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# PHYSIOPATHOLOGICAL EFFECTS OF RAPESEED OIL AND CANBRA OIL IN RATS

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## INTRODUCT ION

In our laboratory, the biological effects of rapeseed oil (RSO) and Canbra oil in a variety of animals are being investigated. The investigations were initiated by the demonstration of Roine et al.(1)that RSO produces interstitial myocarditis in rats and probably also in pigs, and were stimulated by the increasing importance of RSO as an edible oil. This paper deals with the pathological effects of RSO and Canbra oil in rats.

## ANIMALS AND DIETS

Unless stated otherwise 3-week old male Wistar rats were used in the experiments. The rats were fed ad lib. a semisynthetic diet consisting of 25 cal% casein, 77 cal% fat and corn starch and additional vitamins and minerals.

The RSO used was the European RSO; it contained 45-50% erucic acid. The Canbra oil used contained 2% erucic acid.

## RESULTS OF RAPESEED OIL

## FEEDING FOR ONE WEEK

Seven groups of rats were given 50 cal% RSO; daily one group was killed. A group given 50 cal% sunflowerseed oil and killed after 3 days served as control.

On autopsy, paleness of the heart represented the most striking macroscopical change observed in the RSO fed rats. This deviation from the normal colour was already observed after one day and increased in severity after 3 to 6 days. During this period, the heart assumed a creamy yellow colour as shown in Fig. I.





FIGURE I

MACROSCOPY OF THE HEART IN A CONTROL RAT (LEFT) AND A RAT FED 50 CAL% RSO FOR 3 DAYS (RIGHT)



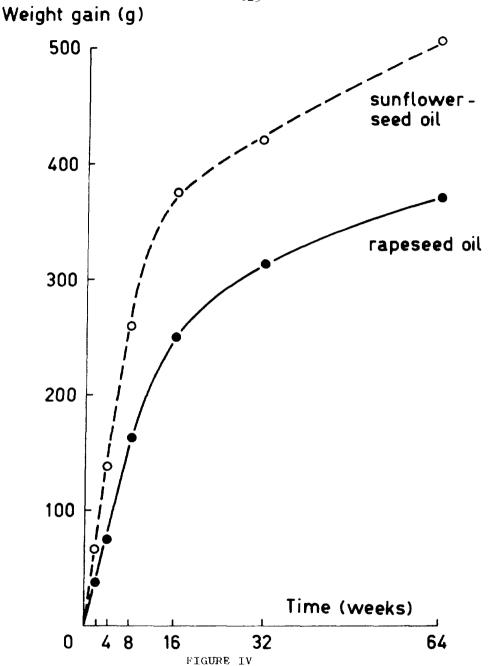
FIGURE II

FATTY ACCUMULATION IN THE MYOCARDIUM OF A RAT FED 50 CAL% RSO FOR 3 DAYS



FIGURE III

FATTY DEPOSITION (VACUOLATION) IN THE SKELETAL MUSCLES OF A RAT FED 50 CAL% RSO FOR 6 DAYS



AVERAGE GAIN IN WEIGHT OF RATS FED 60 CAL% RSO OR SUNFLOWERSEED OIL FOR 64 WEEKS

Microscopically massive fatty deposition in the heart (Fig. II) and the skeletal muscle fibres (Fig. III) was demonstrated in all RSO-fed rats.

In accordance with the autopsy observation the fatty deposition in the heart became most severe after 3 to 6 days. In the skeletal muscles the fatty deposition also increased in severity, a maximum occurring after 6 days. In contrast to the RSO-fed rats, none of the control rats revealed any macroscopical or microscopical changes.

## FEEDING FOR PERIODS UP TO 64 WEEKS

Groups of rats were fed 60 cal% RSO or sunflowerseed oil for 2, 4, 8, 16, 32 or 64 weeks. On all occasions, the growth rate of the RSO-fed rats was lower than that of the sunflowerseed oil-fed rats. (Fig. IV).

On autopsy, heart and adrenals of RSO-fed rats were paler than those of the control rats. However, the colour deviation of the heart was not as pronounced as that observed after 3 days of RSO-feeding. The skeletal muscles of RSO-fed rats were pale after 2 and 4 weeks, but not after 8 or more weeks.

Microscopically, coarse and fine droplet fatty changes of the heart and skeletal muscles, were demonstrated in the animals fed RSO for 2 weeks. The adrenal cortex cells showed fine droplet fatty infiltration and were enlarged. In the myocardium, edema, and sometimes also myolysis and foci of necrosis were observed.

The adrenal changes persisted as such with the course of time but in the heart and skeletal muscles, a decrease in the severity of the fatty accumulation occurred. This decrease was complete in the case of skeletal muscles and after 16 weeks the skeletal muscles regained their normal morphology. In the heart, however, the fatty accumulation never disappeared completely. In addition, focal or diffuse infiltrations of mononuclear cells, histiocytes and proliferation of fibroblasts appeared in the myocardium after 4 and 8 weeks. These changes, increasing in severity and becoming less cellular and more fibrotic in the course of time, were the main pathological lesions observed after 16, 32 and 64 weeks (Fig. V).

