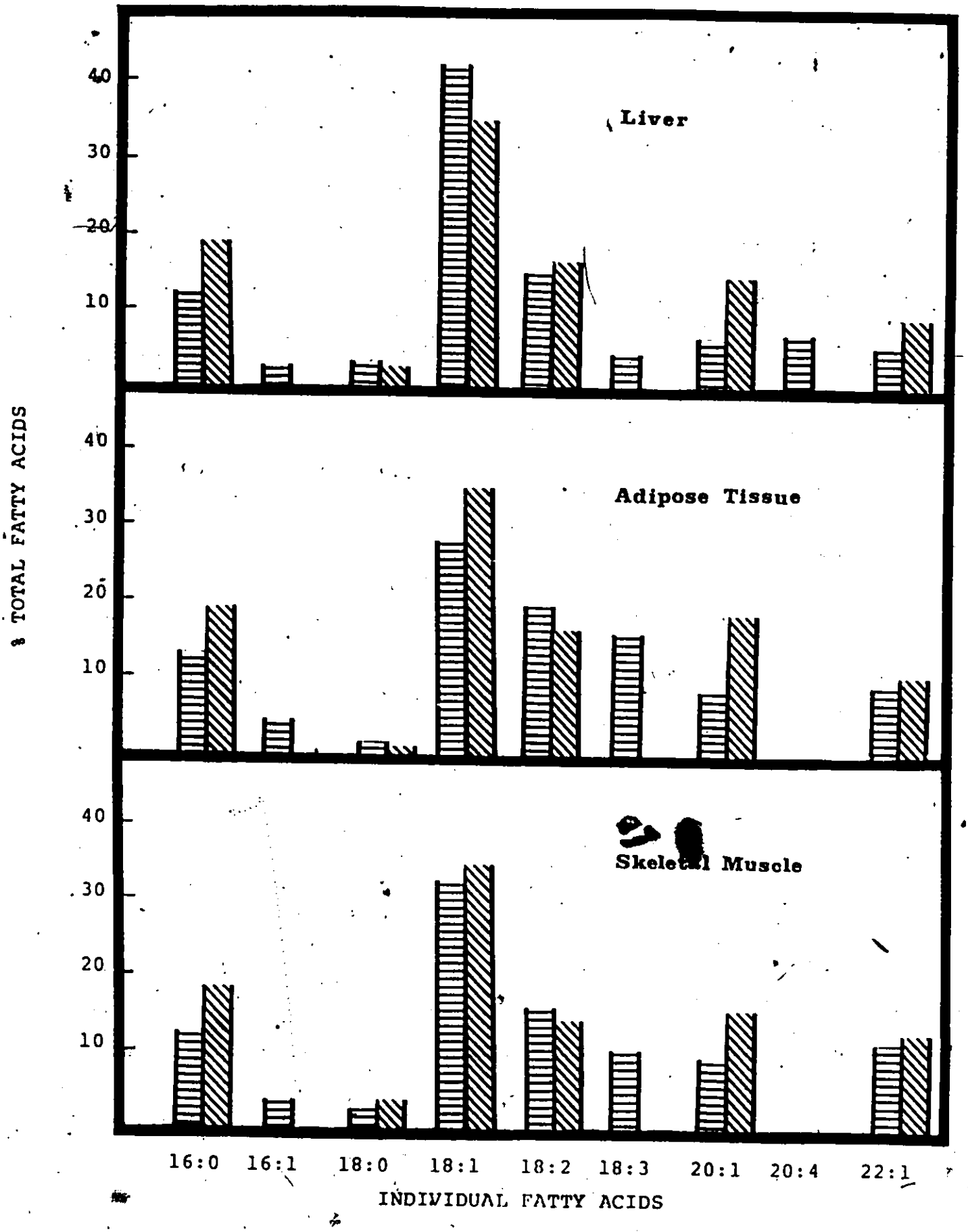


Fig. 9

TISSUE FATTY ACID COMPOSITION



Legend Fig. 10

Weanling rats were fed the mustard seed oil diet for 20 days, then fasted overnight.
Tissues were removed for lipid analysis.
The abscissa indicates the individual fatty acids in tissues, derived from glycerides and FFA. The ordinate indicates the percentage of each of these fatty acids.
The values are means of chromatographic assay run in duplicate.

