Brit. J. Nutr. (1964), **18**, 253, *with 2 plates*

The effect of diet on the level of plasma cholesterol and the degree of atheromatous degeneration in the rabbit

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(*Received* 18 *December* 1963—*Accepted* 30 *January* 1964)

There has been considerable speculation on a possible relationship between a derangement of cholesterol metabolism and atherosclerotic heart disease in man ever since Windaus (1910) reported that human atheromatous aortas contained about six times as much free cholesterol and about twenty times as much esterified cholesterol as normal tissues and Anitschkow (1913) discovered that the inclusion of pure cholesterol in the diet of rabbits resulted in elevated levels of cholesterol in the blood and atheromatous degeneration of the aorta. The claim of many investigators (e.g. Davis, Stern & Lesnick, 1937; Barker, 1939) that atherosclerosis in man is associated with high levels of blood cholesterol stimulated interest in the factors that controlled blood cholesterol levels. In view of the findings of Anitschkow (1913) it is perhaps not surprising that attention was focused on the cholesterol content of the human diet and many authorities (e.g. Engelberg & Newman, 1943) were of the opinion that blood cholesterol levels in man were regulated to a certain extent by the cholesterol content of the diet. However, Keys, Mickelsen, Miller & Chapman (1950) showed that the level of blood cholesterol in man was not influenced by the addition of cholesterol to the diet. In the search for other dietary factors that exerted a regulatory effect, Kinsell, Partridge, Boling, Margen & Michaels (1952) and Groen, Tjiong, Kamminga & Willerbrand (1952) observed that the replacement of animal fat in the human diet by an equivalent amount of vegetable fat resulted in a decrease in blood cholesterol levels. Since then extensive investigations have been carried out on the influence of the type of dietary fat on blood cholesterol levels in man and, although there is by no means complete agreement, the weight of evidence would seem to indicate that fats containing substantial amounts of polyunsaturated fatty acids (either essential or non-essential) produce a marked decrease in blood cholesterol levels whereas those containing predominantly saturated fatty acids have the opposite effect (Kinsell, 1963). The fact that the type of dietary fat has been shown to control the levels of blood cholesterol in patients given cholesterol-free diets (Ahrens, Hirsch, Insull & Peterson, 1958) indicates in man that the regulatory mechanism involves some aspect of the endogenous metabolism of cholesterol. Little progress has been made in the elucidation of this mechanism by experiments with laboratory animals, for until fairly recently it appeared that the blood cholesterol levels of the dog, chick, rabbit and rat could not be altered by the addition of different types of fat to diets unsupplemented with cholesterol (Olsen, 1959). Although there are numerous reports of changes in the blood cholesterol levels of experimental animals when different fats were added to diets

https://doi.org/10.1079/BJN19640025 Published online by Cambridge University Press

supplemented with cholesterol, these changes were not always analogous to those observed in man (Olsen, 1959) and were probably due, in part, to the effect of the various dietary fats on the absorption from the intestine of exogenous cholesterol (Byers & Friedman, 1958). A considerable advance was made when Lambert, Miller, Olsen & Frost (1958) and Wigand (1959) independently showed that the addition of hydrogenated coconut oil or butterfat to a purified diet containing no cholesterol resulted in elevated blood cholesterol levels and aortic atherosis in experimental rabbits. On the other hand, the addition of maize oil to the diet resulted in low blood cholesterol levels and no atherosis.

With the rabbit as an experimental animal, an investigation into the cause of the hypercholesterolaemic and atherogenic effects of diets containing butterfat is now in progress in this laboratory. Since butterfat is a potent source of calories it seemed logical that the first step in this investigation should be a comparison of the atherogenic effect of butterfat with that of an isocaloric amount of starch in order to determine whether the fatty acids as such present in butterfat exert a positive atherogenic effect. The results of these studies are now reported, together with a comparison of the hypercholesterolaemic and atherogenic effects of butterfat with those of other dietary fats. A preliminary account summarizing these experiments has been published (Moore & Kon, 1963).

EXPERIMENTAL

Diets, technique and procedure

Expt 1. Forty male New Zealand White rabbits, obtained at the age of 6 months from a local breeder, were housed in individual cages and were given a commercial rabbit diet for a short period until they became accustomed to their surroundings. The rabbits were then divided into five groups of eight each so that the offspring of the same parents were distributed equally throughout the groups, and during the following 6 weeks (the pre-experimental period) the rabbits were gradually introduced to the five experimental diets. This was achieved by grinding the commercial pellets and successively adding 25, 50 and 75% of the experimental diets and each time re-pelleting the whole. At the end of this pre-experimental period all the rabbits were readily consuming the experimental diets. Throughout this period (weeks 1–6) and the experimental period (weeks 7–37) the rabbits were given food and water *ad lib.*

The basal diet was similar to that used by Funch, Krogh & Dam (1960), and consisted (parts) of wheat starch (Starch Products Ltd, Slough) 16·3, sucrose 10, casein (Lactic acid casein; Glaxo Research Ltd, Greenford) 25·0, wheat straw (ground to pass through a 2 mm sieve) 19·0, methyl cellulose (Celacol M. 450; J. M. Steel & Co. Ltd, London, WC 2) 1·0, potassium acetate 2·5, magnesium oxide 0·5, sodium chloride 0·7, choline chloride 0·5, salt mixture 4·0 and vitamin mixture 0·5; making 80 parts in all. The composition of the salt mixture was (g): calcium lactate pentahydrate 3900, ferric citrate trihydrate 355, dipotassium monohydrogen orthophosphate 2862, tricalcium orthophosphate 1620, monosodium dihydrogen orthophosphate monohydrate 1041, magnesium sulphate 798, sodium chloride 519, cupric sulphate pentahydrate 15, manganese sulphate tetrahydrate 60, potassium iodide 1·5, zinc carbonate

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1·5. The composition of the vitamin mixture was: biotin 50 mg, folic acid 50 mg, *p*-aminobenzoic acid 35 g, thiamine hydrochloride 5 g, riboflavine 5 g, pyridoxine hydrochloride 5 g, calcium pantothenate 5 g, nicotinic acid 8 g, *meso*-inositol 15 g, ascorbic acid 5 g, cyanocobalamin 4 mg, menaphthone sodium bisulphite 750 mg, Rovimix E (250 mg α-tocopheryl acetate/g) 20 g, Rovimix AD (50000 i.u. vitamin A and 5000 i.u. vitamin D_3/g) 20 g; and glucose to 500 g. With the exception of cyanocobalamin, which we obtained from Glaxo Research Ltd, the vitamins were from Roche Products Ltd, Welwyn Garden City.

Table 1. *Expt* 1. *Composition and gross energy value of the diets given to the rabbits*

Percentage composition (by weight):	Group 1	Group 2	Group 3	Group 4	Group 5
Fat	20·0*	20·6†	20·0‡	20·0§	0·8*
Wheat starch	16·3	16·2	16·3	16·3	47·2
Sucrose	10·0	9·9	10·0	10·0	8·2
Casein	25·0	24·8	25·0	25·0	20·4
Wheat straw	19·0	18·9	19·0	19·0	15·5
Methyl cellulose	1·0	1·0	1·0	1·0	0·8
Potassium acetate	2·5	2·5	2·5	2·5	2·0
Magnesium oxide	0·5	0·5	0·5	0·5	0·4
Sodium chloride	0·7	0·7	0·7	0·7	0·6
Choline chloride	0·5	0·5	0·5	0·5	0·4
Salt mixture	4·0	4·0	4·0	4·0	3·3
Vitamin mixture	0·5	0·5	0·5	0·5	0·4
Gross energy value‖ (kcal/100 g diet):	532	532	532	532	433
Percentage of total calories supplied as supplementary fat	35	35	35	35	1·8
Percentage of total calories supplied as starch	13	13	13	13	46
Percentage of total calories supplied as protein	28	28	28	28	28

* Maize oil. † Butter. ‡ Butterfat. § Hydrogenated coconut oil.
‖ Calculated from the values given by Maynard (1937).