The kidneys of the RSO-fed animals showed one or all of the following anomalies: tubular dilatation, casts and foci of scar

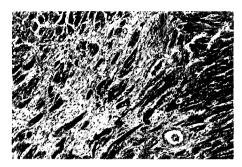
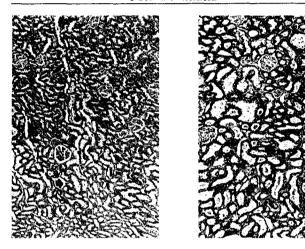


FIGURE V

FIBROTIC CHANGES IN THE HEARTS OF RATS FED RSO FOR 64 WEEKS



F1GURE VI

KIDNEY OF A CONTROL RAT (LEFT) AND OF A RAT FED 60 CAL% RSO FOR 64 WEEKS, SHOWING NEPHROTIC CHANGES (RIGHT)

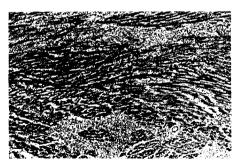


FIGURE VII

EXTENSIVE MONONUCLEAR CELL INFILTRATION IN THE MYOCARDIUM OF A MATURE RAT FED 50 CAL% RSO FOR 6 DAYS

formation. These changes first appeared after 16 weeks and then increased in severity (Fig. VI). The lesions in the heart and kidneys were associated with a significant increase in their weights in comparison with the control group (Table I).

## TABLE I

AVERAGE WEIGHTS (g) OF HEART AND KIDNEYS IN 12 RATS FED 60 CAL% RSO OR SUNFLOWERSEED OIL FOR 64 WEEKS

Dietary Fat	Heart	Kidney
Rapeseed Oil	1.60	3.33
Sunflowerseed Oil	1.41	2.67

The spleen, testes and epididymides of the RSO-fed rats showed no abnormalities but in the liver a slight degree of fatty infiltration was observed after 64 weeks. In the control groups no pathological abnormalities were observed.

## DOSE-RESPONSE STUDY

Three weeks old male Sprague Dawley rats were given for 3 and 6 days 40 cal% diet containing 0, 5, 10, 15, 20, 25, or 30 cal% RSO; the difference was made up by sunflowerseed qil.

Myocardial fatty accumulation was observed in both occasions in all rats fed RSO. The severity of the fatty accumulation increased with the RSO level in the diet. The minimum level of RSO producing fibrosis is being investigated.

## THE EFFECT OF DISCONTINUED RSO FEEDING

In an experiment in which 50 cal% RSO was given for 3 days and then discontinued for 3 more days (a fat-free diet or sunflowerseed oil given instead), it was found that the myocardial fatty accumulation decreased markedly compared with rats fed 50 cal% RSO for 6 days.

## THE EFFECT OF AGE

Male mature rats (11 weeks old) of the Sprague Dawley strain were fed 50 cal% RSO for 6 days. The rats showed a less severe myocardial fatty accumulation than that previously found in 3-week old Wistar rats. However, an extensive mononuclear cell infiltration was observed already after this short period (Fig. VII).

## THE SIMILARITY OF THE LESIONS PRODUCED BY RSO AND GLYCERYL TRIERUCATE

Groups were fed diets containing RSO or glyceryl trierucate (GTE) on isocaloric erucic acid basis for 21 days. Total dietary fat was made isocaloric by adding soybean oil.

Compared with a control group fed a mixture of soybean oil and hardened palm oil, the RSO group and GTE group showed similar degrees of growth retardation (Table II).

## TABLE II

AVERAGE GROWTH OF 12 RATS FED CONTROL DIETS OR DIETS CONTAINING ISOCALORIC AMOUNTS OF ERUCIC ACID AS RSO OR GTE

Cal% Erucic Acid	Group	Growth (g)
17.6 17.6	Rapeseed Oil Trierucate	59.6 56.6
0	Control	113.6

Comparable degrees of the pathological changes previously described were found in the RSO group and the GTE group (Table III).

TABLE III

THE SIMILARITY OF THE LESIONS PRODUCED BY RSO AND GTE

	Heart		Skeletal Muscle	
Group	Frequency	Severity (0-4)	Frequency	Severity (0-4)
Rapeseed Oil Trierucate	11 12	1.6 1.5	12 12	2.6 2.0
Control	0	0	1	0.1

## RESULTS OF CANBRA OIL

Cambra oil is an oil containing little or no erucic acid. Three experiments were done with a Cambra oil containing 2% erucic acid to investigate its nutritional and pathological properties.

In the first experiment groups were fed 60 cal% RSO, sunflowerseed oil or Canbra oil for 2 weeks. No differences were found in food intake and growth between the groups fed the sunflowerseed oil and the Canbra oil (Table IV).

