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# COMPARATIVE PHYSIOLOGICAL EFFECTS OF RAPESEED AND CANBRA OILS IN THE RAT: INFLUENCE OF THE RATIO OF SATURATED TO MONOUNSATURATED FATTY ACIDS

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## INTRODUCTION

In 1960, ROINE et al. (19) first pointed out heart damages (myocarditis) among rats and pigs fed large amounts of rapeseed oil high in erucic acid. We were able to confirm these results in 1967 and 1968 (16) (17) using lower fat concentrations (15 p.100 by weight) in the diet but in experiments over a longer period of time. Similar results were also obtained elsewhere (23). Moreover, we showed that such lesions were non-reversible and persisted in rats fed either rapeseed oil high in erucic acid or Canbra oil, a new type of rapeseed oil (Brassica Napus) without erucic acid (21). None of these lesions were found in the control group receiving peanut oil.

Cardiac lesions (myocarditis) induced by dietary lipids other than rapeseed oil are scarcely found in the literature (1) (13) (24) (15) (20) (2). Widely differing experimental conditions under which these lesions were observed, and particularly the nature and the level of lipids in the diet make it hard to find a common mechanism of action. However ALLAIN (2), giving rats large amounts of ethyl laurate in a choline-free diet, shows that there is a rapid accumulation of heart lipids, mainly free fatty acids, prior to cardiac lesions. The excess of lipids in the heart is associated with a lower rate of fatty acid oxidation by the myocardium.

Experimental myocarditis induced by rapeseed oil in several animal species has not been thoroughly explained as far as we know. According to our previous results it seems that erucic acid should not be the only responsible factor since identical but probably less frequent and severe lesions also appear among rats fed Canbra oil (zero-erucic acid rapeseed oil).

Other fatty acid characteristics common to both oils should therefore be considered. (*See note further below.) It has previously been shown by several authors (12) (14) (3) that an unbalanced ratio of saturated to monounsaturated fatty acids of dietary lipids as found in rapeseed oil might have an unfavourable effect on growth. Such a fatty acid pattern — unusual in other dietary vegetable oils — also exists in Canbra oil, although it does not seem to have the same effect on growth (9).

Other possible effects — particularly on tissue lipid metabolism — of an unbalanced ratio of saturated to monounsaturated fatty acids in the diet have been investigated in the present paper as one approach to the explanation of the observed pathological effects.

*NOTE: The unsaponifiable fraction of the oils, the complete nature of which is not known at the moment, might have possible physiological effects that could also be studied. However, experimental conditions of the present work hardly support such a hypothesis.

## MATERIALS AND METHODS

### I. ANIMALS AND DIETS

Seven groups of one month old Wistar rats, bred in our own colongy, were fed ad libitum for two months on the same type of semi-liquid diet, except for the nature of the dietary lipids which was changed (Table I). Food intakes were recorded daily and rats weighed twice a week.

TABLE I

COMPOSITION OF THE BASAL DIET(1)

| | p. 100 |
|---|---|
| Fat . . . . . | 15 |
| Casein . . . . . | 18 |
| Wheat Starch . . . . | 37 |
| Sucrose . . . . . | 24 |
| Agar-Agar . . . . . | 2 |
| Salt Mixture (Providing 0.9 p.100 of Calcium) | 4 |

(1) Animals are fed ad libitum the above ingredients mixed with distilled water (2/1 W/W) containing an adequate vitamin mixture.