To 80 parts of the basal diet were added: for group 1, 20 parts maize oil (Brown and Polson Ltd, London, WC 1); for group 2, 24 parts butter (equivalent to 20·6 parts dry matter); for group 3, 20 parts butterfat; for group 4, 20 parts hydrogenated coconut oil (Loders and Nucoline Ltd, London, E 16); and for group 5, 1 part maize oil and 41·9 parts wheat starch. The percentage composition and calculated gross energy values of these diets are given in Table 1. The butter used in Expts 1 and 2 was obtained from Wilts United Dairies Ltd, Trowbridge. The butterfat was prepared from butter simply by warming the butter on a water bath at 50°, centrifuging the melted butter and decanting the clear supernatant fat. Since the butter contained 83·5% fat the quantities of butterfat in the diets given to the rabbits of groups 2 and 3 were the same. The high-starch diet given to the rabbits of group 5 was devised so that the linoleic acid:gross energy ratio was the same as that of the diet containing 20% butterfat given to the rabbits of group 3. At the beginning of the experiment no facilities were available for analysing the butterfat and maize oil for linoleic acid so mean values were calculated from the various analytical results reported in the literature (e.g. Hilditch, 1940), i.e. it was assumed that butterfat and maize oil contained

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2·4 and 48·0% linoleic acid respectively. Thus 20 g of butterfat in the diet of the rabbits of group 3 were replaced in the diet given to the rabbits of group 5 by a mixture of 1 g maize oil and 41·9 g wheat starch. The latter mixture supplied 0·48 g linoleic acid and 186·6 kcal gross energy, as did the 20 g butterfat. However, gas chromatographic analysis of the dietary fats at the end of the experiment showed that the assumed linoleic acid contents of the butterfat and maize oil were incorrect (see below).

After mixing, the experimental diets were slightly moistened with water and then passed through the meat-grinding attachment of a Hobart food mixing machine. The resulting pellets were dried overnight at 40–45°. Owing to the danger of oxidative deterioration, only small batches of diet were prepared at a time and kept in the rabbit house.

The rabbits were weighed at the beginning of the pre-experimental period and at weekly intervals during the remainder of the experiment. Although the design of the rabbit cages and food containers did not allow accurate measurement of the food intake of individual rabbits, a record was made of the total amount of diet consumed by the various groups during the experimental period. Immediately before the beginning of the pre-experimental period and at fortnightly intervals during the experimental period a small sample of blood (approximately 2 ml) was taken from the marginal ear vein of each rabbit for the determination of plasma cholesterol. Throughout Expts 1 and 2 blood samples were collected in heparinized tubes. At the end of the experimental period (i.e. after 32 weeks on the experimental diets) large samples of blood (approximately 50 ml) were taken from the marginal ear veins of the rabbits and immediately afterwards the animals were killed by a blow on the head. As rapidly as possible thereafter the liver, kidneys and heart, together with the aorta extending to the point of division into the two common iliac arteries, were removed from each rabbit for chemical analysis or histological examination.

Expt 2. Expt 2 was undertaken in an attempt to confirm and extend the findings of Expt 1. The rabbits used in Expt 2 were seventy New Zealand × Lop Ear males obtained at the age of 4 months from the same source as the animals used in Expt 1. The rabbits were divided at random into five groups of fourteen each and were again housed in individual cages. As described previously, the animals were gradually accustomed to the experimental diets during a pre-experimental period of 6 weeks. At the end of this period the rabbits were consuming the experimental diets readily. As in Expt 1, they were given food and water *ad lib.* throughout the experimental period (weeks 7–42).

The basal diet was exactly the same as that used in Expt 1. To 80 parts of the basal diet were added: for group 1, 20 parts maize oil; for group 2, 20 parts butterfat; for group 3, 10 parts maize oil and 10 parts butterfat; for group 4, 0·47 parts maize oil and 43·1 parts wheat starch; and for group 5, 10·2 parts maize oil and 21·6 parts wheat starch. The percentage composition and calculated gross energy values of these diets are given in Table 2. Gas chromatographic analysis showed that the percentage of linoleic acid in the maize oil and butterfat used in these experiments was 53·0 and 1·26 respectively. Thus 20 g of butterfat and a mixture of 0·47 g maize oil and 43·1 g

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starch each supplied 0·25 g linoleic acid and 186·6 kcal gross energy. Therefore the linoleic acid:gross energy ratio of the diet given to the rabbits of group 2 was the same as of that given to the rabbits of group 4. Similarly, the linoleic acid:gross energy ratios were the same for the diets given to the rabbits of groups 3 and 5. These ratios can be calculated from values in Tables 1 and 2.

Table 2. *Expt 2. Composition and gross energy value of the diets given to the rabbits*

Percentage composition (by weight):	Group 1	Group 2	Group 3	Group 4	Group 5
Fat	20·0*	20·0†	10·0* 10·0†	0·4*	9·2*
Wheat starch	16·3	16·3	16·3	48·1	33·9
Sucrose	10·0	10·0	10·0	8·1	9·0
Casein	25·0	25·0	25·0	20·2	22·4
Wheat straw	19·0	19·0	19·0	15·4	17·0
Methyl cellulose	1·0	1·0	1·0	0·8	0·9
Potassium acetate	2·5	2·5	2·5	2·0	2·2
Magnesium oxide	0·5	0·5	0·5	0·4	0·5
Sodium chloride	0·7	0·7	0·7	0·6	0·6
Choline chloride	0·5	0·5	0·5	0·4	0·5
Salt mixture	4·0	4·0	4·0	3·2	3·6
Vitamin mixture	0·5	0·5	0·5	0·4	0·5
Gross energy value‡ (kcal/100 g diet):	532	532	532	427	472
Percentage of total calories supplied as supplementary fat	35	35	35	0·8	18
Percentage of total calories supplied as starch	13	13	13	48	30
Percentage of total calories supplied as protein	28	28	28	28	28

* Maize oil. † Butterfat.
‡ Calculated from the values given by Maynard (1937).

During the experiment, the weights of individual rabbits and the total weights of food consumed by the various groups of rabbits were recorded. Small samples of blood were taken from the marginal ear vein of each rabbit at intervals of 8 weeks and then a final interval of 4 weeks for cholesterol determination. After 36 weeks on the experimental diets large samples of blood were taken from the marginal ear veins of the rabbits. Immediately afterwards the animals were killed and various tissues were removed for analysis and examination as in Expt 1.

Treatment of tissues and methods of analysis

After the removal of adventitious fatty tissue, the aortas and portions of the heart were fixed in 4% (w/v) formaldehyde in 0·9% (w/v) sodium chloride saturated with calcium carbonate. Each aorta was then stained with Sudan IV (0·05%, w/v, in ethanol–acetone–water 35:35:30, v/v/v). In Expt 1 the degree of lipid degeneration of the intimal surface was judged as described by Wigand (1959). To assess the degree of lipid degeneration of the aortas obtained from the rabbits of Expt 2 the following more detailed procedure was adopted. The intimal surface of each aorta was subdivided into a number of anatomically defined regions: (1) the semilunar valves, (2) the

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junction of the aorta with the coronary arteries, (3) the aortic arch, (4) the junction of the innominate artery with the aorta, (5) the ductus arteriosus, (6) the thoracic aorta, (7) the antero-renal abdominal aorta, (8) the postero-renal abdominal aorta. An arbitrary value of 10 was allocated to each of these regions in the most markedly involved aorta (from rabbit no. 12 in group 4 of Expt 2). With this particular aorta as a standard for comparison, the degrees of lipid degeneration in the various regions of the remaining aortas were graded, account being taken of both the area covered and the intensity of the Sudan IV staining of the atheromatous lesions. In addition to wax sections, frozen sections of the aorta and coronary tissues were prepared by gum-syrup and gelatin infiltration techniques for detailed examination of the lesions. The sections were submitted to the standard staining procedures or examined without further staining under a polarizing microscope.