Fig. 10

TISSUE FATTY ACID COMPOSITION

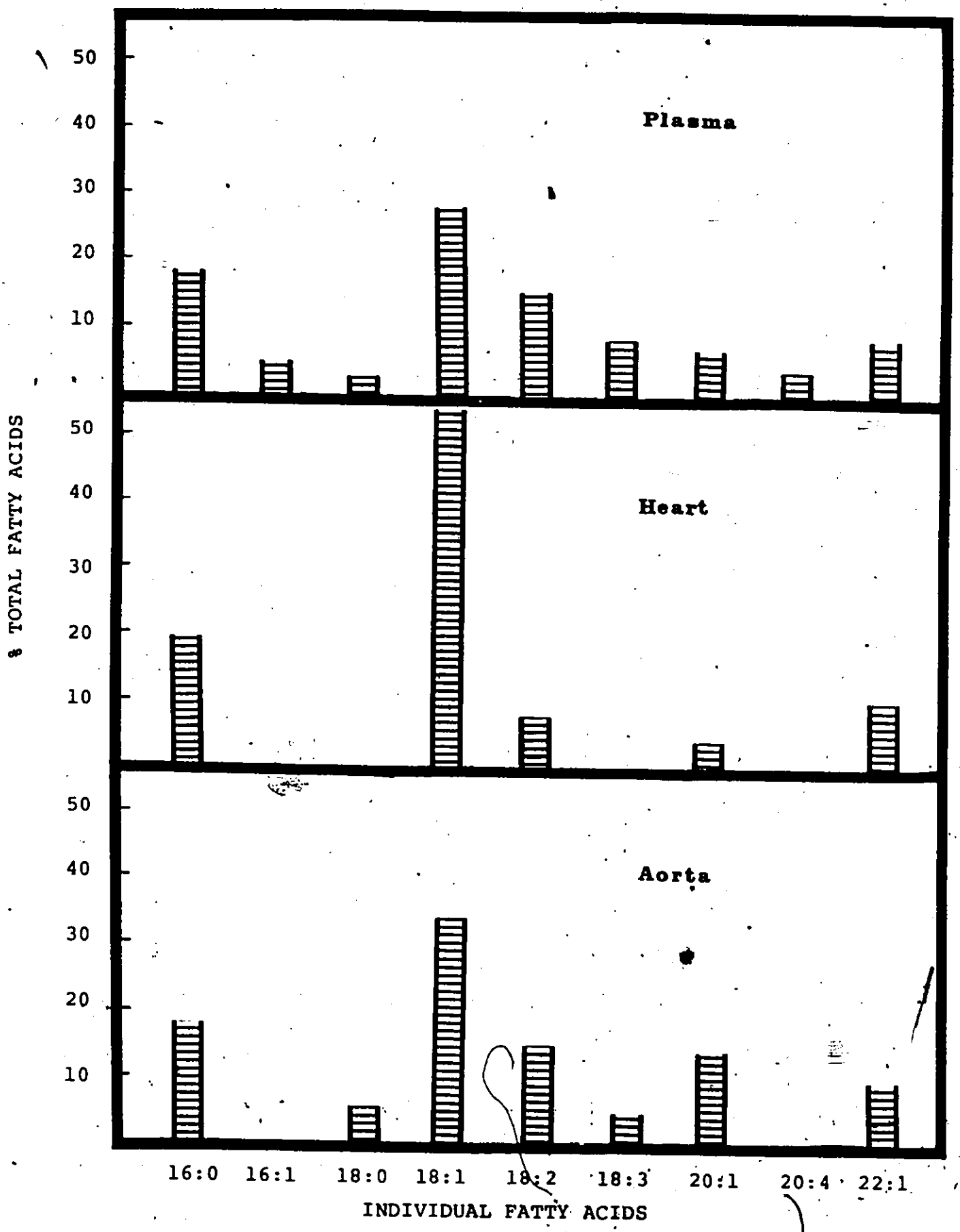
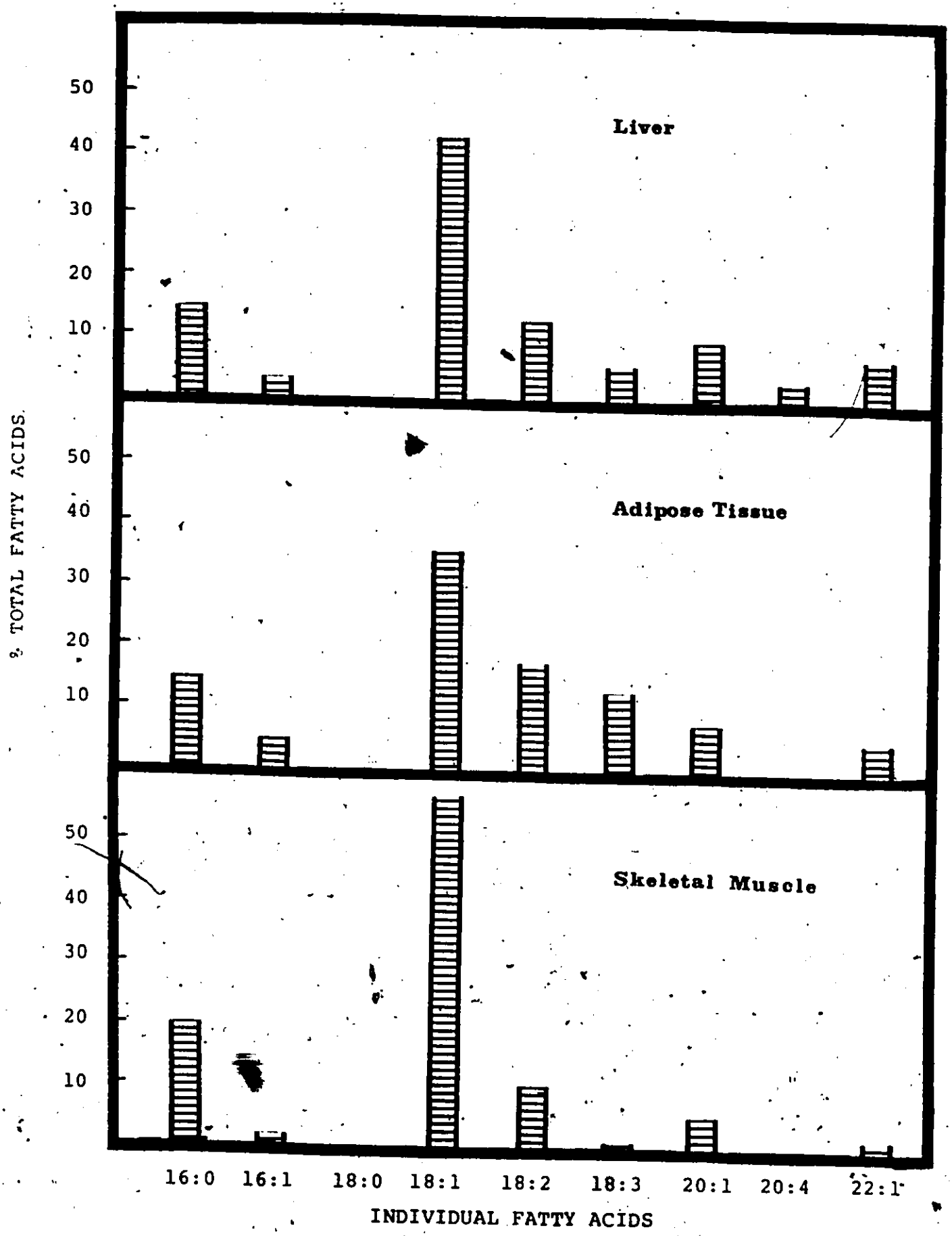


Fig. 10

TISSUE FATTY ACID COMPOSITION



regimen to six weeks significantly decreased cardiac TG concentration to half the amount reached after one week of treatment (Table 1, page 59).

Estimation of triglyceride in liver did not show a statistically significant difference in TG content between rats fed either diet for a period of one week.

Absolute values increased after six weeks of consumption of a corn oil diet (Table 1).

In the case of skeletal muscle triglyceride, values from rats fed the corn oil diet for one week were very close to those from rats fed mustard seed oil diet for the same duration of time. After six weeks of treatment, all of the values remained in the same range (Table 1).