TABLE II

DIETARY LIPIDS AND THEIR FATTY ACID COMPOSITION

| Dietary Lipids | p.100 by Weight of Lipids in the Diet | Rats/ Group | IDENTIFIED FATTY ACIDS (p.100) | | | | | | | | | | R = Saturated / Monoun-saturated |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | C16:0 | C18:0 | C18:1 | C18:2 | C18:3 | c20:0 | C20:1 | C22:0 | C22:1 | C24:0 | |
| Peanut Oil | 15 | 5 | 10.0 | 4.0 | 58.2 | 20.5 | - | 1.8 | 1.8 | 2.5 | - | 1.6 | R ≃ 1/3 |
| Rapeseed Oil | 15 | 5 | 3.1 | 1.1 | 13.8 | 14.5 | 6.4 | 1.0 | 10.0 | 0.9 | 44.7 | - | R ≃ 1/11 |
| Canbra Oil(1) | 15 | 5 | 3.9 | 2.1 | 60.0 | 19.9 | 9.8 | - | 1.8 | - | 1.9 | - | R ≃ 1/9 |
| Mixture of 1 Tripalmitin + 3 Triolein $P_1O_3$ | 15 | 9 | 22.2 | - | 59.7 | 8.2 | - | - | - | - | - | - | R ≃ 1/3 |
| Mixture of 1 Tripalmitin + 7 Triolein $P_1O_7$ | 15 | 9 | 10.2 | - | 71.6 | 8.3 | - | - | - | - | - | - | R ≃ 1/7 |
| Mixture of 1 Tripalmitin + 2 Triolein + 7 Trierucin $P_1O_2E_7$ | 15 | 9 | 8.3 | - | 15.4 | 7.5 | - | - | - | - | 57.6 | - | R ≃ 1/9 |
| Mixture of 1 Tripalmitin + 3 Trierucin $P_1E_3$ | 15 | 9 | 18.9 | - | - | 7.1 | - | - | - | - | 59.7 | - | R ≃ 1/3 |

(1) Canbra Oil = "Zero Erucic Acid" Rapeseed Oil (Brassica Napus)

P = TRIPALMITIN O = TRIOLEIN E = TRIERUCIN

Seven different dietary lipids were investigated in the present experiment (Table II). Three mixtures of simple synthetic triglycerides ($P_1O_3$, $P_1O_2E_7$, $P_1O_7$) were constituted to match the ratio of saturated to monounsaturated fatty acids of the three dietary oils, and to study their physiological effects on the rat. The fourth mixture ($P_1E_3$) must be regarded as an extra control group as well as $P_1O_3$, except that erucic acid was substituted for oleic acid. Gadoleic acid ($C_{20:1}$) which accounts for 10 p.100 in rapeseed oil was associated here with erucic acid ($C_{22:1}$) in group $P_1O_2E_7$. Linoleic acid was added to the four mixtures. Because of the lack of absolute purity of the fatty acids in the synthetic triglycerides (93 p.100 and up) and of the commercial linoleic acid used in the experiment, it may be observed that the levels of the saturated and monounsaturated fatty acids, and particularly of the linoleic acid in the four diets containing the synthetic triglyceride mixtures do not completely reflect the composition of the dietary oils with respect to these fatty acids (see Table II). This aspect will make it impossible to assess some of the data comparing synthetic triglyceride groups with the dietary oil groups.

## 2. HISTOLOGICAL STUDIES

Animals were sacrificed by decapitation at the end of the two-month experiment. Whole tissues and organs or part of them (fat depots, liver, heart, kidneys, brain, testicles) were immediately removed, weighed and fixed first in Bouin's solution for 48 hours and then in 10 p.100 formalin prior to microscopic studies. Detailed histological techniques were described elsewhere (17).

## 3. BIOCHEMICAL STUDIES

Lipids of tissues and organs were extracted according to FOLCH, et al (10). Purified lipid extracts were weighed and submitted to further analyses such as gas-liquid chromatography of total fatty acids and fractionation into lipid classes by means of column chromatography and thin-layer chromatography (5). These determinations are still underway at the present time. Therefore, only partial results will be presented here.

# RESULTS AND DISCUSSION

## 1. FOOD INTAKE AND GROWTH OF THE RATS

### (a) DIETARY OILS

As observed in our previous experiments, it must be noted that 15 p.100 by weight of rapeseed oil, high in

TABLE III

FOOD INTAKES (g/rat/day)

A. DIETARY OIL GROUPS

| | Peanut Oil | Rapeseed Oil | Canbra Oil |
|---|---|---|---|
| 1st Week | 11.7 | 13.3 | 12.4 |
| 2nd Week | 13.2 | 13.8 | 13.5 |
| 3rd Week | 14.3 | 15.0 | 15.3 |
| 4th Week | 16.9 | 17.4 | 17.8 |
| 5th Week | 17.4 | 17.1 | 17.5 |
| 6th Week | 16.3 | 15.9 | 16.8 |
| 7th Week | 16.8 | 17.0 | 17.1 |
| 8th Week | 17.0 | 16.3 | 16.3 |
| 9th Week | 17.0 | 16.5 | 15.5 |