The liver, kidneys and remainder of the heart from each rabbit were homogenized in chloroform–methanol (2:1, v/v) and the lipid was extracted and purified by the method of Folch, Lees & Stanley (1957). The lipid contained in the plasma from the final blood samples was extracted and purified by an adaptation of the Folch *et al.* (1957) technique described by Nelson & Freeman (1959). In addition, all lipid extracts were taken to dryness under reduced pressure in a rotary film evaporator connected to a supply of nitrogen. The dry residues were rapidly taken up in chloroform, filtered and then stored in chloroform–methanol (2:1, v/v) in a cold room.

In the early stages of Expt 1 the total cholesterol content of the plasma derived from the fortnightly blood samples was determined by the method of Abell, Levy, Brodie & Kendall (1952), but in view of the very low plasma cholesterol levels of the rabbits given the 20% maize-oil diet, the colour reagent of Abell *et al.* (1952) was subsequently replaced for the remainder of Expt 1 and throughout the whole of Expt 2 by the more sensitive reagent described by Brown (1959). The total cholesterol content of the liver, heart and kidney extracts was determined by the method of Abell *et al.* (1952).

RESULTS

Weights of rabbits and food intake

During Expt 1, two rabbits died in each of groups 3, 4 and 5. Post-mortem examination at the local Veterinary Investigation Centre of the Ministry of Agriculture, Fisheries and Food indicated that deaths were caused by either pneumonia or enteritis and could in no way be connected with dietary treatment. During Expt 2, two rabbits died in each of groups 1 and 4, one in each of groups 2 and 3 and four in group 5. These deaths were all attributed to 'middle ear disease'. In both experiments values (e.g. liver weights, plasma cholesterol levels) obtained for these rabbits up to the time they died were eliminated from the mean values presented.

The live weights of the rabbits immediately before and during the pre-experimental and experimental periods of Expts 1 and 2 are summarized in Tables 3 and 4 respectively. In Expt 1, the rabbits tended to lose weight during the pre-experimental period (weeks 1–6). Between weeks 6 and 14 most of the rabbits, with the exception of those given the hydrogenated coconut-oil diet (group 4, Expt 1), regained the weight

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they had lost during the pre-experimental period and then maintained a fairly constant weight and appeared in good condition for the remainder of the experiment. The rabbits given the hydrogenated coconut-oil diet continued to lose weight throughout the experiment, and towards the end of the feeding period certain of the animals in this group were in a rather poor condition similar to that described and illustrated by Wigand (1959) for New Zealand White rabbits given for 15 weeks a diet containing 8% hydrogenated coconut-oil. The rabbits in Expt 2 for some obscure reason became

Table 3. *Expt* 1. *Weights* (*kg*) *of the rabbits*

(Mean values with their standard errors)

Group no. and diet	No. of rabbits/ group	0 weeks	6 weeks	14 weeks	22 weeks	30 weeks	37 weeks
1, 20% maize oil	8	3·88±0·23	3·65±0·23	3·90±0·22	3·92±0·21	3·97±0·17	4·09±0·19
2, 24% butter	7	3·65±0·32	3·12±0·27	3·53±0·29	3·45±0·28	3·51±0·28	3·68±0·24
3, 20% butter-fat	6	4·11±0·33	3·65±0·20	3·91±0·23	3·91±0·23	3·87±0·25	3·97±0·23
4, 20% hydro-genated coconut oil	6	4·16±0·18	3·47±0·05	3·44±0·09	3·32±0·10	3·21±0·12	3·15±0·11
5, High-starch	6	3·98±0·24	3·59±0·14	3·93±0·13	3·92±0·13	3·88±0·11	3·93±0·15

Table 4. *Expt* 2. *Weights* (*kg*) *of the rabbits*

(Mean values with their standard errors)

Group no. and diet	No. of rabbits/ group	0 weeks	6 weeks	14 weeks	22 weeks	30 weeks	42 weeks
1, 20% maize oil	12	4·09±0·18	4·03±0·19	4·26±0·19	4·43±0·18	4·23±0·19	4·38±0·18
2, 20% butter-fat	13	4·23±0·14	3·94±0·14	4·11±0·14	4·17±0·14	4·25±0·16	4·29±0·17
3, 10% maize oil, 10% butterfat	13	4·09±0·14	3·99±0·16	4·31±0·16	4·43±0·18	4·49±0·19	4·52±0·17
4, High-starch	12	3·80±0·24	3·79±0·19	3·86±0·22	4·09±0·24	4·18±0·24	3·99±0·25
5, 10% maize-oil-starch	10	3·70±0·15	3·83±0·24	4·11±0·21	4·20±0·23	4·32±0·25	4·35±0·25

used to the experimental diets far more readily than those in Expt 1 and consequently did not show an appreciable loss in weight during the pre-experimental period.

The values for total dry-matter and gross energy intakes between weeks 6 and 37 in Expt 1 and between weeks 6 and 42 in Expt 2 are given in Table 5. These values can only be regarded as approximate as no account was taken of food wastage. However, it is most unlikely that food wastage varied from group to group, and in any event it was certainly less than 5% of the total amount of diet given to the rabbits. It would therefore seem justifiable to consider the values given in Table 5 in a comparative, if not in an absolute, sense. Apart from the rabbits given the hydrogenated coconut-oil diet (group 4, Expt 1), it is noteworthy that the gross energy intake of the remaining

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groups of rabbits was relatively constant. The fact that the gross energy intake of the rabbits of group 4 in Expt 1 was somewhat lower than that of the other groups undoubtedly provides in part an explanation for the loss in weight of the rabbits given the hydrogenated coconut-oil diet. Another possibility that must be considered in this respect, however, is that the hydrogenated coconut-oil was poorly digested by the rabbits. Although Paul & McCay (1942) have found digestibility coefficients as high as 91% for hydrogenated vegetable oils when included in the diet of rabbits at a level of 6%, it cannot be assumed that the hydrogenated coconut oil is digested to this extent when it is included in the diet at a level of 20%.

Table 5. *Expts 1 and 2. Daily dry-matter and gross-energy intakes of the rabbits during the experimental period*

Expt 1 (intake/rabbit)			Expt 2 (intake/rabbit)		
Group no. and diet	Dry matter (g)	Energy (kcal)	Group no. and diet	Dry matter (g)	Energy (kcal)
1, 20% maize oil	99	527	1, 20% maize oil	95	505
2, 24% butter	98	522	2, 20% butterfat	95	505
3, 20% butterfat	96	510	3, 10% maize oil, 10% butterfat	97	515
4, 20% hydrogenated coconut oil	85	452	4, High-starch	117	502
5, High-starch	116	502	5, 10% maize-oil–starch	107	505

Plasma cholesterol levels

No blood samples were taken during either of the pre-experimental periods since it was considered that any variation in plasma cholesterol levels observed in the course of the change from commercial to experimental diets (i.e. a period when there was considerable variation in food intake between the groups and a general but variable loss in body-weight) would be extremely difficult to interpret. Statistical analysis of the results for plasma cholesterol seemed superfluous since the effects of dietary treatment on plasma cholesterol levels in both experiments were self-evident.