OXIDATION OF OCTANOIC ACID BY SKELETAL MUSCLE IN VITRO

The effect of both high fat diets on the fatty acid oxidation in skeletal muscle tissue was determined (Fig. 11).

When expressed as picomoles of $^{14}CO_2$ produced per gram of wet tissue per hour, it appeared that there was no

significant difference in the oxidation rate after feeding either diet for one week. After three weeks of ingestion of corn oil diet, octanoic acid oxidation increased significantly and remained so after six weeks of treatment.

On the other hand, oxidation of octanoic acid was unchanged after three and six weeks of mustard seed oil consumption and no significant differences were observed relative to the length of the feeding period (Figure 11).

Legend Fig. 11

Incubation in vitro of skeletal muscle tissue from rats fed either test diet, with labelled octanoic acid. The test diets were given for one, three or six weeks.

Rats on a mustard seed oil diet.

Rats on a corn oil diet.

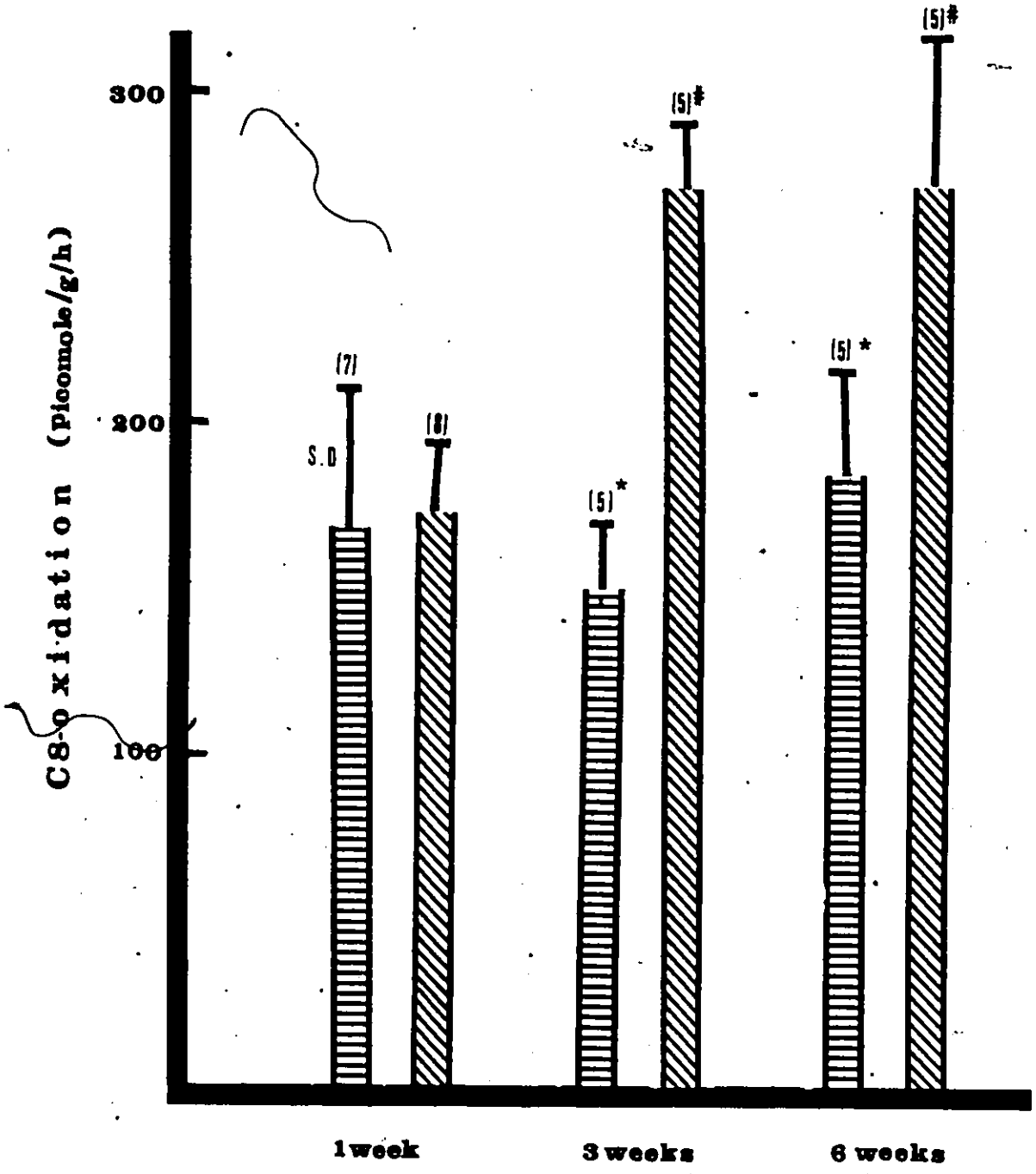
S.D. Standard deviation

Number in parentheses is the number of determination

* Significant change compared to values from corn oil-fed rats.

\# Significant change compared to values of one week-fed-corn oil-rats.

Fig.11



Incubation in vitro of skeletal muscle with labelled octanoic acid.

DISCUSSION

Fully refined seed oils are composed almost entirely of triglycerides. These oils have in common a very high ratio of unsaturated to saturated fatty acids, and the oils differ primarily in the unsaturated fatty acid composition. Corn oil consists primarily of linoleic acid (18:2) with less oleic acid (18:1), while mustard seed oil is higher in oleic acid than linoleic acid. Peculiar to mustard seed oil (and rapeseed oil) are long-chain monoenoic acids, i.e. gadoleic acid (20:1) and erucic acid (22:1), which can comprise more than 50% of the total fatty acids. The fatty acid composition of tissues in animals fed a normal diet, consists of approximately 50% saturates and is considerably different from the fatty acid composition of most vegetable oils which consist of approximately 90% mono- or poly-unsaturates. Nevertheless, prolonged feeding of vegetable oils at 20% by weight of a balanced diet to laboratory animals produces no ill effects, except some biochemical and histological lesions that resulted from the feeding of a high erucic acid-containing rapeseed or mustard seed oil (Kramer et al. 1973).

Effects of diets on Plasma Lipids

In rats fed a mustard seed oil diet, the fatty acid composition of plasma resembled that of mustard seed oil and was directly proportional to the alimentary state of the rats. The results showed both with the high erucic acid and the low erucic acid diet that the percentage of plasma erucic acid was very similar to that of the respective diet. Furthermore, the duration of feeding did not affect the results.

