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Human Nutrition

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Nutritional evaluation of hydrogenated fats

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In continuation of previous work, oils hardened in different ways were investigated by means of a biological method analogous to that applied to the evaluation of the safety of food additives and defined as a 'sub-acute oral toxicity study' (Thomasson & Gottenbos, 1957; Gottenbos & Thomasson, 1965; de Jongh & Beerthuis, 1965).

Experimental details

Groups of twenty-four male and twenty-four female rats consumed during 12 weeks adequate diets in which 54% of the calories came from experimental fat. Moreover, 6% of the calories derived from sunflower-seed oil in order to cover the requirement for essential fatty acids.

The fatty acid compositions of the dietary fats including the sunflower-seed oil are shown in Table 1.

As control, unhardened soya-bean oil was used, the effect of which was compared with that of five industrially hardened fats and with that of one naturally hardened fat, butterfat. Soya-bean oil is rich in linoleic acid (55%). The three hardened

Table 1. *Percentage fatty acid composition of dietary fats*

Type of experimental fat	Fatty acids				
	Saturated	Monoene		Diene	
		<i>cis</i>	<i>trans</i>	<i>cis-cis</i>	<i>trans</i>
Soya-bean oil (control)	15	24	0	55	0
Hydrogenated soya-bean oil (L5)	21	12	54	6	4
Hydrogenated soya-bean oil (L15)	16	47	1	16	19
Hydrogenated soya-bean oil (L40)	15	22	14	41	7
Hydrogenated linseed oil	16	20	40	6	16
Hydrogenated olive oil	24	39	29	6	1
Butterfat	53	24	8	7	3

soya-bean oils were prepared by hydrogenation in different ways, which is reflected in the differences in their fatty acid compositions. Hardened linseed oil and hardened olive oil were used as representatives of oils originally rich in linolenic acid and oleic acid respectively.

Food intake

The mean food intake per animal during the 12 weeks was 910 g for the males and 745 g for the females. This represented 305 and 245 g of dietary fat, respectively. There appeared to be hardly any differences between the experimental groups.

The *trans*-acid intake, on the other hand, varied considerably for the different groups. Apart from the group given unhardened soya-bean oil containing no *trans*-acids, it was lowest in the groups that received butterfat, hardened soya-bean oil L15 or L40 (25–60 g/12 weeks), moderate in the group given hardened olive oil (70–95 g) and highest in the group that received hardened linseed oil and hardened soya-bean oil L5 (130–175 g/12 weeks).

Body-weight

The mean gain in weight in 12 weeks was 258 g for the males and 137 g for the females; no significant differences were found between the experimental groups.

Faecal fat

In all groups the amount of fat excreted in the faeces was less than 5% of that eaten, being within the limits of normal excretion. Some differences seemed to exist between the groups, and there was a positive correlation between the consumption of *trans*-acids and the percentage of fat excreted in the faeces.

Water consumption

The mean water consumption (mg/day per cm² body surface) of males and females was 6.6 and 5.8 mg respectively. No significant differences between the groups appeared to exist, except that the females given hardened linseed oil drank less water. However, it is doubtful whether this finding could be reproduced.

Rectal temperature

The means were 38.8° for the males and 39.0° for the females. There were no significant differences between the experimental groups.

Kidney function

18 h concentration test. The specific gravity of the urine after the animals had been deprived of water for 18 h was for males and females 1.067 and 1.068 respectively. No disturbances of the concentration capacity of the kidneys occurred in any of the experimental groups.

Urine aspartate transaminase. The values varied between 11 and 23 Wróblewski units/ml (La Due, Wróblewski & Karmen, 1954), being higher for males than for females. Under pathological conditions the level is increased. However, the values found for the groups given hardened oils were generally lower than those of the controls.

Liver function

Serum alanine transaminase. The serums appeared to contain an average of 20 Wróblewski units/ml (Wróblewski & La Due, 1956) and there were no indications that one of the groups differed from the other.

Alkaline phosphatase. The values were between 2 and 3.5 m-moles/l. and no differences occurred between the experimental groups. Males showed higher levels than females.

Blood

Haemoglobin. The mean content was about 16 mg/100 ml. There were no differences between the groups.

Haematocrit. The values were constant with means per group of 50 or 51%.