B. SYNTHETIC TRIGLYCERIDE GROUPS

| | $P_1O_3$ | $P_1O_7$ | $P_1O_2E_7$ | $P_1E_3$ |
|---|---|---|---|---|
| 1st Week | 11.1 | 10.1 | 8.3 | 11.4 |
| 2nd Week | 14.3 | 11.4 | 11.5 | 13.1 |
| 3rd Week | 16.0 | 14.4 | 13.9 | 15.3 |
| 4th Week | 19.3 | 17.8 | 16.7 | 18.7 |
| 5th Week | 20.0 | 18.0 | 17.1 | 19.2 |
| 6th Week | 19.8 | 18.4 | 16.5 | 19.0 |
| 7th Week | 20.0 | 18.3 | 17.8 | 19.2 |
| 8th Week | 17.9 | 17.4 | 15.8 | 18.2 |
| 9th Week | 17.6 | 16.7 | 16.7 | 17.4 |

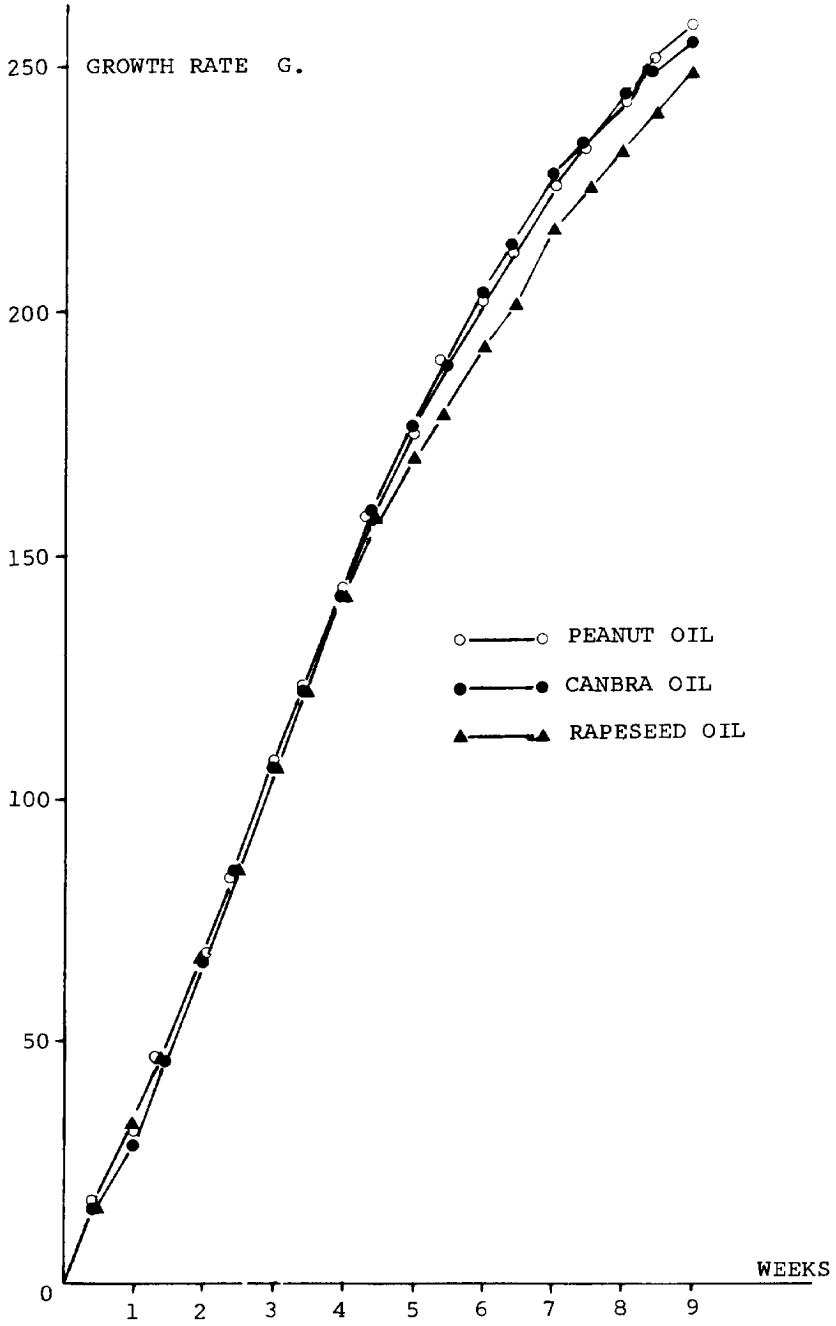



FIGURE IA

GROWTH RATE OF MALE RATS FED 15% BY WEIGHT (30 CAL %) PEANUT, RAPESEED OR CANBRA OIL

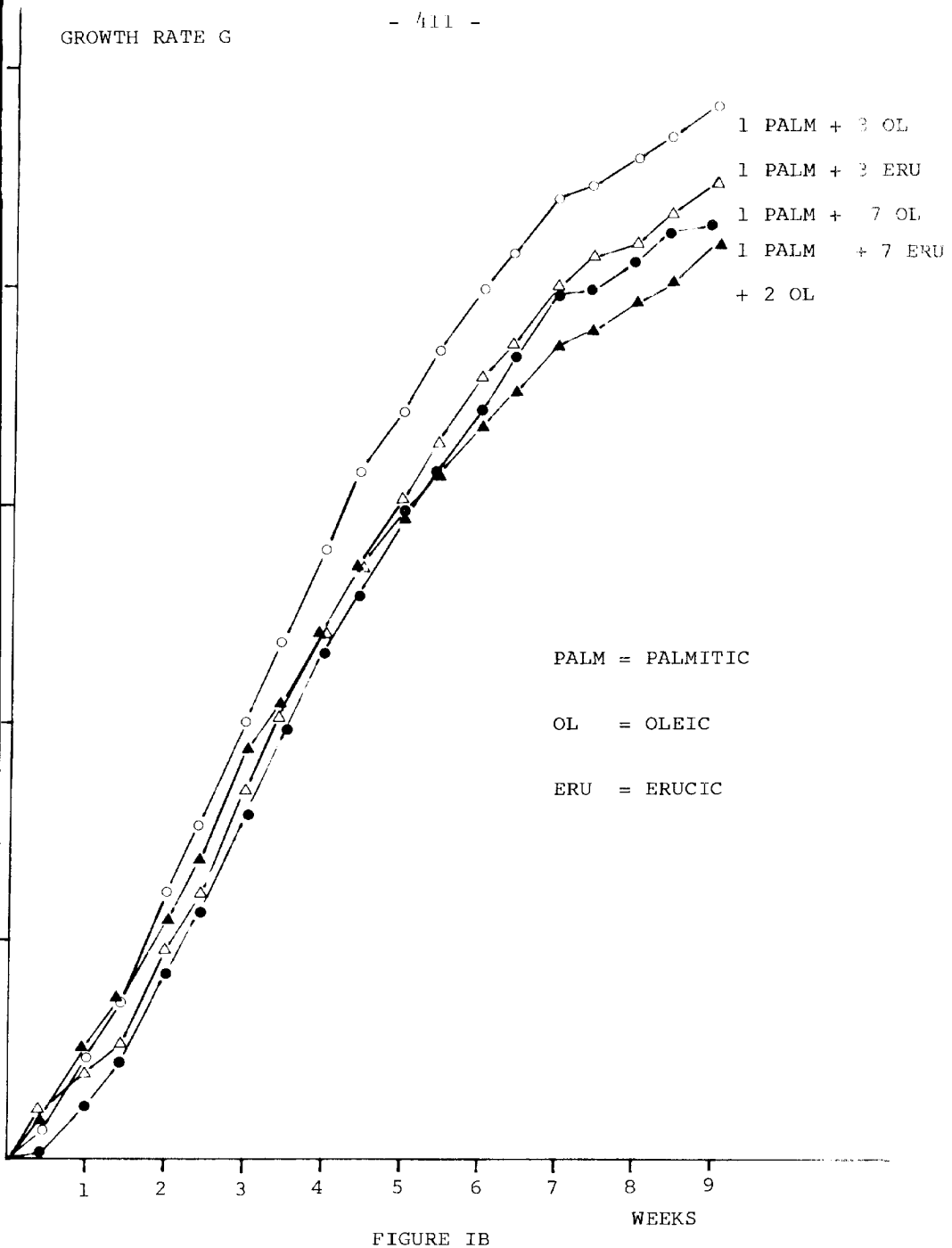



FIGURE IB

GROWTH RATE OF MALE RATS FED 15% BY WEIGHT (30 CAL %) DIFFERENT TRIGLYCERIDE MIXTURES

erucic acid, in the diet does not involve a significant decrease in food consumption as compared with the control group (Table IIIA). Food consumption of rats fed Canbra oil is also similar to those of the 2 other groups. The growth rate of the animals is comparable in all groups, however, there is a slight decrease among rats fed rapeseed oil high in erucic starting in the 4th week of the experiment (Figure IA). This result confirms our previous findings(17) in which we observed a significant decrease in body weight when rats fed rapeseed oil high in erucic acid were getting older.

