# THE EFFECT OF DIETARY FATS AND SYNTHETIC GLYCERIDES ON THE PRODUCTION OF ATHEROSCLEROSIS AND THROMBOSIS IN THE RAT

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THROMBOSIS and atherosclerosis can be produced independently in the rat by feeding a diet containing 2 different fats (Gresham and Howard, 1960). Thus rats fed with butter cholesterol, cholic acid and thiouracil produce thrombosis. If butter is replaced by arachis oil, no thrombosis is seen but lesions are produced, closely resembling those of human atherosclerosis. Butter differs from arachis oil in having a high content of saturated fatty acids but a low content of linoleic acid. The present work is concerned with effects of feeding, natural and synthetic fats with widely different fatty acid compositions.

## MATERIALS AND METHODS

*Source of fats.*—Butter and beef fat were the normal retailed products. Hydrogenated arachis oil (m.p. 34°) was kindly supplied by the British Oil and Cake Mills Ltd., Erith, Kent. Arachis oil was obtained from British Houses, Poole, Dorset; maize oil from the Clyde Oil Extraction Co., Glasgow.

*Synthetic glycerides.*—Glyceryl mono-oleate distearate was prepared according to the method of Wheeler, Riemenschneider and Smith (1940). Oleic acid (33 g., British Drug Houses), stearic acid (666 g., British Drug Houses), glycerol (102 g.) and *p*-toluene sulphonic acid (10 g.) were heated under oxygen free nitrogen at 120–130° for 5 hr. The mixture on cooling was added to 3 l. petroleum ether (b.p. 40–60°) and washed with 70 per cent ethanol containing 4 g. sodium hydroxide, followed by 4 further washes with 70 per cent ethanol only. After washing 3 times with water, the petroleum ether layer was dried over sodium sulphate and the solvent removed at 100° under nitrogen. Final traces were removed by vacuum pump. This method gives a mixture of symmetrical and unsymmetrical triglycerides and no attempt was made to purify the product further.

The other glycerides were prepared in a similar manner using the following quantities: glyceryl dioleate mono stearate:—oleic acid (666 g.) stearic acid (333 g.); glyceryl trioleate:—1 kg. oleic acid; glyceryl trilinoleate:—1 kg. linoleic acid.

Linoleic acid and methyl linoleate were prepared by standard methods (Biochemical Preparations, 1955).

*Animals and experimental design.*—The animals and basic diet used (Table I) were similar to those described previously (Gresham and Howard, 1960). Groups of 5–10 rats were fed the basic diet containing different fats as described below. Table II shows the different fats used together with their fatty acid composition (Groups 1–5). Three groups of rats were fed mixtures, *viz.* Group 6: butter and arachis oil mixed (1 : 1); Group 7: butter containing 8 per cent (W/W) methyl linoleate (thus producing a total linoleic acid content approximating that of arachis oil); Group 8 was fed arachis oil for 8 weeks and then changed to butter. Synthetic fats given were glyceryl trilinoleate (Group 9), glyceryl mono-oleate distearate (Group 10), glyceryl dioleate mono stearate (Group 11), and glyceryl trioleate (Group 12).

*Pathological examination.*—Animals were allowed to continued on the diets until death occurred. Necropsy and treatment of tissues was as described previously (Gresham and Howard, 1960).

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TABLE I.—*Basic Diet*

| Active ingredients* | g. |
|---|---|
| Fat | 400 |
| Cholesterol | 50 |
| Cholic acid | 20 |
| Thiouracil | 3 |

* Other ingredients were : sucrose 169 g., casein 200 g., choline chloride 10 g., salts 40 g., cellophane flaked film 100 g., magnesium oxide 5 g., inositol 2 g., thiamine hydrochloride 16 mg., pyridoxine hydrochloride 16 mg., folic acid 10 mg., calcium pantothenate 40 mg., biotin 0·6 mg., $B_{12}$ 0·05 mg., nicotinamide 0·2 g. ; a weekly supplement to each rat comprising 1000 i.u. vitamin A and 40 i.u. vitamin D supplied as one drop halibut liver oil, 2 mg. α-tocopheryl acetate and 0·05 mg. 2-methyl-1 : 4 naphthoquinone.

TABLE II.—*Different Fats Used and their Composition* (per cent mole)*

| Group | Fat | Fatty acids | | |
|---|---|---|---|---|
| | | Saturated | Oleic acid | Linoleic acid |
| 1 | Butter | 65 | 30 | 5 |
| 2 | Beef fat | 55 | 43 | 2 |
| 3 | Hydrogenated arachis oil | 25 | 73 | 2 |
| 4 | Arachis oil | 20 | 55 | 25 |
| 5 | Maize oil | 15 | 25 | 60 |

* Hilditch (1956)

## RESULTS

### *Different fats*

Results are given in Table III.