Non-electrolyte haemolysis. The time in which 75% haemolysis occurred in a thiourea medium varied between 30 and 40 sec. The values for males were higher than those for females. Although no significant differences between the groups were found, the values for the groups given industrially hardened fats were in general somewhat lower than those for the controls. The biological significance of this observation may become clear in future research.

White blood cells. Neither total nor differential counts indicated pathological changes, except that values for the total white cell counts for the group given hardened soya-bean oil L40 and for the butterfat group were slightly higher than for the controls. The number of white cells was distinctly higher in males than in females. The percentage of lymphocytes was high and between narrow limits, 89–93%.

Coagulation. This was determined by means of a thrombelastograph and exclusively in males. The *r* values (time after which the coagulation begins) seemed to be longer in animals fed on hardened oils than in controls, although the differences were not significant.

Platelet adhesiveness. The method of Hellem, Ödegaard & Skålhegg (1963) (without addition of ADP) was used. Some indication was found that the platelets of the females fed on hardened soya-bean oils were more adhesive than those of the controls. However, the number of animals tested was limited (three to four per group) and the difference was not significant and not found in the males.

Post-mortem examination

Autopsies were carried out on all rats. The weights of heart, liver, kidneys, adrenals, spleens and testes were determined. Moreover, a histological investigation was carried out. None of these studies provided evidence of any unfavourable effect of the fats used.

The composition of body fat at the end of the experiment was also determined. Highly significant correlations appeared to exist between the saturated fatty acids, monoenoic, polyenoic acids and *trans*-acids, respectively, of the dietary fats and the body fats.

Summary

On applying several biological criteria, including food intake, body-weight, fat content of faeces, water consumption, rectal temperature, kidney function

(concentration test, aspartate transaminase content), liver function (alanine transaminase content, alkaline phosphatase content), blood (Hb, haematocrit, haemolysis, white blood cells, coagulation, platelet adhesiveness), post-mortem examinations and body fat composition, no indications were obtained that a 12-week feeding period with high amounts of hydrogenated fats of extremely divergent compositions induces abnormal changes in rats.

REFERENCES

- de Jongh, H. & Beerthuis, R. K. (1965). *Bibliotheca Nutritio Dieta*, Fasc. no. 7, p. 137.
 Gottenbos, J. J. & Thomasson, H. J. (1965). *Bibliotheca Nutritio Dieta*, Fasc. no. 7, p. 110.
 Hellem, A. J., Ødegaard, A. E. & Skålhegg, B. A. (1963). *Thromb. Diath. haemorrh.* **10**, 61.
 La Due, J. S., Wróblewski, F. & Karmen, A. (1954). *Science, N.Y.*, **120**, 497.
 Thomasson, H. J. & Gottenbos, J. J. (1957). *Verh. K. vlaam. Acad. Geneesk. Belg.* **19**, 369.
 Wróblewski, F. & La Due, J. S. (1956). *Proc. Soc. exp. Biol. Med.* **91**, 569.

Heat-induced changes during processing and use of edible fats

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Many recorded studies have combined to confirm that oxidized, heated fats, when given to test animals, produce toxic signs ranging from growth retardation to death. There is general agreement that the toxic components are concentrated in the fraction of the fat which does not form a urea adduct, but there remains considerable uncertainty of the chemical nature of the toxic substances. That this should be so is in no way surprising, since the chemical processes involved are extraordinarily complex, and the problems of separation and isolation of the various chemical entities almost unsurmountable.

These chemical processes may be generally described as cyclization and polymerization, and the ease with which they proceed increases with the degree of unsaturation of the fat. Fats which are commonly used for edible purposes do not readily undergo these reactions; and a criticism which may be made of a number of recorded nutritional and toxicological studies is that to provide sufficient material for feeding experiments oils have been treated under drastic conditions far removed from those encountered in normal use, or that more highly unsaturated oils such as linseed and tung oil, not commonly regarded as edible oils, have been used.

Our interest lay in the extent to which the reactions of cyclization and polymerization occurred during the refining of glyceride oils for edible purposes. Subsequently our investigations were extended to oils used in deep frying, since this operation is probably the most drastic treatment to which an edible oil is subjected. These latter studies are in their early stages, and represent no more than a preliminary survey to provide the basis for a systematic investigation of such factors as the influence of frying conditions on fat deterioration, and the extent to which changes in the bulk fat are reflected in the fat content of fried foods.

Our work began with cottonseed oil (CSO), chosen because it combines a high degree of unsaturation with good oxidative stability, and for this reason is used in