(b) SYNTHETIC TRIGLYCERIDES

Such diets are well accepted by our animals in all groups even though a short adaptation period is noticeable in groups $P_1O_7$and $P_1O_2E_7$ (Table IIIB). Moreover the rats seem to consume more food at the end of the experiment than rats fed dietary oils. From the growth rates we obtained (Figure IB)we may conclude that the highest weight gains were obtained in group $P_1O_3$

$$\left(R = \frac{\text{Saturated Fatty Acids}}{\text{Monounsaturated Fatty Acids}} = 1/3\right)$$

and the other growth performances may be summed up as follows:

$$P_1O_3 > P_1E_3 > P_1O_2E_7 \simeq P_1O_7$$

$$P_1O_3 > P_1O_7$$

($>$ means significant differences at $P = 0.05$ and $\simeq$ means no significant differences).

On the basis of all the data shown in Figures IA and IB the following statements can be made:

- When the dietary linoleic acid supply is as high as 15 to 20 p.100 of the total fatty acids, as in the 3 dietary oils, there is no growth depressing effect due to an unbalanced ratio of saturated to monounsaturated fatty acids in the diet (Figure IA). We have shown that, for instance, Canbra oil which has the same amounts of saturated and monounsaturated fatty acids as rapeseed oil, ensures as good a gain in weight as peanut oil. Consequently the growth-retarding effect of rapeseed oil seems to be directly related to its high content of erucic acid.

TABLE IV

ORGAN WEIGHTS (g p.100 g OF BODY WEIGHT)

A. DIETARY OIL GROUPS

| Organs | Months on Diet | Rats/ Group | DIETARY LIPIDS | | |
|---|---|---|---|---|---|
| | | | Peanut Oil | Rapeseed Oil | Canbra Oil |
| Liver | 2 | 5 | 3.45 | 3.65 | 3.43 |
| Heart | 2 | 5 | 0.30 | 0.32 | 0.32 |
| Kidney(1) | 2 | 5 | 0.32 | 0.34 | 0.37** |
| Testicle(1) | 2 | 5 | 0.42 | 0.42 | 0.44 |
| Brain | 2 | 5 | 0.55 | 0.56 | 0.56 |
| Epididymal Fat(1) | 2 | 5 | 1.24 | 1.11 | 1.23 |

** - Significant at $P \leq 0.01$ (Peanut oil as reference group)

B. SYNTHETIC TRIGLYCERIDE GROUPS

| Organs | Months on Diet | Rats/ Group | DIETARY LIPIDS | | | |
|---|---|---|---|---|---|---|
| | | | $P_1O_3$ | $P_1E_3$ | $P_1O_7$ | $P_1O_2E_7$ |
| Liver | 2 | 9 | 4.16 | 3.97 | 4.11 | 4.18 |
| Heart | 2 | 9 | 0.31 | 0.35** | 0.32 | 0.36** |
| Kidney(1) | 2 | 9 | 0.47 | 0.40 | 0.42 | 0.43 |
| Brain | 2 | 9 | 0.58 | 0.62 | 0.62 | 0.64** |
| Testicle(1) | 2 | 9 | 0.43 | 0.43 | 0.47 | 0.50 |
| Epididymal Fat(1) | 2 | 9 | 1.10 | 1.10 | 1.06 | 0.97 |

** - Significant at $P \leq 0.01$ ($P_1O_3$ as reference group)

(1) Only one organ or tissue out of 2 was used for weight determination.

- When the dietary linoleic acid level decreases to 10 p.100 or less of the total fatty acids the growth of the animal will be influenced by the erucic acid concentration as well as by the ratio of saturated to monounsaturated fatty acids in the diet. This last aspect is in agreement with previous work(12)(14). A one-third ratio of tripalmitin and triolein (or trierucin) promotes better weight gains than the corresponding unbalanced ratios of tripalmitin and triolein ($R=\frac{1}{7}$) or of tripalmitin and triolein plus trierucin ($R=\frac{1}{3}$). On the other hand, erucic acid does have a growth-retarding effect as compared to oleic acid, even when both acids are given to animals with saturated fatty acids in balanced ratios ($R=\frac{1}{3}$).