After an overnight fast, the relative amount of erucic acid in the plasma clearly diminished regardless of the previous duration of the special diet treatment (one or three weeks). This is in agreement with the results of Vasdev and Kako (1978). This dramatic reduction of circulating erucic acid concentration indicated that erucic acid is rapidly eliminated from the plasma. It is well known that in the fasted state, fatty acids of the adipose tissue and liver supply the plasma. Since the results of this study showed that they contained only a small amount of erucic acid, they could not supply plasma with much erucic acid. Therefore, plasma of the fasted rats contained considerably less erucic acid than did the

plasma of the non-fasted rats.

As for the rats under the corn oil regimen for one and three weeks, the results showed no significant difference in plasma composition between the fasting and non-fasting state. This could be explained by the adipose tissue storage of the different fatty acids in the same percentage as that in the diet. When food was withdrawn, adipose tissue supplied the plasma with fatty acids.

Effects of diets on Adipose Tissue Lipids

The results of the present study, in combination with other reports (Beare-Rogers 1977; Boucrot and Bezard 1973; Walker 1972; Vasdev and Kako 1978), showed that following a mustard seed oil regimen, the percentage of erucic acid in adipose tissue was very low despite its high proportion in the plasma. This could be due to discrimination of the acylation reaction in the adipose tissue, whereby erucic acid could be excluded as the acyl donor for the reaction leading to TG formation. The present work does not provide direct evidence to support this assumption. However, it is well known that glycerol-3-phosphate acylation reaction by the

subcellular fractions of the liver is substrate specific (Monray et al. 1972; Yamashita et al. 1975). Also, Zaror-Behrens et al. (1976) demonstrated that the rate of such a reaction with erucoyl-CoA was 1/11 of that with oleoyl-CoA. Consequently, it is possible that the acylation reaction of the adipose tissue also discriminates against erucic acid.

Another possibility would be that TG lipolysis occuring in adipose tissue may favor erucic acid, thus resulting in a comparably low erucic acid concentration of this tissue. However, the fact that plasma erucic acid was almost in the same proportion as that found in mustard seed oil suggests that lipoprotein lipase hydrolyzes plasma triglycerides without strong fatty acid specificity. Such a view is supported by the results obtained by Morley et al. (1977), who reported that lipoprotein lipase of both bovine milk and rat plasma did not show substrate specificity. According to another report (Brockerhoff et al. 1967), pancreatic lipase too, does not discriminate against erucic acid during the hydrolysis of triglycerides. Thus a more plausible explanation for the fact that erucic acid

is excluded from the adipose tissue could be that this fatty acid is unsuitable for the acylation reaction leading to triglyceride formation(Boucrot 1973), rather than triglyceride containing erucic acid being preferentially hydrolyzed.

When rats were fed a corn oil diet for one and three weeks the fatty acid composition of the adipose tissue resembled very much the corn oil composition. This may be explained by the fact that all fatty acids involved had "normal" chain length; thus, the rates of acylation for the different fatty acids were probably the same.

Effects of diets on Liver Lipids

Generally, the liver appears to be capable of metabolizing a large influx of dietary long chain fatty acids. In this study, its relative content of erucic acid was usually in the range of 5-10%. This is in agreement with many other authors (Kramer 1975; Beare-Rogers 1977; Neat 1981; Bremer 1982).

Also, the low percentage of erucic acid was accompanied by a high percentage of oleic acid. According to Ong (1977)

and Bremer (1982), liver seems to be the most active organ in the conversion of erucic acid to oleic acid in the intact animal.

Like other fatty acids, the 22:1 fatty acids are oxidized by liver mitochondria in the presence of ATP, CoA and carnitine, but the oxidation of erucate compared to palmitate is slow (Christiansen 1978). However, it has been established recently that besides the "classical" β-oxidation system of mitochondria, liver peroxisomes contain a different cyanide-insensitive β-oxidation system (Lazarow 1978).

The peroxisomal β-oxidation system in rat liver is induced by the intake of 22:1 fatty acid-containing diets (Neat 1980, Neat 1981), by fasting (Ishii 1980) and by clofibrate (Osumi 1979).

Unlike mitochondrial β-oxidation, peroxisomal β-oxidation does not oxidize fatty acids to completion (Lazarow, 1978) and is unable to oxidize short chain fatty acids (Bremer 1982). Thus, it could be suggested that the low

percentage of 22:1 in the fatty acids of the liver is probably due to the activity of the liver peroxisomal β-oxidative system in chain-shortening long chain erucic acid.

When the concentration of triglyceride in liver was compared between rats on a mustard seed oil diet and on a corn oil diet, the difference in concentration was not statistically significant. Furthermore, the length of the feeding period (one, three or six weeks) did not affect the results significantly. These observations seem consistent since the liver was not affected by the high erucic acid diet.

In the fasted state, triglycerides are mobilized from the adipose tissue, transported as free fatty acids in the blood, and then redeposited as triglycerides in the liver, where the initial stages of much of the fat degradation begin.

In this study, the difference in the composition of liver fatty acids between fed and fasted state could be attributed to the transfer of adipose tissue triglyceride to the liver after an overnight fast.

Effects of diets on Aortic Lipids

Feeding a high fat-high erucic acid diet increased the proportion of erucic acid in the total fatty acids of aorta FFA and glycerides, whereas feeding a high fat-high linoleic acid-diet increased the proportion of linoleic acid in similar lipid fractions in aorta. The general pattern of fatty acid distribution in the aorta was similar to that in the plasma. This is in agreement with the results of other authors (Kako and Vasdev, 1980).

In the fasted state, the percentage of erucic acid dropped significantly as it did in plasma. This could be explained by the short supply of lipoprotein triglyceride or FFA in plasma with erucic acid. Neither adipose tissue nor liver stored erucic acid in the feeding state; therefore because neither adipose tissue nor liver could supply tissues with erucic acid when animals were fasted, a drop in erucic acid was expected and found in all tissues specially those that had a relatively high percentage of erucic acid. This decrease was accompanied by an increase in oleic and palmitic acid probably due to erucic acid conversion to shorter chain fatty acids.