The mean plasma cholesterol values for the five groups of rabbits in Expt 1 are shown in Fig. 1. The mean plasma cholesterol level of the rabbits of group 1 (given the 20% maize-oil diet) during the whole of the experimental period was 30·0 mg/100 ml as compared with 35·7 mg/100 ml obtained for these rabbits immediately before the pre-experimental period began. For both groups 2 and 3, replacement of the commercial diet by diets containing butter and butterfat respectively resulted in pronounced increases in the levels of plasma cholesterol. Throughout the whole of the experimental period the mean plasma cholesterol values were: for group 2, 138 mg/100 ml; and for group 3, 118 mg/100 ml; as compared with 34·6 and 34·5 mg/100 ml respectively for these two groups of rabbits when they were given the commercial diet. Replacement of the commercial diet by the experimental diet containing 20% hydrogenated coconut oil (group 4) immediately resulted in a twofold increase in the mean plasma cholesterol level, which then remained relatively constant until the 29th

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week of the experiment. From the 29th week to the end of the experiment there was a dramatic increase in the plasma cholesterol levels (from 112 to 315 mg/100 ml) of the rabbits in group 4. The mean plasma cholesterol level for the whole of the experimental

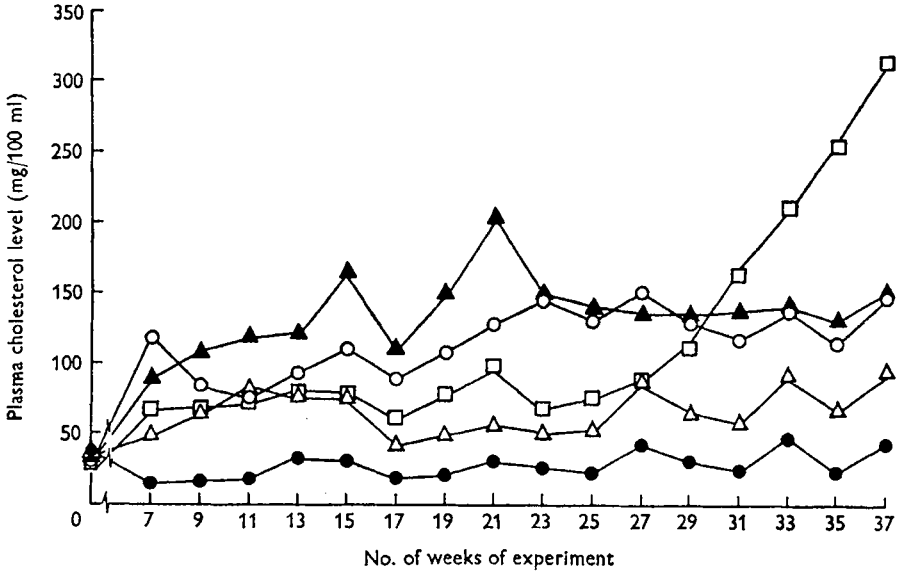

Fig. 1. Expt 1. Mean plasma cholesterol levels of the rabbits immediately before the beginning of the pre-experimental period and at intervals during the experiment. The mean standard error for each plot was for group 1 (●—●) ±4·4, for group 2 (▲—▲) ±26·3, for group 3 (○—○) ±26·7, for group 4 (□—□) ±22·9, and for group 5 (△—△) ±15·6. The numbers of rabbits in each group are given in Table 3.

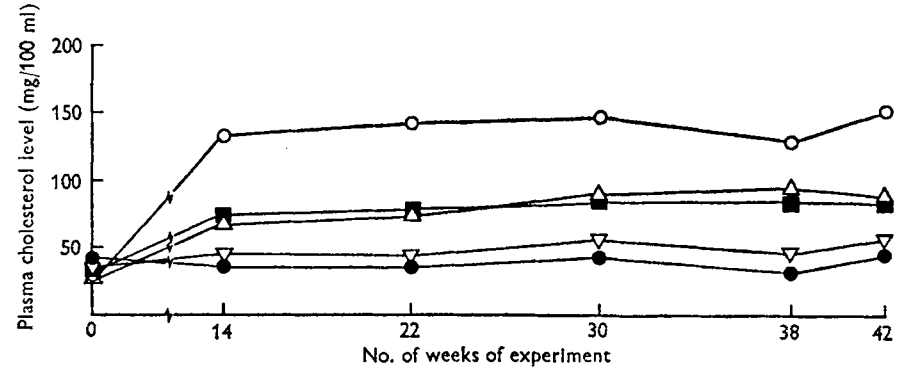

Fig. 2. Expt 2. Mean plasma cholesterol levels of the rabbits immediately before the beginning of the pre-experimental period and at intervals during the experiment. The mean standard error for each plot was for group 1 (●—●) ±9·2, for group 2 (○—○) ±13·4, for group 3 (■—■) ±9·8, for group 4 (△—△) ±10·0, and for group 5 (▽—▽) ±4·9. The numbers of rabbits in each group are given in Table 4.

period was for this group 119 mg/100 ml. For the rabbits of group 5 given the high-starch diet, the mean plasma cholesterol level for the whole of the experimental period was 67·6 mg/100 ml as compared with 34·6 mg/100 ml found when these rabbits were given the commercial diet. It is noteworthy that the plasma cholesterol level of one of

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the rabbits of this group was abnormally low throughout the whole of the experiment. The plasma cholesterol level of this rabbit (no. 6 of group 5) when given the commercial diet was 19·5 mg/100 ml, a level that was not altered by the change from the commercial to the experimental diet, and the mean plasma cholesterol level of this animal during the whole of the experimental period was also 19·5 mg/100 ml. In addition to the changes in plasma cholesterol levels that were brought about by dietary treatment, certain changes occurred during the experimental period that were clearly independent of diet. For instance, there was a decrease in the mean plasma cholesterol levels of all groups from the 15th to the 17th week of the experiment. At present no explanation can be put forward to account for such overall changes in plasma cholesterol levels.

The mean plasma cholesterol levels of the five groups of rabbits in Expt 2 are shown in Fig. 2. The plasma cholesterol level of rabbit no. 1 of group 1 given the 20% maize-oil diet was abnormally high compared with the levels of the remainder of the group. This particular rabbit when given the commercial diet had a level of plasma cholesterol of 73·6 mg/100 ml, and its mean plasma cholesterol level for the whole of the experimental period was 130 mg/100 ml. Excluding these results for rabbit no. 1, the mean plasma cholesterol level of the rabbits of group 1 during the whole of the experimental period was 32·1 mg/100 ml, a value almost identical with that obtained for the rabbits of group 1 in Expt 1. For the rabbits of group 2 given the 20% butterfat diet, the plasma cholesterol results were very similar to those found for the rabbits of groups 2 and 3 of Expt 1. Although the diet containing 10% butterfat and 10% maize oil gave rise to elevated plasma cholesterol levels in the rabbits of group 3, these levels were considerably less than those observed during the experimental period for the rabbits of group 2 given the 20% butterfat diet. It is important to note that for the whole of the experimental period the mean plasma cholesterol levels of the rabbits of groups 3 and 4 were very similar (81·6 and 84·2 mg/100 ml respectively). The plasma cholesterol levels of the rabbits in group 5 given the 10% maize-oil–starch diet (mean value for the whole experimental period 49·7 mg/100 ml) were slightly higher than those observed for the rabbits in group 1 given the 20% maize-oil diet, but the difference between these two groups was relatively small.

It is noteworthy that during the experimental periods the mean plasma cholesterol level of the rabbits of group 4 in Expt 2 (84·2 mg/100 ml) was somewhat higher than the corresponding level for group 5 in Expt 1 (67·6 mg/100 ml). Although the amount of maize oil included in both the high-starch diets given to these two groups of animals was small, it is perhaps not without significance that the diet given to the rabbits of group 4 in Expt 2 contained only 0·4% maize oil, whereas that given to the rabbits of group 5 in Expt 1 contained 0·8% (Tables 1 and 2).

The cholesterol contents of various tissues

The weights and cholesterol contents of the hearts, livers and kidneys of the rabbits in Expt 1 are given in Table 6. There were no substantial differences in the mean weights of the livers and kidneys in the five groups of rabbits. Although heart weight seemed to vary somewhat with dietary treatment, this variation was reduced when the mean weights of the hearts were expressed as a proportion of the mean body-weights

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(0·17 for group 1, 0·16 for group 2, 0·17 for group 3, 0·18 for group 4 and 0·16 for group 5). It is clear from Table 6 that the cholesterol contents of the livers, hearts and kidneys of the rabbits in group 1 (low plasma cholesterol levels) were not greater than the cholesterol contents of these tissues in groups 2, 3 and 4 (high plasma cholesterol levels). Nevertheless, it is doubtful if the cholesterol contents of the livers of the rabbits in group 1 were significantly lower than in the remaining groups (Moore & Williams, 1963).