  The effect of erucic acid in rapeseed oil on rat growth seems to be predominant.

## 2. ANATOMICAL STUDIES

### (a) ORGAN WEIGHTS

Unlike our previous experiments, it was hardly possible to notice significant differences in the weights of tested organs and tissues. We may merely mention significantly larger hearts in animals fed diets containing Trierucin (Table IVB) which were not found this time with rapeseed oil (Table IVA). Unbalanced ratios of saturated to monounsaturated fatty acids do not seem to affect organ weights dramatically one way or another.

### (b) HISTOLOGICAL STUDIES

Once again cardiac damages (myocarditic lesions) were observed among rats fed rapeseed oil or Canbra oil (Table V). Considering all the data we possess so far on the subject, it seems that lesions are more frequent and severe among animals fed rapeseed oil

TABLE V

HEART DAMAGES DEPENDING ON THE NATURE OF DIETARY LIPIDS

| Dietary Lipids | No. of Rats | Months on Diet | Definite Myocarditis | Not Clearly Identified Myocarditis | Normal Histology |
|---|---|---|---|---|---|
| Peanut Oil | 5 | 2 | 0 | 0 | 5 |
| Rapeseed Oil | 5 | 2 | 4 | 0 | 1 |
| Canbra Oil | 5 | 2 | 2 | 0 | 3 |
| $P_1O_3$ | 9 | 2 | 0 | 1 | 8 |
| $P_1O_7$ | 9 | 2 | 0 | 0 | 9 |
| $P_1O_2E_7$ | 9 | 2 | 0 | 2 | 7 |
| $P_1E_3$ | 9 | 2 | 6 | 0 | 3 |

high in erucic acid than among animals receiving Canbra oil. Sclerosis seems to appear in long-term experiments (rats held on the diet for 12 months). No particular lesions were observed in any other organs studied (kidneys for example). The results we got with diets containing different mixtures of triglycerides do not confirm completely those obtained with dietary oils (Table V). It seems, that on the one hand, even when rats receive 19 p.100 of tripalmitin ($P_1E_3$ group), erucic acid has a major effect on myocarditic lesions since heart damage was found this time only in animals fed trierucin containing diets. On the other hand, lesions do not appear in group $P_1O_7$ which was supposed to have a saturated and monounsaturated fatty acid pattern comparable to Canbra oil.

Slight but possibly significant changes between the fatty acid composition of the triglyceride mixtures and the dietary oils, as mentioned earlier, and probably also an inadequate length of the experiment (the rats were 3 months old when sacrificed) might explain these apparent differences.

## 3. BIOCHEMICAL STUDIES

### (a) TOTAL LIPID CONTENT OF LIVER AND HEART

After 2 months of feeding the experimental diets, no fatty livers or hearts were found in animals fed either rapeseed oil or Canbra oil as compared to rats receiving peanut oil. Lipid content of organs of animals fed different triglyceride mixtures also does not differ from one group to another (Table VI).

TABLE VI

TOTAL LIPID CONTENT OF LIVER AND HEART

(g p.100 g of tissue)

| Groups | Liver | Heart |
|---|---|---|
| Peanut Oil | 6.2 | 3.5 |
| Rapeseed Oil | 5.9 | 4.1 |
| Canbra Oil | 6.4 | 3.5 |
| $P_1O_3$ | 5.7 | 3.8 |
| $P_1E_3$ | 5.5 | 3.8 |
| $P_1O_7$ | 5.4 | 3.8 |
| $P_1O_2E_7$ | 5.3 | 4.1 |

(b) FATTY ACID COMPOSITION OF DIFFERENT TISSUE LIPIDS IN RATS FED DIETARY OILS[x]

Total fatty acids of tissue lipids (heart, liver, muscle, brain, epididymal and abdominal fats) have been qualitatively and quantitatively determined by gas-liquid chromatography and compared with those of the dietary lipids.

- Deposition of characteristic fatty acids of rapeseed oil in several rat tissues.