In contrast with aorta from rats on a mustard seed oil diet, similar tissue from rats on a corn oil diet did not show a significant difference in fatty acid composition between the fasted and fed state. Linoleic acid derived from the diet accumulated increasingly in rat tissues.

Effects of diets on Skeletal Muscle Lipids

Skeletal muscle tissue, unlike cardiac tissue, had a relatively low percentage of erucic acid and a relatively high percentage of oleic acid after one week on the mustard seed oil diet.

After three weeks, conversion of erucic acid to shorter monoenoic acids was more noticeable.

Skeletal muscle tissue did not accumulate triglycerides compared to a similar tissue after a corn oil diet. This is in agreement with other studies (Mersel et al. 1978; Vles 1975). Triglycerides values after one to six weeks of mustard seed oil diet were not significantly different from those after consuming a corn oil diet.

The reason could be that because of the skeletal muscle tissue dependence on fatty acid oxidation for its energy requirements, this tissue has a relatively high cellular oxidative enzyme activity.

Effects of diets on Cardiac Lipids

The results of this study make it evident that a diet high in erucic acid mainly affected the heart.

The main points of the results observed in rats hearts on a mustard seed oil diet may be summarized as follows:

1. After one week on the diet, the fatty acid composition of heart glycerides and FFA showed a disproportionately large amount of erucic acid compared to the fatty acid composition of the test diet (Figure 2, p.50).

2. By the third week of the experiment, myocardial fatty acid composition began to change, erucic acid decreasing and oleic acid increasing. According to several investigators (Abdellatif 1972; Kramer et al. 1978; Kako and Vasdev 1979), this trend was continued with the extension of the feeding period

to 16 weeks.

3. The myocardial triglyceride content increased several fold within one week of the commencement of the diet, but decreased markedly by the sixth week of the regimen (Table 1, page 59).

Fatty acid composition of the plasma examined after three weeks on a mustard seed oil diet was nearly identical to that examined after one week. Also, erucic acid content of the glycerides and free fatty acids of both liver and adipose tissue was much lower than that of the heart. Since the heart uses the fat coming from the liver and adipose tissue and transported in the plasma, and since the heart was exposed to the same erucic acid concentration in plasma after one and three weeks on the diet, it is likely that the marked changes in lipid composition of the cardiac tissue were not caused by alteration in body lipid metabolism, but were the result of an adaptation by the heart itself.

There are four mechanisms which may account for erucic acid accumulation in the heart:

i) A greater rate of uptake and esterification of erucic acid by the cardiac cell, compared to other fatty acids.

ii) A slow rate of erucic acid chain shortening inside the cells.

iii) A slow rate of erucic acid oxidation to CO_2 inside the cells.

iv) A slow lipolysis of intracellular erucic acid-containing triacyl glycerol.

It has been shown that erucic acid uptake occurs at a similar rate to that of other fatty acids, in proportion to its concentration in the medium. It is mainly incorporated into triacyl glycerols (Samuel et al. 1976; Pinson et al. 1976; Vasdev et al. 1977) (i).

Long chain fatty acids with 18 or more carbons are known to be shortened in the intact animal, while fatty acids with 16 or less carbons seems to be completely oxidized once the oxidation has started. Chain shortening has been shown for erucic acid in rats hearts but it is done at a slow rate (Vasdev and Kako 1976) (ii).

It has also been reported that erucic acid is completely oxidized by tissues other than heart (Craig and Beare 1967; Boucrot and Bezard 1973) but the ability of the heart to oxidize this fatty acid is limited (Christophersen and Bremer, 1972; Christiansen et al. 1977; Vasdev and Kako, 1976). These authors reported that erucic acid was poorly oxidized by heart mitochondria relative to shorter chain fatty acids (iii).

Conflicting results are found between studies dealing with lipolysis of erucic acid-containing TGs (iv). Chi Ming and Kummerow (1977) found high levels of cleavage for triglycerides rich in erucic acid whereas Mersel et al. (1978) noticed an absence of cleavage of these triglycerides by the heart.

In conclusion, the most plausible explanation for the proportion of erucic acid in the heart after consuming a mustard seed oil diet for one week is the low cardiac ability to oxidize completely or partially this long chain fatty acid, erucic acid.

The results of this study showed a decrease in erucic acid percentage in cardiac tissue after three weeks of feeding and an increase in oleic acid in the same tissue. Similar results were also found by other investigators (Vasdev and Kako, 1978; Norseth et al. 1979; Bremer et al. 1982). Data are interpreted to suggest some adaptive changes in the heart toward the diet. It seems probable that erucic acid undergoes an increased degradation (chain-shortening) with removal of 2-carbon units from the carboxyl end of the chain and with partial stabilization of the molecule at oleic acid (Vasdev et al. 1978). Norseth et al.(1979) and Bremer et al.(1982) suggested that the increased chain shortening could be explained by some increase in mitochondrial oxidative capacity and by an increased peroxisomal activity in the heart.

Within a few days of being on a mustard seed oil diet, rats developed a substantial cardiac lipidosis. The accumulated heart lipids were almost exclusively triglycerides. The results of this study showed that this accumulation reached an extreme magnitude after one week (9.2 mg/g vs. 1.18 mg/g in corn oil-fed-rats hearts).

This difference compares favourably with previously published results (Abdellatif et al. 1970; Hung et al. 1977; Norseth J. 1979).

However, the cardiac lipidosis regressed after one week of treatment despite the continuous consumption of the diet (Table 1, page 59). By the sixth week, it reached half the amount found after one week (9.2 mg/g after the first week vs. 4.6 mg/g after the sixth week). Furthermore, according to other investigators (Beare-Rogers et al. 1971; Kako and Vasdev 1979) cardiac triglycerides fall to control values when the diet is administered for a longer duration.

In comparison, rats on a corn oil diet did not accumulate triglycerides in their hearts. Cardiac triglyceride content did not change significantly after one, three or six weeks of treatment. The reason is that all fatty acids involved in this diet are fatty acids with normal chain length.

The accumulation of triglycerides in the myocardium of rats on a mustard seed oil diet is obviously an imbalance between the net input and the oxidation of fatty acids.

The following mechanisms could be held responsible for this accumulation:

i) An increased uptake of fatty acids by the heart.

ii) An increased uptake of lipoprotein TG by the heart.

iii) An increased TG synthesis in the heart.

iv) A decreased hydrolysis of cardiac tissue TGs.