Table 6. *Expt* 1. *Cholesterol contents of the various tissues of the rabbits*

(Mean values with their standard errors)

	Group 1 (20% maize-oil diet)	Group 2 (24% butter diet)	Group 3 (20% butter-fat diet)	Group 4 (20% hydrogenated coconut-oil diet)	Group 5 (high-starch diet)
Liver					
Weight of fresh tissue (g)	93·6 ± 9·9	96·3 ± 8·1	101 ± 13	93·8 ± 5·8	98·0 ± 13
Cholesterol content (mg/100 g fresh tissue)	287 ± 14	614 ± 102	609 ± 122	533 ± 45	438 ± 79
Total liver cholesterol (mg)	268 ± 23	590 ± 99	615 ± 96	500 ± 61	430 ± 95
Heart					
Weight of fresh tissue (g)	7·0 ± 0·4	6·0 ± 0·4	6·7 ± 0·7	5·8 ± 0·3	6·3 ± 0·2
Cholesterol content (mg/100 g fresh tissue)	126 ± 3	155 ± 7	155 ± 13	147 ± 17	143 ± 8
Total heart cholesterol (mg)	8·8 ± 0·5	9·4 ± 0·9	10·2 ± 1·0	8·5 ± 0·9	9·0 ± 0·2
Kidneys					
Weight of fresh tissue (g)	17·4 ± 1·0	17·8 ± 0·9	17·9 ± 1·1	17·9 ± 1·0	17·1 ± 0·8
Cholesterol content (mg/100 g fresh tissue)	376 ± 12	346 ± 10	336 ± 12	324 ± 20	379 ± 10
Total kidney cholesterol (mg)	65·0 ± 5·3	61·2 ± 4·6	60·0 ± 4·3	57·8 ± 4·8	64·7 ± 1·9

The degeneration of the aorta and coronary arteries

The nature of the lesions

Two distinct types of vascular degeneration were observed in the rabbits in both experiments. The first type of lesion, a sclerotic degeneration of the media, was present in about 20% of all the experimental rabbits, but was not associated with dietary treatment in either experiment. The macroscopic appearance of these lesions was very similar to that described and illustrated by Kobernick & Hashimoto (1963). Generally, this medial degeneration was confined to the aortic arch, but occasionally lesions of this type were also observed in the region of the ductus arteriosus and along the abdominal aorta. The microscopic appearance of the lesions in a portion of the aortic arch of a rabbit given the diet containing 20% butterfat in Expt 1 is illustrated in Pl. 1 *a*, *b*. The early stages of this type of lesion are distinguished by a decided thickening of the media and a diffuse deposition of collagen in its central layers. As the lesion develops, hyaline cartilage appears with characteristic cartilage cells contained in cyst-like spaces (Pl. 1 *a*). Calcification occurs and further degeneration is often characterized by the appearance in the centre of the lesion of a cavity containing true bone marrow cells. The cavity may be surrounded by an area of genuine bone containing typical osteocytes (Pl. 1 *b*). Pl. 1 *b* in fact shows all the stages in bone

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metaplasia within the media of the aorta from the early sclerotic stage to complete ossification with bone marrow formation. This type of lesion was not characterized by lipid involvement and would appear to be a form of Mönkeburg's medial calcification or senile sclerosis. We have also observed medial degeneration of the aortas in about 20% of a group of rabbits of similar age, breed and strain to those used in Expt 2 but that had been given an ordinary commercial rabbit diet. A much higher incidence of this 'spontaneous' involvement has been reported by Kesten (1935) who found medial sclerosis in the aortas of 54% of a group of 125 rabbits that had been given a normal commercial diet.

The second type of lesion was confined almost exclusively to the intima and was certainly associated with dietary treatment. Macroscopically, these intimal plaques were similar to those described by Wigand (1959). Histological examination revealed the presence of considerable amounts of lipid situated between the internal elastic membrane and the endothelial surface. This sudanophilic material appeared to be present either extracellularly as fine droplets scattered throughout the ground substance or intracellularly as accumulations in lipid-laden phagocytes. Occasionally, sudanophilic phagocytes were observed in the media subjacent to the internal elastic membrane beneath an intimal plaque. Anisotropic lipid crystals were observed when sections of the plaques were viewed under a polarizing microscope. Examples of this type of lesion in tissues of rabbits given the high-starch diet (group 5) in Expt 1 are illustrated in Pl. 2*a*, *b*, *c*. Pl. 2*a* shows a fairly large plaque projecting into the lumen of a coronary artery. When this section is examined at higher magnification (Pl. 2*b*) the dense accumulation of lipid between the internal elastic membrane and the layer of endothelial cells is clearly evident. A similar lesion in the intima of the thoracic aorta is shown in Pl. 2*c*. This second type of lesion would therefore appear to be a form of atheroma or atherosis.

The extent of atheromatous degeneration

Expt 1. The degrees of severity of the atheromatous lesions in each of the aortas obtained from the rabbits in Expt 1 are given in Table 7. The aortas were graded in a manner similar to that described by Wigand (1959), i.e. 0, perfectly normal appearance; +, isolated small plaques mainly in the aortic arch; + +, abundant medium-sized confluent plaques in all regions of the aorta; + + +, large areas of the aorta completely covered with confluent plaques. As may be seen from Table 7, atheroma was virtually absent in the group of rabbits given the 20% maize-oil diet. Nevertheless, it was possible to detect minute sudanophilic plaques, only just visible to the naked eye, at the junction of the aorta and anterior mesenteric artery in three of the rabbits in group 1 (graded > 0). Pronounced atheromatous lesions were observed in the aortas of most of the rabbits in groups 2, 3, 4 and 5. Although the number of rabbits in each group was relatively small and the technique employed to grade the severity of the lesions was only semi-quantitative, it appeared that the degree of atheromatous degeneration of the aortas in these four groups of rabbits was about the same. The aorta of one of the animals in each of groups 2, 4 and 5 appeared perfectly normal. However, only in rabbit no. 6 of the group given the high-starch diet was this

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absence of aortic atheroma associated with an abnormally low plasma cholesterol level (see p. 262). At all stages of Expt 1, the plasma cholesterol levels of rabbit no. 6 in group 2 and of rabbit no. 5 in group 4 were as high as the levels observed for other rabbits in these two groups in which extensive degeneration of the aortas had occurred.

Table 7. *Expt* 1. *Degrees of severity* of the atheromatous lesions for the aortas of the individual rabbits*

Group 1 (20% maize-oil diet)	Group 2 (24% butter diet)	Group 3 (20% butterfat diet)	Group 4 (20% hydrogenated coconut-oil diet)	Group 5 (high-starch diet)
o	++	+	+	+
o	+++	+++	+	+
o	+	+	+++	++
> o	+	+++	++	++
> o	++	+++	o	+++
o	o	+	+++	o
> o	+++			
o				

* Arbitrary scale (see p. 264).