Animals given beef fat and hydrogenated arachis oil died first after 4–8 weeks. Those given butter, arachis and maize oil lived from 10–14 weeks. Butter, beef fat and hydrogenated arachis oil produced thrombosis of the cardiac chambers, aorta and coronary arteries, myocardial infarction (Fig. 1) and large clusters of lipid filled macrophages at the apex of the heart, on the endocardial surface of the mitral valve and on the aortic intima. These 3 fats have a somewhat similar composition consisting chiefly of glycerides containing saturated fatty acids linked to oleic acid.

TABLE III.—*Distribution of Lesions in Rats given Different Fats*

| Group | Fat in diet | No. of animals | Range of survival time (weeks) | Atherosclerosis | Lipid-filled Macrophages | Thrombosis | Myocardial infarction |
|---|---|---|---|---|---|---|---|
| 1 | Butter | 25 | 11–17 | 0 | 18 | 13 | 6 |
| 2 | Beef fat | 7 | 4–8 | 0 | 6 | 2 | 1 |
| 3 | Hydrogenated arachis oil | 6 | 4–10 | 0 | 2 | 3 | 1 |
| 4 | Arachis oil | 8 | 10–18 | 7 | 1 | 0 | 0 |
| 5 | Maize oil | 7 | 11–22 | 3 | 1 | 2 | 0 |

Those fed arachis oil showed no thrombosis or infarction and few lipid filled macrophages. In contrast atherosclerotic plaques were produced in the proximal aorta (Fig. 2*a*) and coronary arteries. These plaques consisted chiefly of fibrous

material and contained lipid at the base. Plaque formation appears to be associated with fats containing a high proportion of linoleic acid since rats given hydrogenated arachis oil (containing a negligible amount of linoleic acid) did not show atherosclerosis. Butter and beef fat also have low linoleic acid contents.

Likewise, maize oil (60 per cent linoleic acid) produced atherosclerotic plaques but these contained much more lipid than with arachis oil (Fig. 2*b*). An unexpected result was the production of intra cardiac thrombosis and thrombi related to the aortic atherosclerotic plaques. Animals given arachis oil did not develop thrombosis.

*Mixture of fats*

The results are shown in Table IV.

Butter and arachis oil mixed in equal amounts produced the sum of lesions given by each independently: atherosclerosis in addition to thrombosis and infarction. As was expected a similar result was seen when rats were fed arachis oil for 8 weeks and then changed to butter. Addition of methyl linoleate to butter, produced atherosclerosis in addition to thrombosis and infarction which occurred with butter alone. This result would support the view that linoleic acid is essential for the production of atherosclerotic plaques.

TABLE IV.—*Distribution of Lesions in Rats given Mixtures of Fats*

| Group | Mixture of fats | No. of animals | Range of survival time (weeks) | Athero-sclerosis | Lipid-filled macro-phages | Throm-bosis | Myo-cardial infarction |
|---|---|---|---|---|---|---|---|
| 6 | Butter and arachis oil (1 : 1) | 7 | 9–24 | 3 | 1 | 1 | 2 |
| 7 | Butter and methyl linoleate (8 per cent) | 4 | 7–20 | 3 | 1 | 2 | 2 |
| 8 | Arachis oil for 8 weeks followed by butter | 7 | 12–18 | 4 | 1 | 5 | 1 |

*Synthetic glycerides*

The results are shown in Table V.

Linoleic acid alone, when given as glyceryl trilinoleate, did not however produce atherosclerosis. This result would suggest that its effect depends on the presence of saturated fatty acids such as occur in small proportion in maize and arachis oil. Two animals showed an accumulation of lipid filled macrophages but no other lesions were seen.

Two glycerides (group 10 and 11), which simulate those occurring in butter and beef fat, produced thrombosis and aggregates of lipid filled macrophages.