The increased level of triglycerides which was observed following the first week of feeding the mustard seed oil diet could be due to an increased fatty acid uptake by the heart (i). However, it is difficult to explain the decrease in the heart TG level in the subsequent weeks by a decrease of fatty acid uptake for the following reasons:

(a) The TG content of the heart relatively decreased by the third week despite the fact that the fatty acid composition of the plasma did not change during the same period, and

(b) Kako and Vasdev (1979) found that hearts isolated from the rats fed for one week took up exogenous fatty acids during perfusion at a rate similar to that of the hearts of rats fed for three weeks.

Therefore, it is unlikely the mechanism (i) above is the cause of the observed metabolic changes in the heart.

The magnitude of triglyceride uptake by the heart is controlled by the activity of cardiac lipoprotein lipase and by the level of plasma very low density lipoprotein TG (ii). However, many investigators (Jansen et al. 1975; Vasdev and Kako, 1978) demonstrated that the activity of this enzyme and the very low density lipoprotein TG level were both increased in the first week of feeding mustard seed oil diet and remained elevated during the whole feeding period. This finding may explain the lipidosis observed in one week-fed rats of this study, but is incompatible with the decrease of cardiac triglyceride content after a few weeks.

By exclusion therefore, the observed TG change could be attributed to the change in the rate of TG synthesis (iii) and/or in the rate of TG hydrolysis (iv) in the heart. In support of these hypotheses, Christophersen and Bremer (1972) concluded that erucic acid inhibits the oxidation of other fatty acids. The activated fatty acids therefore accumulate and are channelled into other pathways which are relatively less inhibited by erucic acid, such as those involved in triglyceride synthesis. The fact

that the triglycerides which accumulate in the heart of the erucic acid-fed-rats contain not only erucic acid but also the other fatty acids related to the diet, supports this idea. Furthermore, Kako and Vasdev (1979) reported that the hearts of rats consuming the mustard seed oil diet for a week oxidized palmitic or erucic acid at a reduced rate and esterified these acids at an accelerated rate. Thus, mechanism (iii) is a possible cause of cardiac triglyceride accumulation.

Finally, as noted earlier in the discussion, the role of the intracellular cardiac lipase on TG hydrolysis is still subject to controversy. Conflicting results are found in the literature (iv). While Kramer et al. (1973) showed a negligible trierucin hydrolysis by rat heart lipase, Mersel et al. (1978) noticed an absence of cleavage by the heart for these TGs. However, Chi Ming et al. (1977) suggested that trierucin would be the best substrate for TG lipase because its melting point is below body termperature.

In short, cardiac lipolysis following a mustard seed oil diet seems to be caused by the presence of a high concentration of erucic acid in the heart that appears to enhance the esterification of fatty acids through mechanisms as yet unknown.

The decrease of the triglyceride level in the heart after prolonged feeding of mustard seed oil diet is probably due to adaptive changes in the heart itself.

In the process of this adaptation, it seems probable that the ability of the heart to oxidize erucic acid to shorter chain fatty acids (i.e., fatty acids normally found in the body) increases.

Norseth et al. (1979), Kako et al. (1979) and Bremer et al. (1982) reported an increased chain shortening of erucic acid in the heart as a result of three weeks feeding of a high erucic acid diet. The conversion of erucic acid to a shorter chain fatty acid ester would reduce the level of erucic acid, and fatty acid esterification would consequently be no longer accelerated. As a result of such an adaptation, the

heart TG level would return gradually to the control level despite the continuous consumption of the same diet.

Effects of diets on fatty acid oxidation in Skeletal Muscle Tissue

Skeletal muscle tissue taken randomly from legs of rats on a mustard seed oil diet or corn oil diet for one, three or six weeks, was incubated with octanoic acid. Many other investigators have used octanoic acid for similar types of experiments (Geyer 1949, Fritz et al. 1958, Knittle et al. 1965). According to Geyer (1949), the use of water-soluble octanoate is a valid and useful tool for the study of fat metabolism in vitro: the importance of the use of octanoate lies in the fact that most of the water-soluble forms of the fatty acids which contain 12 or more carbon atoms are relatively toxic to surviving cells, while the non-toxic forms are so water-insoluble that they have difficulty in entering the cell. Moreover, the high sensitivity and accuracy with which the radio-activity can be determined allow even small levels of oxidation to be detected and measured.

Skeletal muscle tissue was chosen to be studied furthermore for several reasons. First, lipid oxidation is the predominant metabolic process in this tissue. Second, there is a paucity of information regarding the metabolism of fatty acids in general in voluntary skeletal muscle in comparison to the number of studies on cardiac muscle. Finally, because cardiac tissue is the most affected tissue by the high erucic acid diet and because heart is a muscle too, it seemed logical to investigate other muscles i.e., the skeletal muscles.

It is known that skeletal muscle is made up of individual muscle fibers and that each muscle fiber is a single, multi-nucleated, long cell. Thus, the action of mincing the skeletal muscle tissue breaks up the fibers and the octanoate has easy access to the enzyme system; therefore the oxidation rates are not limited by membrane transport. For this reason, different fatty acids enter the cells at a similar rate which is limited by diffusion.

The results obtained after incubating skeletal muscle with octanoic acid gave similar rates of octanoic acid oxidation in tissues from rats fed either the mustard seed oil diet or the corn oil diet for a period of one week.

While the oxidation rate of octanoic acid remained the same after one, three or six weeks of consuming the mustard seed oil diet, it increased markedly when the diet contained corn oil.

From these results, one could suggest that mustard seed oil diet did not inhibit fatty acid oxidation nor reduce it. Although corn oil diet seemed to clearly increase oxidation rate, it does not look as though mustard seed oil diet lowered it. Since it was previously observed that erucic acid was in a low percentage in skeletal muscle tissue after a mustard seed oil diet and since this was explained by the possible conversion of erucic acid to oleic acid, one could probably suggest that whatever pathological effect erucic acid could have, it was eliminated by its rapid degradation to shorter chain fatty acids, mainly oleic acid.