Table 8. *Expt* 2. *Degrees of severity* of the atheromatous lesions for the separate regions of the aortas (mean values) and for the whole aortas (mean values with their standard errors) of the rabbits*

Region of aorta	Group 1 (20% maize-oil diet)	Group 2 (20% butterfat diet)	Group 3 (10% maize-oil, 10% butterfat diet)	Group 4 (high-starch diet)	Group 5 (10% maize-oil–starch diet)
Semilunar valves	1·1	3·5	1·6	2·5	0·1
Coronary–aorta junction	1·6	4·8	2·3	5·8	1·2
Aortic arch	1·0	2·4	1·3	3·8	0·3
Innominate–aorta junction	0·3	1·5	0·6	2·2	0·4
Ductus arteriosus	0·9	2·6	1·2	2·8	0·2
Thoracic aorta	0·2	1·2	0·8	2·0	0·0
Antero-renal abdominal aorta	0·4	2·4	0·2	2·2	0·1
Postero-renal abdominal aorta	0·2	0·5	0·1	1·5	0·0
Whole aorta	5·6 ±4·0 (1·61±0·8)†	18·9±5·3	8·1±4·3 (2·0±0·6)†	22·9±7·5	2·3 ±1·3 (1·32±0·6)†

* Arbitrary scale (see below).
† Excluding atypical rabbits (see text p. 266).

Expt 2. In Expt 2, an attempt was made to devise a more quantitative method of grading the severity of the aortic lesions. It seemed that the assessment of the degree of atheromatous degeneration could be sharpened somewhat if the aortas could be compared with a standard. The most highly involved aorta, i.e. that of rabbit no. 12 of the group given the high-starch diet (group 4), was chosen as a standard for comparison. Over 90% of the total intimal surface of this aorta was covered with confluent atheromatous plaques. In addition, various regions of the aortas (see p. 257) were graded separately on a scale of 0–10 to determine whether the distribution of the lesions varied with dietary treatment. A value of 10 was allocated to each region of the

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aorta of rabbit no. 12 of group 4. A value of 0 was allocated to any particular region when examination under an ordinary hand lens revealed the complete absence of sudanophilic plaques. A value representing the severity of the lesions for the whole aorta was obtained from the sum of the values for each separate region of that particular aorta, a value of 80 being derived for the aorta of rabbit no. 12 of group 4. The mean degrees of severity of the atheromatous lesions in the aortas of the rabbits in Expt 2 are given in Table 8. The incidence of aortic atheroma in eleven of the twelve rabbits given the 20% maize-oil diet was again very low. The aorta of rabbit no. 1 in this group was, however, highly involved. If the aorta of this particular rabbit is excluded, the mean degree of severity of the atheromatous lesions in the whole aorta for the remaining eleven animals in group 1 was 1·6 with a standard error of ±0·8. Attention is drawn to the fact that the plasma cholesterol levels of rabbit no. 1 of the group given the 20% maize-oil diet were abnormally high (see p. 262). In agreement with the findings of Expt 1, extensive atheromatous degeneration of the aorta was observed in the rabbits of group 2 given the 20% butterfat diet and in group 4 given the high-starch diet. Again, the incidence (the aortas of nine animals in each group were highly involved) and the degree of degeneration appeared to be the same in groups 2 and 4. One rabbit in each of groups 2 (no. 13) and 4 (no. 1) was atypical in that aortic lesions were virtually absent. In neither animal was the absence of atheroma associated with an abnormally low plasma cholesterol. Although the results presented in Table 8 show that the incidence of aortic atheroma in groups 3 and 5 was certainly reduced when compared with that in groups 2 and 4, it is perhaps difficult to draw definite conclusions about the extent of this reduction. The mean value for the whole aorta obtained for the rabbits of group 3 may be spuriously high since there was considerable involvement of the aortas in two of the rabbits in this group. (Abnormally high plasma cholesterol levels were observed in only one of these animals.) The aortas of the remaining eleven rabbits in group 3 were only slightly involved (the mean degree of severity for the whole aorta was 2·0, with a standard error of ±0·6). Similarly the aorta of one of the rabbits in group 5 was involved to a much greater extent (the plasma cholesterol level of this animal was in fact somewhat less than the group mean) than the others, and if the value for this aorta is excluded the mean degree of severity of the atheromatous lesions in the whole aorta for the remaining nine rabbits of the group was 1·3 with a standard error of ±0·6. Thus it appears that the degree of atheromatous degeneration of the aortas of the majority of the rabbits in groups 3 and 5 was in fact little different from that observed for the rabbits of group 1 given the 20% maize-oil diet. The results in Table 8 also indicate that there was no obvious effect of diet on the distribution of atheromatous lesions in the various regions of the aorta.

Since the detection of sudanophilic plaques in the coronary arteries involves histological techniques, it is difficult to arrive at any quantitative assessment of the degree of atheromatous degeneration in these arteries. Nevertheless, examination of sections of the coronary arteries has so far indicated that the incidence of coronary atheroma was similar to the incidence of aortic atheroma in the various groups of rabbits.

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DISCUSSION

It was originally intended that a purified basal diet should be used in the experiments since the work of Lambert *et al.* (1958) and Wigand (1959) had shown that such diets were acceptable to rabbits. After considerable preliminary investigations it became our experience that rabbits did not readily accept diets that contained any of the available forms of purified cellulose as a source of roughage. The possibility of using a purified basal diet was therefore abandoned and finely ground wheat straw was eventually chosen as a source of roughage. It was also our experience that the type of diet used in these experiments was not readily accepted unless offered in pellet form. This presented a further problem, that of pelleting high-fat diets. After various trials with different additives, it was found that reasonably stable pellets could be obtained when 1% methyl cellulose was included in the diet.

In both Expts 1 and 2 (Figs. 1 and 2) pronounced hypercholesterolaemia was observed in the rabbits given the diets containing 20% butterfat, whereas the plasma cholesterol levels of the rabbits given the diets containing 20% maize oil remained low and in fact were not very different from the levels observed for these rabbits when they were given an ordinary commercial diet. Similar differences in serum cholesterol levels have been reported by Wigand (1959) who compared rabbits given diets containing 8% butterfat with those given a diet containing 8% maize oil and by Funch *et al.* (1960) who compared rabbits given diets containing 24% butter with those given diets containing 20% arachis oil. However, in the experiments of Wigand (1959) and Funch *et al.* (1960) the diets containing butter or butterfat gave rise to a degree of hypercholesterolaemia that was far in excess of that found by us for the rabbits of groups 2 and 3 in Expt 1 and for those of group 2 in Expt 2. The reason for this difference is at present obscure, but it is pointed out that although the diets used by Funch *et al.* (1960) were very similar to those used by us there was one fundamental difference. Our basal diet was only semi-pure and contained wheat straw as a source of roughage, whereas the purified basal diet of Funch *et al.* (1960) contained a form of cellulose as a source of roughage. Whether this dissimilarity in basal diet could possibly account for the differences in blood cholesterol levels would seem to be worthy of investigation. Comparison of the changes in plasma cholesterol levels found for the rabbits given the diets containing 20% butterfat with those for the rabbits given the diets containing 20% hydrogenated coconut oil (Fig. 1) seems to indicate some essential difference in the mechanisms of the hypercholesterolaemic effects of these two dietary fats. The most marked changes in the plasma cholesterol levels occurred in the rabbits of group 4 (Expt 1) only after the hydrogenated coconut-oil diet had been given for about 20 weeks. Other investigators have found high levels of blood cholesterol in rabbits given coconut-oil (Gottenbos & Thomasson, 1961) or hydrogenated coconut oil (Lambert *et al.* 1958; Wigand, 1959). Apart from the differences in absolute levels, the findings of Lambert *et al.* (1958), Wigand (1959), Funch *et al.* (1960) and Gottenbos & Thomasson (1961) together with those now presented all establish that rabbits develop hypercholesterolaemia when given purified or semi-purified diets with no added cholesterol but containing certain of the more saturated