TABLE IV

AVERAGE GROWTH AND FOOD INTAKE OF 12 RATS FED 60 CAL% SUNFLOWERSEED OIL, RSO OR CANBRA OIL FOR 2 WEEKS

Dietary Fat	% Erucic	Food Consumption (g)		Growth (g)	
(60 ca1%)	Acid in Oil	lw	2w	lw	2w
Sunflower- seed Oil Canbra Oil	0 2	6.2	9.8 9.9	33.8	70.8 67.7
Rapeseed Oil	49	5.4	5•5	17.6	25.6

Neither did these groups show pathological changes contrary to the group fed the RSO which showed the characteristic lesions.

In the second experiment, the rats were fed 50 cal% Canbra oil for 3 days. No pathological changes were observed.

Finally, Rocquelin's experiment  $^2$  which showed that Canbra oil caused myocardial lesions, was repeated. Groups were fed 30 cal% sunflowerseed oil, Canbra oil or RSO for  $2^{l_1}$  weeks.

The results shown in Tables V and VI indicate that Canbra oil does not retard the growth; neither does it possess pathogenic properties as RSO does.

TABLE V

GROWTH OF 12 RATS FED 30 CAL% SUNFLOWERSEED OIL,
RSO OR CANBRA OIL FOR 24 WEEKS

Dietary Fat (30 cal%)	% Erucic Acid in Oil	Growth (g)
Sunflower- seed Oil Canbra Oil	0 2	375 366
Rapeseed 0il	46	348 <sup>x</sup>

x Significantly lower (P ≤ 0.10)

TABLE VI

PATHOLOGICAL CHANGES IN 12 RATS FED 30 CAL%
SUNFLOWERSEED OIL, RSO OR CANBRA OIL FOR 24 WEEKS

Dietary Fat (30 cal%)	Mild Myocardial Fibrosis or Edema	Mild Nephrosis
Sunflower- seed Oil Canbra Oil	1	3 4
Rapeseed 0il	9	9

The mild pathological changes observed in the Canbra oil group are of the same nature and severity as those in the sunflowerseed oil group. They can rightly be designated as spontaneous. These changes showed a higher incidence in the RSO group.

The sunflowerseed oil and the Canbra oil groups did not differ in organ weight either. Only the RSO group showed significant differences (Table VII).

TABLE VII

AVERAGE WEIGHT OF HEART AND KIDNEY IN 12 RATS FED 30 CAL%
SUNFLOWERSEED OIL, CANBRA OIL OR RSO FOR 24 WEEKS

Dietary Fat (30 cal%)	Heart (g)	Kidney (g)
Sunflower- seed Oil Canbra Oil	1.24 1.21	2.50 2.48
Rapeseed 0il	1.31 <sup>x</sup>	2.79 <b>*</b>

★ - Significantly higher (P<0.05)</pre>

It is concluded that Canbra oil produces no pathological abnormalities in rats, and that the pathological effects of conventional RSO are caused by its erucic acid content.

## ACKNOWLEDGEMENT

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## REFERENCES

- Roine, P., Uksilla, E., Tier, H. and Rapola, J.: Histopathological changes in rats and pigs fed rapeseed oil. Z. Ernährungswiss. 1: 118-124 (1960).
- Rocquelin, G. et Cluzan, R.: L'huile de Colza riche en acide érucique et huile de Colza sans acide érucique: Valeur nutritionelle et effects physiologiques chez le rat. l - Effects sur la croissance, l'efficacité alimentaire et l'état de différents organes. Ann. Biol. Anim. Biochim. Biophys. 8: 395-406 (1967).

#### SUMMARY

The pathogenicity of rapeseed oil (RSO) and Canbra oil in weaned rats was investigated in a series of short and long-term experiments using sunflowerseed oil as the control fat. RSO produced fatty deposition in the heart and skeletal muscle fibres already after one day of feeding which became massive after 3-6 days when the heart showed a creamy yellow color. After that the fatty changes regressed and the skeletal muscles regained normal histology after 16 weeks. However, in the heart the decrease of fatty accumulation was associated with multifocal mononuclear cell infiltration and interstitial connective tissue formation. Nephrotic changes were also observed in the kidney after 16 weeks. The heart and kidneys were heavier in rats fed RSO for 64 weeks than in control rats.

The fatty accumulation of the heart produced by feeding RSO for 3 days regressed rapidly and markedly when feeding of the oil was either discontinued or the RSO replaced by sunflowerseed oil.

In mature rats, the feeding of RSO produced an extensive mononuclear cell infiltration in the myocardium after 6 days.

Contrary to RSO, Canbra oil did not produce growth retardation when it was fed for 2 weeks. Neither did it produce any pathological changes in the heart or skeletal muscles after 3, or 14 days or 24 weeks feeding.