Erucic acid ($C_{22:1}$), gadoleic acid ($C_{20:1}$) and to some extent linolenic acid ($C_{18:3}$) are the 3 unusual dietary fatty acids found in rapeseed oil. According to previous data (9)(22)(8)(7)(6)(4) less erucic and gadoleic acids are found in tissues of different animal species than in rapeseed oil. However, these two acids are still present in appreciable amounts in depot fats, heart and muscle of 3-month old rats (Table VII). Linolenic acid is also found in depot fats and in lower concentrations in other tissues.

- Total saturated, monounsaturated, linoleic and arachidonic acids in lipid tissues of animals fed dietary oils (Figure II).

x - The results obtained with triglyceride mixtures are not yet all available and will not be given in the present paper.

TABLE VII

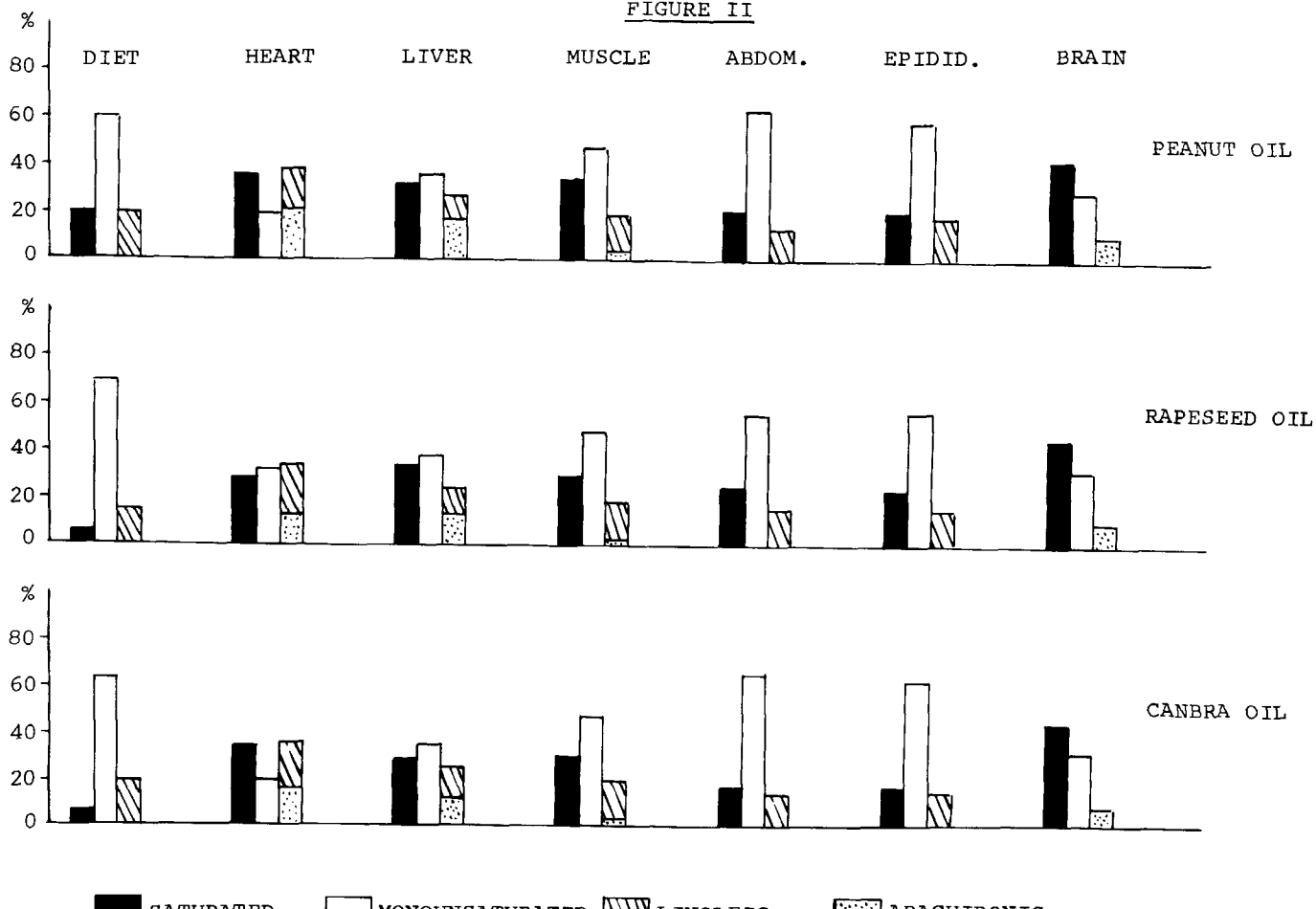
DEPOSITION OF ERUCIC ($C_{22:1}$) GADOLEIC ($C_{20:1}$) AND LINOLENIC ($C_{18:3}$) ACIDS IN VARIOUS TISSUE LIPIDS OF 3-MONTH OLD MALE RATS FED 30 CAL.p.100 RAPESEED OIL IN THE DIET