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fats such as butterfat or coconut oil, whereas rabbits given diets containing the unsaturated vegetable oils such as arachis oil or maize oil do not. It is noteworthy that in Expt 2 the mean plasma cholesterol level for the rabbits in group 5 (given the diet in which half of the supplementary starch in the diet given to the rabbits of group 4 was replaced by an isocaloric amount of maize oil) was almost mid-way between the levels observed for the rabbits of group 4 and for those of group 1. Comparison of the blood cholesterol levels for the rabbits of groups 1, 2 and 3 revealed that an analogous reduction in plasma cholesterol levels occurred when half of the butterfat in the diet given to the rabbits of group 2 was replaced by an equivalent amount of maize oil. In changeover experiments with individual rabbits, Wigand (1959) found that the replacement of hydrogenated coconut oil in the diet by maize oil resulted in a dramatic reduction in the blood cholesterol levels in less than 3 weeks. The fundamental mechanisms underlying the hypercholesterolaemic effect of saturated fat and the hypocholesterolaemic effect of unsaturated fat are as yet undetermined and it is clear that there is a need for more extensive research into this problem. It has been suggested that the differences in blood cholesterol levels in animals given these two types of dietary fat might simply be due to a shift in the partition of cholesterol between the blood and tissues. When rats were given a diet containing 20% coconut oil the levels of cholesterol in the plasma were higher but the levels of cholesterol in the liver were lower than in rats given a diet containing 20% maize oil (Avigan & Steinberg, 1958). Gerson, Shorland & Adams (1961) have shown with rats that the addition of 2 or 10% maize oil to a low-fat diet led to a decrease in the concentration of serum cholesterol that was accompanied by an increase in the concentration of cholesterol in such tissues as the heart and liver. If the plasma volume of a rabbit is taken as 45 ml/kg bodyweight (Altman & Dittmer, 1961), it may be calculated approximately that the total amounts of cholesterol circulating in the plasma in the rabbits of groups 1 and 3 (Expt 1) were 75 and 255 mg respectively. Thus to account for the differences in plasma cholesterol levels in these two groups of animals, those given the 20% maize-oil diet should contain about 180 mg more cholesterol in their tissues than those given the 20% butterfat diet. Although it is conceivable that 180 mg of extra cholesterol could easily be accommodated in the tissues of a rabbit weighing 4 kg, there was certainly no evidence that this extra cholesterol was deposited in the liver, heart and kidney (Table 6) or in the perinephric and subcutaneous adipose tissues (Moore & Williams, unpublished observations). Wigand (1959) found that the level of cholesterol in the livers of rabbits given a diet containing 8% maize oil was only about one-third of that in the livers of rabbits given a diet containing 8% butterfat. Clearly, other tissues must be examined but, nevertheless, it seems unlikely that the hypocholesterolaemic effect of maize oil and the hypercholesterolaemic effect of butterfat could be explained by a shift in the partition of cholesterol between the plasma and tissues of the rabbit.

There is no indication that the effects of different dietary fats on blood cholesterol levels can be explained on the grounds of an altered rate of cholesterol synthesis by the liver, for experiments with slices (Mukherjee & Alfin-Slater, 1958) and homogenates (Wood & Migicovsky, 1958) have shown that cholesterol synthesis in the livers of rats

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given diets containing cottonseed oil or maize oil was in fact greater than in the livers of rats given diets containing coconut oil or hydrogenated coconut oil. There is considerable confusion in the literature on the possibility of relating the control of blood cholesterol levels by certain dietary fats to an altered excretion of sterols and bile acids in the faeces. Part of this confusion is undoubtedly due to the difficulties encountered in the analysis of the complicated mixtures of sterols and bile acids that are normally found in faeces. Wilson & Siperstein (1959) injected [4-^{14}C]cholesterol intravenously into rats that were given diets containing either lard or maize oil. Although there was no consistent difference in the excretion of ^{14}C as bile acids or digitonin-precipitable neutral sterols in the faeces of the two groups, there was a markedly higher excretion of ^{14}C in the neutral sterols not precipitable with digitonin in the faeces of the rats given the maize-oil diet. At variance with these findings are those of Byers & Friedman (1958) who found that rats given walnut oil (iodine value 132) in the diet excreted larger quantities of both cholesterol and bile acids in the bile than did rats given a diet containing coconut oil. Equally conflicting reports have appeared from studies with man. For instance, in isotope experiments with male patients, Hellman & Rosenfeld (1959) have found that the fall in plasma cholesterol levels that was associated with a change from a diet containing butter to one containing maize oil was accompanied by an increased excretion of sterols, whereas there was no change in the excretion of bile acids in the faeces. On the other hand, the work of Gordon, Lewis, Eales & Brock (1957) and of Haust & Beveridge (1958) appeared to indicate that the respective hypocholesterolaemic effects of sunflower-seed oil and maize oil were due not to an increased excretion of sterols but to an increased excretion of bile acids. In the only comprehensive study with rabbits that seems to have been reported, Hellström, Sjövall & Wigand (1962) have shown that the differences in blood cholesterol levels between groups given diets containing hydrogenated coconut oil or maize oil were associated neither with an increased bile acid excretion nor with an increased sterol excretion in the faeces.

It would seem unlikely that the relationship between blood cholesterol levels and type of dietary fat is the result of an effect of dietary fat on the absorption of cholesterol from the intestine, for most of the available evidence (e.g. Byers & Friedman, 1958) indicates that the inclusion in the diet of unsaturated fat results in a greater absorption of cholesterol than does the inclusion of an equivalent amount of saturated fat.

Yet another facet of this problem on which there is considerable uncertainty is the identity of the specific components in the various dietary fats that are responsible for the raising or lowering of blood cholesterol levels. Many investigators in this field (e.g. Kinsell, Michaels, Friskey & Splitter, 1958; Keys, Anderson & Grande, 1958; Malmros & Wigand, 1957; Ahrens, Insull, Hirsch, Stoffel, Peterson, Farquhar, Miller & Thomasson, 1959) concluded that the hypocholesterolaemic effect of a number of vegetable and fish oils was most probably due to the polyunsaturated acids contained in the saponifiable fraction of these oils. Nevertheless, Beveridge, Connell, Haust & Mayer (1959) maintain that the presence of plant sterols such as sitosterol or other substances in the unsaponifiable matter of maize oil accounts for much of its hypocholesterolaemic activity. Beveridge *et al.* (1959) and Funch, Kristensen & Dam

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(1962) consider it possible that the nature of the fatty acids in the saponifiable fraction and the presence of cholesterol in the non-saponifiable fraction both contribute to the hypercholesterolaemic effect of butterfat.

The results of our experiments showed that rabbits given diets containing 24% butter, 20% butterfat or 20% hydrogenated coconut oil developed extensive atheromatous degeneration of the aorta and coronary arteries. Rabbits given diets containing 20% maize oil developed little or no atherosis. These findings are in complete agreement with those reported by Lambert *et al.* (1958), Wigand (1959) Funch *et al.* (1960) and Gottenbos & Thomasson (1961) for rabbits given similar diets with no added cholesterol. The substitution of half of the butterfat by an equivalent amount of maize oil reduced the degree of atherosis in the majority of the rabbits in group 3 (Expt 2) to a level that was little different from that found in the rabbits given the diet containing 20% maize oil. Similar observations have been made by Funch *et al.* (1962). As far as we are aware, a direct comparison of the atherogenic effect of the non-polyunsaturated fatty acids of butterfat with that of an isocaloric amount of starch in diets containing no added cholesterol has not been reported previously by other investigators in this field. It was of interest therefore to find that the extent of aortic atherosis in the rabbits given the high-starch diets (group 5 of Expt 1 and group 4 of Expt 2) was about the same as in the rabbits given the diets containing 20% butterfat (group 3 of Expt 1 and group 2 of Expt 2). This finding does not lend any support to the opinion of Funch *et al.* (1962) that the small amount of cholesterol present in butterfat is partly responsible for its atherogenic effect. Thus it would appear that saturated fat (or more correctly non-polyunsaturated fat) *per se* does not exert a positive atherogenic effect and that the atherogenic properties of the high-starch and high-butterfat diets may be ascribed simply to their lack of polyunsaturated fatty acids. With experimental rabbits, therefore, it is tempting to consider whether the atherogenic property of a particular diet (containing no supplementary cholesterol) may possibly be determined by its polyunsaturated fatty acid:calorie ratio, irrespective of whether the major portion of the calories is supplied as fat or as carbohydrate. It would seem inappropriate to discuss this possible relationship any further until more information is available on the atherogenic properties of a variety of diets with widely differing polyunsaturated fatty acid: calorie ratios. Moreover, in view of the lack of fundamental knowledge on the basic nutrition of the rabbit, this tentative relationship must necessarily be put forward in its simplest terms, i.e. in terms of gross energy. Should this hypothesis receive any support from future experiments then it is likely that a more precise relationship might be found between the atherogenic properties of a diet and its digestible polyunsaturated fatty acid:digestible energy ratio or its digestible polyunsaturated fatty acid : metabolizable energy ratio. Since it is not impossible that the atherogenic effect of a particular diet may also be determined in part by the level of intake, the influence of food restriction on the atherogenic properties of various diets is also a problem worthy of investigation. It is essential to note that, unlike the diets used in the experiments of Malmros & Wigand (1959), the diets used in our study were not deficient in essential fatty acids. The only diet that did not contain any maize oil whatsoever was that given to the rabbits of group 4 in Expt 1 and even this diet was not completely