As for the increasing oxidation noticed after the corn oil diet was continued for three and six weeks, this might be explained by a certain effect of the high percentage of linoleic acid reported earlier in the discussion. With its high percentage in the skeletal muscle tissue, the essential fatty acid could have

enhanced the β-oxidation of fatty acids through different possible mechanisms that are not the subject of this work.

In conclusion, the metabolic activity vis-ā-vis the oxidation of lipids was not affected by the mustard seed oil diet and did not significantly change.

GENERAL CONCLUSION

In this work, three main different effects of feeding diets containing high erucic acid were observed:

1. Cardiac tissue showed a large incorporation of erucic acid into the lipid fraction after one or three weeks.

   There were signs of adaptation after three weeks in that erucic acid levels were somewhat lower than after one week and appeared to be more labile since there was a great reduction at three weeks following an overnight fast.

2. There was a large increase in total TG content of

heart muscle following one or three weeks on a high erucic acid diet. This did not occur in adipose tissue nor skeletal muscle. Again there was evidence of adaptation since the highest level (about 8 x control) was found after one week and was about half this amount after six weeks.

3. There was significant increase in the ability of skeletal muscle to oxidize fatty acids as shown by the oxidation of octanoate in rats fed corn oil but not erucic acid containing oil. This appeared to be related to an adaptive process since it occured between one and three weeks on the corn oil diet.

The first two observations are not new findings as such, but this is the first time the effects of the diets are studied in the whole animal in the same work under the same circumstances. This gives an advantage of looking at the metabolism as a whole and discussing the interrelations between one organ and another in the same animals.

As for the third observation, it is a new finding that could not be found elsewhere in the literature. Thus, the results could not be compared with other results.

More research needs to be done on this point for a better understanding and a better evaluation of its importance.

APPENDIX

FATTY ACID COMPOSITION OF THE TEST DIETS (%)

FA	Mst. s.o.d.*	Corn o.d. **	Must. s.o.d. (2) ***
16:0	3.95	9.7	3.44
18:0	1.95	1.4	-
18:1	23.25	25.9	40.22
18:2	18.74	62.1	23.35
20:1	25.54	0.9	19.73
22:1	26.52	-	13.23

* Mustard seed oil diet

** Corn oil diet

*** Mustard seed oil diet with moderate erucic acid content

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

(Derived from glyceride and FFA)

RATS FED MUSTARD SEED OIL DIET FOR ONE WEEK

FA	Plasma	Heart	Aorta
16:0	7.28	3.67	14.82
16:1	-	-	3.54
18:0	-	1.51	5.11
18:1	28.86	22.22	27.25
18:2	15.25	13.74	14.13
18:3	9.44	4.51	6.35
20:1	8.98	14.38	9.99
20:4	4.10	-	-
22:1	26.15	39.95	18.78

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

(Derived from glyceride and FFA)

RATS FED MUSTARD SEED OIL DIET FOR ONE WEEK

FA	Liver	Adipose Tissue	Skeletal Muscle
16:0	12.53	13.17	12.88
16:1	3.47	4.59	3.40
18:0	3.94	2.03	2.51
18:1	42.17	28.10	32.43
18:2	14.94	19.32	17.27
18:3	4.43	16.15	10.31
20:1	6.39	8.17	9.62
20:4	6.11	-	-
22:1	5.98	8.45	11.49

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

(Derived from glyceride and FFA)

RATS FED CORN OIL DIET FOR ONE WEEK

FA	Plasma	Heart	Aorta
16:0	17.58	20.43	19.26
18:0	5.34	10.35	15.18
18:1	22.17	21.91	25.75
18:2	45.59	34.04	32.18
20:1	-	-	-
20:4	9.27	12.63	7.58

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

(Derived from glyceride and FFA)

RATS FED CORN OIL DIET FOR ONE WEEK

FA	Liver	Adipose Tissue	Skeletal Muscle
16:0	17.71	13.07	13.72
16:1	-	4.58	6.95
18:0	5.09	1.83	-
18:1	18.88	21.72	23.81
18:2	43.16	54.40	52.23
20:1	-	2.10	-
20:4	15.13	2.28	3.28

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

(Derived from glyceride and FFA)

RATS FED MUSTARD SEED OIL DIET FOR THREE WEEKS

FA	Plasma	Heart	Aorta
16:0	9.21	4.33	13.34
16:1	-	-	-
18:0	3.37	1.61	4.1
18:1	29.27	35.22	29.36
18:2	15.24	11.57	15.03
18:3	9.03	2.22	6.69
20:1	8.81	14.13	12.91
20:4	-	-	-
22:1	25.02	30.90	18.59

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

(Derived from glyceride and FFA)

RATS FED MUSTARD SEED OIL DIET FOR THREE WEEKS

FA	Liver	Adipose Tissue	Skeletal Muscle
16:0	8.66	16.84	18.23
16:1	1.60	3.15	1.98
18:0	1.69	-	2.70
18:1	48.16	35.84	48.82
18:2	13.33	18.94	10.99
18:3	4.07	15.53	3.47
20:1	6.93	6.75	6.71
20:4	5.74	-	-
22:1	9.79	2.93	7.07

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

(Derived from glyceride and FFA)

RATS FED CORN OIL DIET FOR THREE WEEKS

FA	Plasma	Heart	Aorta
16:0	15.93	28.64	21.38
16:1	-	-	-
18:0	4.29	8.35	7.96
18:1	21.99	19.59	24.12
18:2	47.60	40.91	43.75
20:1	-	-	-
21:4	10.17	2.49	2.79

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

(Derived from glyceride and FFA)

RATS FED CORN OIL FOR THREE WEEKS

FA	Liver	Adipose tissue	Skeletal Muscle
16:0	17.71	14.54	21.08
16:1	-	3.09	1.63
18:0	2.37	0.56	1.72
18:1	21.66	21.22	23.33
18:2	53.47	59.89	51.00
20:1	-	0.49	-
20:4	4.76	0.20	1.21

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

RATS FED MUSTARD SEED OIL DIET WITH A MODERATE ERUCIC ACID CONTENT

FA	Plasma	Heart	Aorta
16:0	11.99	7.27	12.79
18:0	3.90	3.33	7.88
18:1	38.72	34.03	35.51
18:2	20.09	15.39	19.12
20:1	12.61	11.37	11.46
22:1	12.68	28.59	13.17