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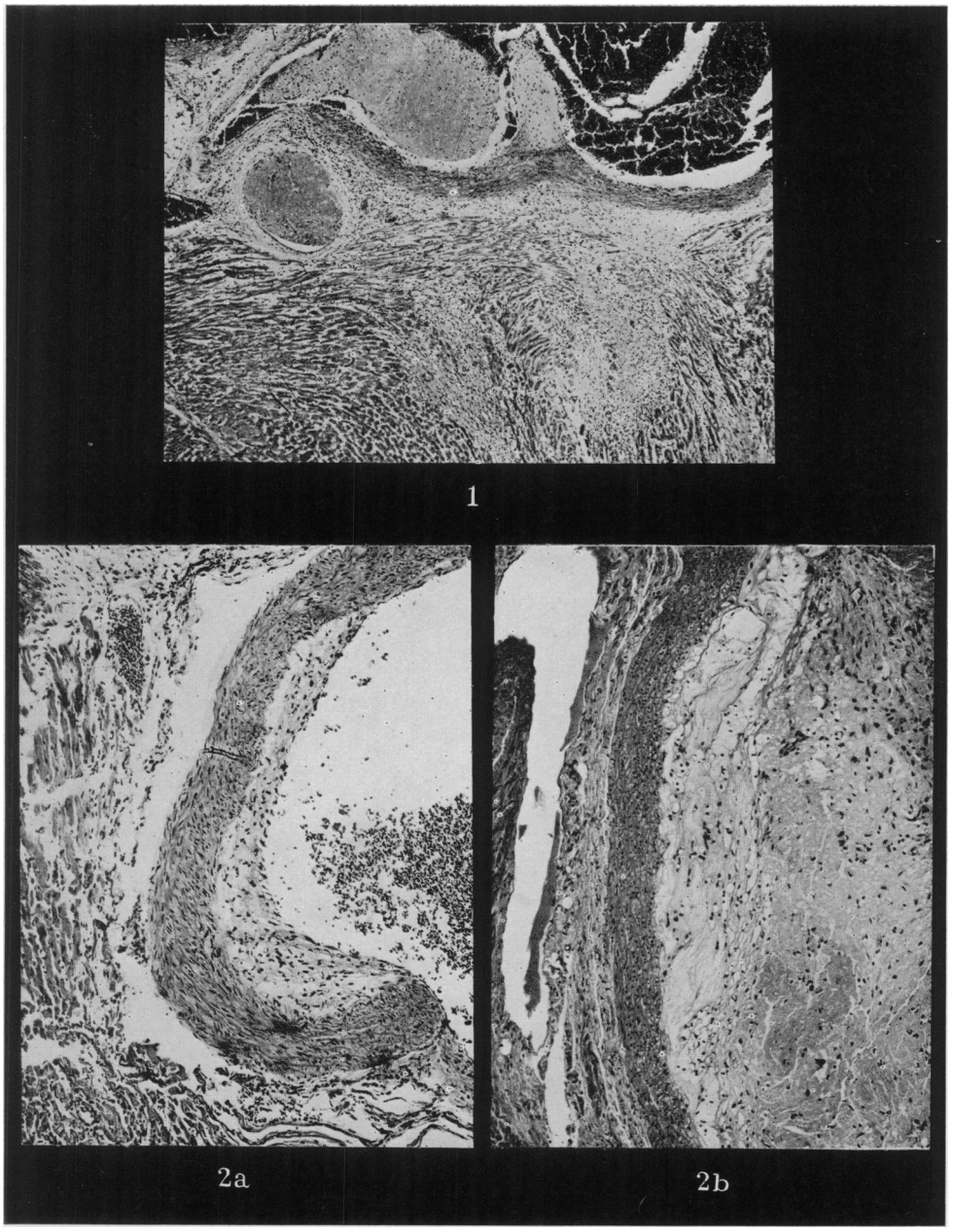
EXPLANATION OF PLATE.

FIG. 1.—Heart of rat given hydrogenated arachis oil showing thrombosis in the aorta and coronary artery and a septal infarct. (H. and E. × 33.)

FIG. 2*a*.—Aorta of rat given arachis oil showing fibrous plaque with small deposition of lipid in base. (H. and E. × 78.)

FIG. 2*b*.—Aorta of rat, given maize oil showing plaque with abundant lipid. (H. and E. × 82.)



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2a

2b

Gresham and Howard.

TABLE V.—*Distribution of Lesions in Rats Given Synthetic Glycerides*

| Group | Glyceride | No. of animals | Range of survival time (weeks) | Athero-sclerosis | Lipid filled macro-phages | Throm-bosis | Myo-cardial infarction |
|---|---|---|---|---|---|---|---|
| 9 | Trilinoleate | 5 | 8–9 | 0 | 2 | 0 | 0 |
| 10 | Mono-oleate-distearate | 10 | 2–4 | 0 | 8 | 6 | 0 |
| 11 | Di-oleate mono-stearate | 5 | 4–5 | 0 | 4 | 2 | 0 |
| 12 | Tri-oleate | 8 | 5 survived 16 weeks | 0 | 0 | 0 | 0 |

More thrombosis was seen with the glyceride containing the greater amount of saturated fatty acid (group 10). Myocardial infarction was not seen but it should be noted that these animals died after only a short period, on the diet. The essential component for thrombosis is a saturated fatty acid since no lesions were observed if oleic acid was given alone as glyceryl trioleate (group 12).