(IN p.100 OF METHYL ESTERS)

| Tissue | $C_{20:1}$ | $C_{22:1}$ | $C_{18:3}$ |
|---|---|---|---|
| Epididymal Fat | 6.6 | 6.9 | 5.4 |
| Abdominal Fat | 7.2 | 7.4 | 5.2 |
| Liver | 1.7 | 1.4 | 1.1 |
| Heart | 3.1 | 7.6 | 1.8 |
| Brain | 3.5 | 1.1 | 1.0 |
| Muscle (Gastrocnemius) | 5.6 | 5.7 | 2.3 |

Although dietary oils have different fatty acid composition, they hardly change the level of total saturated and monounsaturated fatty acids of depot fats (mainly composed of triglycerides) from one group to another. Similar observations were made with more complex tissue lipids, except for cardiac fatty acids of rats fed rapeseed oil, which definitely differed from those of animals receiving peanut oil or Canbra oil in their diets. In this particular tissue of animals fed rapeseed oil less of the saturated and more of the unsaturated acids were found. Slight differences in the polyunsaturated fatty acids ($C_{18:2}$ and $C_{20:4}$) of some tissues (liver and heart) seem also to exist between the control group and rats fed either rapeseed oil or Canbra oil (Table VIII). BEARE et al(3) previously mentioned that similar results were obtained only with rapeseed oil high in erucic acid and assumed that in the presence of this acid conversion of linoleic to arachidonic acid might decrease in liver tissue. Since the level of arachidonic acid in liver and heart seems also to be lowered when Canbra oil is present in the diet, other fatty acids common to both, rapeseed and Canbra oils might interfere with this conversion(11). An evaluation of the complete fatty acid profile of a tissue obviously requires quantitative determination of the different tissue lipid classes and their fatty acid composition. Such investigation, particularly of liver and cardiac lipids is still under study in our laboratory and will be published later.

FIGURE II




TOTAL SATURATED, MONOUNSATURATED AND POLYUNSATURATED FATTY ACID CONTENT(%) OF DIETARY OILS AND VARIOUS RAT FISSUES

TABLE VIII

LINOLEIC AND ARACHIDONIC ACID CONTENTS
(p.100 OF TOTAL FATTY ACIDS) IN LIVER AND HEART

| | LINOLEIC ACID ($C_{18:2}$) | | ARACHIDONIC ACID ($C_{20:4}$) | |
|---|---|---|---|---|
| Dietary Fat | Liver(1) | Heart(2) | Liver(1) | Heart(2) |
| Peanut Oil | 9.8 | 16.6 | 17.8 | 21.8 |
| Canbra Oil | 11.9 | 18.3 | 13.0 | 16.9 |
| Rapeseed Oil | 10.2 | 19.6 | 12.5 | 12.9 |

(1) Mean values from 2 pools of respectively 2 and 3 rats.
(2) Values from 1 pool of 5 rats.

All specific effects exerted on an organism by the nature and level of the fatty acids of rapeseed oil and Canbra oil are not yet known. Although erucic acid from rapeseed oil seems to have a definite effect on food efficiency and digestibility(18) other fatty acids which are commonly present in rapeseed and Canbra oils could have other specific effects on the fatty acid pattern of tissue lipids and, therefore, on their metabolic functions. Present knowledge of this aspect is not adequate to make definite statements.

The unbalanced ratio of saturated to monounsaturated fatty acids which characterizes both oils does not decrease growth rate of animals fed 15 p.100 by weight of Canbra oil. How it affects cardiac metabolism and also is responsible for myocarditic lesions, is not apparent from our present investigation. Still, cardiac lesions do appear when Canbra oil is given to rats under our experimental conditions and this requires further investigation in the months ahead.

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