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

RATS FED MUSTARD SEED OIL DIET FOR SIX DAYS THEN FASTED OVERNIGHT

FA	Plasma	Heart	Aorta
16:0	20.49	8.45	17.94
16:1	-	-	-
18:0	5.3	4.21	-
18:1	29.87	24.00	34.53
18:2	18.97	12.09	19.78
18:3	2.29	0.57	-
20:1	10.96	14.26	14.1
20:4	2.63	1.19	-
22:1	9.45	35.20	13.6

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

RATS FED MUSTARD SEED OIL DIET FOR SIX DAYS THEN FASTED OVERNIGHT

FA	Liver	Adipose Tissue	Skeletal Muscle
16:0	19:67	19:21	18:55
16:1	-	-	-
18:0	3.32	2.38	3.4
18:1	34.74	34.26	34.05
18:2	17.66	16.89	15.15
18:3	-	-	-
20:1	15.25	18.09	16.57
20:4	-	-	-
22:1	9.33	9.14	12.25

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

RATS FED MUSTARD SEED OIL DIET FOR TWENTY DAYS THEN FASTED OVERNIGHT

FA	Plasma	Heart	Aorta
16:0	18.88	19.89	18.00
16:1	6.58	-	-
18:0	3.93	-	4.89
18:1	28.22	57.29	33.54
18:2	16.36	7.80	15.22
18:3	8.56	-	4.60
20:1	7.53	4.46	14.25
20:4	4.39	-	-
22:1	8.54	10.00	9.50

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

RATS FED MUSTARD SEED OIL DIET FOR TWENTY DAYS THEN FASTED OVERNIGHT

FA	Liver	Adipose Tissue	Skeletal Muscle
16:0	15.25	15.47	20.06
16:1	3.7	5.22	2.12
18:0	-	-	-
18:1	42.51	34.95	57.74
18:2	12.71	17.43	10.49
18:3	5.71	13.06	1.84
20:1	9.91	8.22	5.22
20:4	3.54	-	-
22:1	6.63	5.63	1.94

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

RATS FED CORN OIL DIET FOR SIX DAYS THEN FASTED OVERNIGHT

FA	Plasma	Heart	Aorta
16:0	18.59	20.19	19.92
18:0	4.24	10.91	4.02
18:1	21.28	25.48	29.77
18:2	42.99	31.87	41.47
20:1	-	-	-
20:4	12.88	11.44	4.80

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

RATS FED CORN OIL DIET FOR SIX DAYS THEN FASTED OVERNIGHT

FA	Liver	Adipose Tissue	Skeletal Muscle
16:0	17.35	16.27	20.69
18:0	2.53	2.05	6.71
18:1	21.25	27.54	30.12
18:2	47.49	53.12	41.49
20:1	-	-	-
20:4	11.36	1.00	0.96

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

RATS FED CORN OIL DIET FOR TWENTY DAYS THEN FASTED OVERNIGHT

FA	Plasma	Heart	Aorta
16:0	19.08	27.93	21.90
16:1	3.18	-	-
18:0	5.28	4.41	6.00
18:1	17.49	30.13	27.47
18:2	40.32	35.79	42.50
20:1	-	-	-
20:4	14.59	1.74	2.13

EFFECT OF DIET ON TISSUE FATTY ACID COMPOSITION (%)

RATS FED CORN OIL DIET FOR TWENTY DAYS THEN FASTED OVERNIGHT

FA	Liver	Adipose Tissue	Skeletal Muscle
16:0	18.38	12.73	20.15
16:1	-	3.77	-
18:0	5.12	1.47	1.30
18:1	15.72	22.29	26.21
18:2	47.95	58.81	52.13
20:1	-	-	-
20:4	12.79	0.89	0.25

Reagents Used for Tissue Triglyceride Estimation.

1. Chloroform:methanol (2:1)
2. Pure solvent upper phase ($CHCl_3$: MethOH: H_2O- 3:48:47)
3. KCl (0.74% in H_2O)
4. Alcoholic KOH (0.4%). 2 grams KOH were dissolved in 95% ethyl alcohol and diluted to 100 ml. Then on the day of use, 10 ml stock were diluted to 50ml with 95% ethanol.
5. Sulfuric acid (0.2N). 3 ml concentrated H_2SO_4 in 500 ml distilled water.
6. Sodium metaperiodate. 53.5 mg in 10 ml distilled water.
7. Sodium bisulfite. 10% w/v.
8. Thiourea. 5%
9. Chromotropic acid 0.2%. (Chromotropic acid is 4,5 - dihydroxy - 2,7 - naphthalene disulfonic acid). Two grams of chromotropic acid (2.24 gm of sodium salt) were dissolved in 200 ml of distilled water stored in a brown bottle. The reagent was stable for about two weeks. Separately 600 ml of concentrated H_2SO_4 were added to 300 ml distilled water. Before assays, diluted H_2SO_4 and aqueous chromotropic acid were mixed together in a ratio of 80:20.
10. Triglyceride standard. The stock standard was prepared

with 0.5 gram tripalmitin in 100 ml chloroform. For working standard, 1 ml stock was diluted to 100 ml with chloroform (5 mg% or 50 µg/ml). For the standard curve, 0.5 ml, 1 ml, 1.5 ml and 2 ml of the working standard were used.

Calculation of triglyceride content in tissue

$$\frac{\text{O.D. saponified unknown - O.D. unsaponified unknown}}{\text{O.D. saponified stendard - O.D. unsaponified standard}} \times \frac{\text{amount of TG in standard x dilution}}{\text{weight of tissue x 1000}} = \text{mg of TG/mg tissue}$$

(Triglyceride = TG)

(Optical density = O.D.)

The standard curve showed a linear relationship between the optical density and the amount of TG in the standard.

Solution used for incubation

In Study II, the following solution was used for incubation:

100 ml Krebs-Ringer solution + 28.84mg C_8 + 2ml 0.1N NaOH

Practical way of preparing 100 ml of Krebs-Ringer solution:

1. 100 ml NaCl 0.90%
2. 4 ml KCl 1.15%
3. 1 ml KH_2PO_4 2.11%
4. 0.5 ml $MgSO_4.7H_2O$ 3.82%
5. 21 ml $NaHCO_3$ 1.30%
6. Aeration
7. 1.5 ml $CaCl_2$ 1.22%
8. Discard 28 ml solution
9. 0.26g glucose

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