## DISCUSSION

Our previous results (Gresham and Howard, 1960) showed that the production of atherosclerosis or thrombosis and myocardial infarction is dependent on the type of fat in the diet. Thus arachis oil together with agents which produce hypercholesterolaemia, cholesterol, cholic acid and thiouracil (Thomas and Hartroft, 1959) produces atherosclerosis whereas replacement of arachis oil by butter produces thrombosis and infarction. In the present experiments the nature of these differences has been clarified. Animal fats such as butter or beef fat which contain chiefly saturated fatty acid linked with oleic acid produced thrombosis in the cardiac chambers, aorta and coronary arteries causing myocardial infarction. Two glycerides resembling the components of animal fats, namely glyceryl mono-oleate distearate and dioleate mono-stearate were found to produce thrombosis. The essential component was shown to be a saturated fatty acid since oleic acid alone as the trioleate was ineffective. Moreover thrombosis was greater in the glyceride containing the greater amount of saturated fatty acid, namely, glyceryl mono-oleate distearate.

Vegetable oils such as arachis and maize, which contain a high content of linoleic acid produced atherosclerosis closely resembling that seen in man. Linoleic acid appears to be important in plaque formation since no plaques were seen after hydrogenation of arachis oil, a procedure which removes linoleic acid. Moreover the addition of methyl linoleate to butter produces atherosclerosis in addition to thrombosis. It should be noted however that glyceryl trilinoleate alone is without effect and indicates the necessity for the presence also of saturated fatty acids. Current investigations are concerned with the effect of glycerides containing linoleic linked to saturated fatty acids.

Our results are in accord with the recent work of Böttcher, Woodford, Romeny-Wachter, Ter Haar, Houte and Bodsma van Gent (1960) who showed conclusively that human atherosclerotic plaques have a high content of linoleic acid which increases with the severity of the disease. In contrast the results offer no support

for the hypothesis of Sinclair (1956) who believes that atherosclerosis is caused by a relative deficiency of essential fatty acids such as linoleic.

An unexpected finding was that maize oil could also cause thrombosis in addition to atherosclerotic. This oil contains little saturated fatty acids and of these only a negligible amount is linked to oleic (Hilditch, 1956). Arachis oil does not produce thrombosis but although the fatty acid content of the two oils are similar the manner in which they are linked in the glycerides is markedly different (Hilditch, 1956). A further investigation into the factors involved in this difference would seem to be of value and is being undertaken.

SUMMARY

Rats were given a diet containing 40 per cent fat, 5 per cent cholesterol, 2 per cent cholic acid and 0·3 per cent thiouracil, and the effects of natural and synthetic fats on thrombosis and atherosclerosis investigated. Butter, beef fat, hydrogenated arachis oil and synthetic glycerides containing stearic and oleic acids produced thrombosis. Arachis oil produced atherosclerosis without thrombosis. Mixtures of butter and arachis oil, butter supplemented with 8 per cent methyl linoleate, and maize oil produced both lesions. None was observed with glyceryl trioleate and trilinoleate. It was concluded that saturated fatty acids are thrombogenic and that linoleic acid in conjunction with saturated fatty acids is atherogenic.

## REFERENCES

'Biochemical Preparations'—(1955) London (Chapman and Hall) p. 86.

Böttcher, C. J. P., Woodford, F. P., Romeny-Wachter, C., Ter Haar, C. H., Houte, F. and Bodsma van Gent, C. M. Van.—(1960) *Lancet*, i, 1378.

Gresham, G. A. and Howard, A. N.—(1960) *Brit. J. exp. Path.*, **41**, 395.

Hilditch, T. P.—(1956) 'The Chemical Constitution of Natural Fats'. London (Chapman and Hall), pp. 365, 372.

Sinclair, H. M.—(1956) *Lancet*, i, 381.

Thomas, N. A. and Hartroft, W. S.—(1959) 'Circulation', **19**, 65.

Wheeler, D. H., Riemenschneider, R. W. and Sando, C. E.—(1940) *J. biol. Chem.*, **132**, 681.