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devoid of linoleic acid (Moore & Williams, 1963). Fatty acid analysis of the lipids extracted from the various tissues of the experimental rabbits (Moore & Williams, 1963 and unpublished observations) has certainly revealed no evidence of essential fatty acid deficiency. For instance, the fatty acids present in the adipose tissues of the rabbits given the 20% hydrogenated coconut-oil diet contained as much as 20% (molar) of linoleic acid.

The relationship between the concentration of the various plasma lipids and the extent of atheromatous degeneration in the rabbits of both experiments will be discussed fully in a communication to be submitted later. At present it is perhaps sufficient to point out that the relationship between plasma cholesterol levels and atheromatous degeneration, if indeed such a relationship exists, is by no means a simple one. Admittedly, high plasma cholesterol levels were associated with extensive atherosis in the rabbits given the high-butter or high-butterfat diets and low plasma cholesterol levels were associated with a low incidence of aortic atherosis in the rabbits given the high-maize-oil diets. On the other hand, the plasma cholesterol levels observed for the rabbits of groups 3 and 4 in Expt 2 were identical, yet there was a pronounced difference in the degree of atheromatous involvement in these two groups of rabbits.

SUMMARY

1. The main purpose of the experiments with rabbits now reported was to compare the hypercholesterolaemic and atherogenic effects of diets containing butterfat with those containing a mixture of maize oil and starch that supplied amounts of linoleic acid and calories equivalent to those supplied by the dietary butterfat. The hypercholesterolaemic and atherogenic effects of diets containing butterfat were also compared with those containing maize oil, hydrogenated coconut oil and mixtures of maize oil and butterfat.

2. In Expt 1, groups of male rabbits (six to eight per group) were given *ad lib.* for a period of 31 weeks a diet consisting of 80 parts of a basal ration to which were added: for group 1, 20 parts of maize oil; for group 2, 24 parts of butter; for group 3, 20 parts of butterfat; for group 4, 20 parts of hydrogenated coconut oil; and for group 5, 1 part of maize oil and 42 parts of wheat starch. In Expt 2, groups of male rabbits (ten to thirteen per group) were given *ad lib.* for a period of 38 weeks a diet consisting of 80 parts of the basal ration to which were added: for group 1, 20 parts of maize oil; for group 2, 20 parts of butterfat; for group 3, 10 parts of maize oil and 10 parts of butterfat; for group 4, 0·47 parts of maize oil and 43 parts of wheat starch, and for group 5, 10·2 parts of maize oil and 21·6 parts of wheat starch.

3. Plasma cholesterol levels were determined in all the rabbits at various intervals during both experiments. At the end of the experiments the animals were killed and the degree of atheromatous degeneration was determined after the aortas had been stained with Sudan IV. The lesions found in the aorta and coronary arteries were also examined histologically. The livers, hearts and kidneys of the rabbits in Expt 1 were analysed for cholesterol.

4. Elevated levels of plasma cholesterol and extensive atheromatous degeneration

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were observed in the rabbits given the diets containing 24% butter, 20% butterfat, or 20% hydrogenated coconut oil. Extensive atheromatous degeneration was also observed in the aortas of the rabbits given the high-starch diets although only a moderate degree of hypercholesterolaemia was observed in these animals. The replacement of half of the butterfat by maize oil resulted in a pronounced decrease both in the plasma cholesterol levels and in the incidence and severity of the atheromatous lesions. A similar reduction in plasma cholesterol levels and in the degree of aortic atherosis was also noted when half of the supplementary starch was replaced by an isocaloric amount of maize oil.

5. There was no simple relationship between plasma cholesterol level and aortic atherosis since the plasma cholesterol levels of the rabbits given the high-starch diet (extensive atherosis) were exactly the same as those of the rabbits given the diet containing 10% maize oil and 10% butterfat (little atherosis).

6. The levels of cholesterol in the livers, hearts and kidneys of the rabbits in Expt 1 were not consistent with the suggestion that the raising or lowering of blood cholesterol levels by different dietary fats is simply due to a change in the partition of the cholesterol between plasma and tissues.

7. The results of these experiments are discussed and it is tentatively suggested that the polyunsaturated fatty acid:calorie ratio of the diet might be of considerable importance in determining its atherogenic properties.

We gratefully thank Dr S. K. Kon for his help and encouragement, Miss J. Carrinci, Mr E. V. Ruby and Mr G. Quelch for their valuable technical assistance, Dr W. G. Siller and Dr F. Hampson for their advice in the interpretation of the histological sections and Mr D. R. Westgarth for his suggestions regarding the presentation of the results. These studies were supported by a grant from the Butter Information Council.

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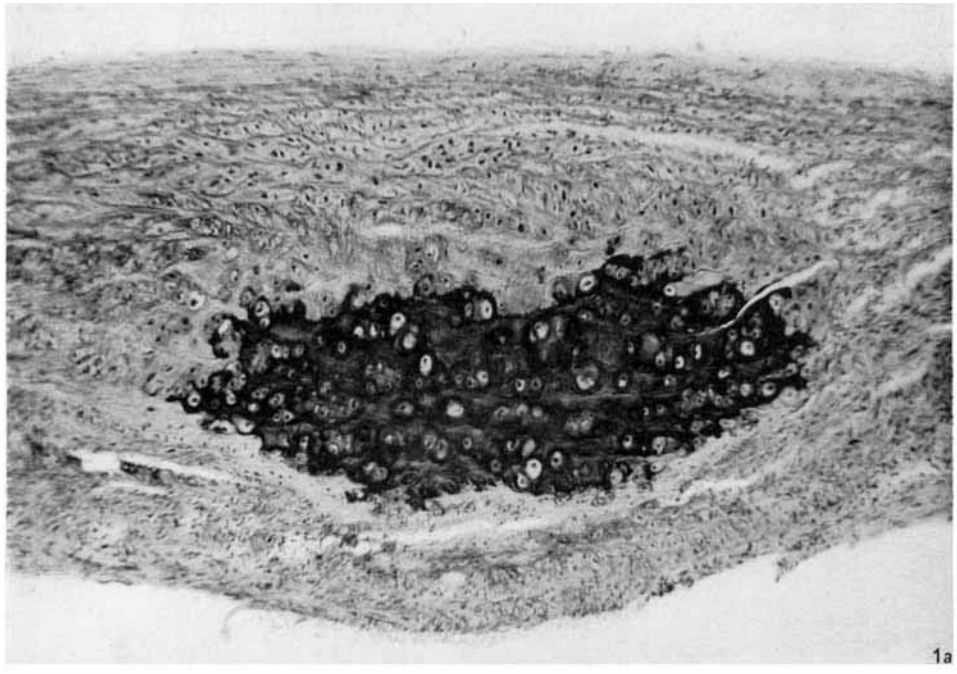

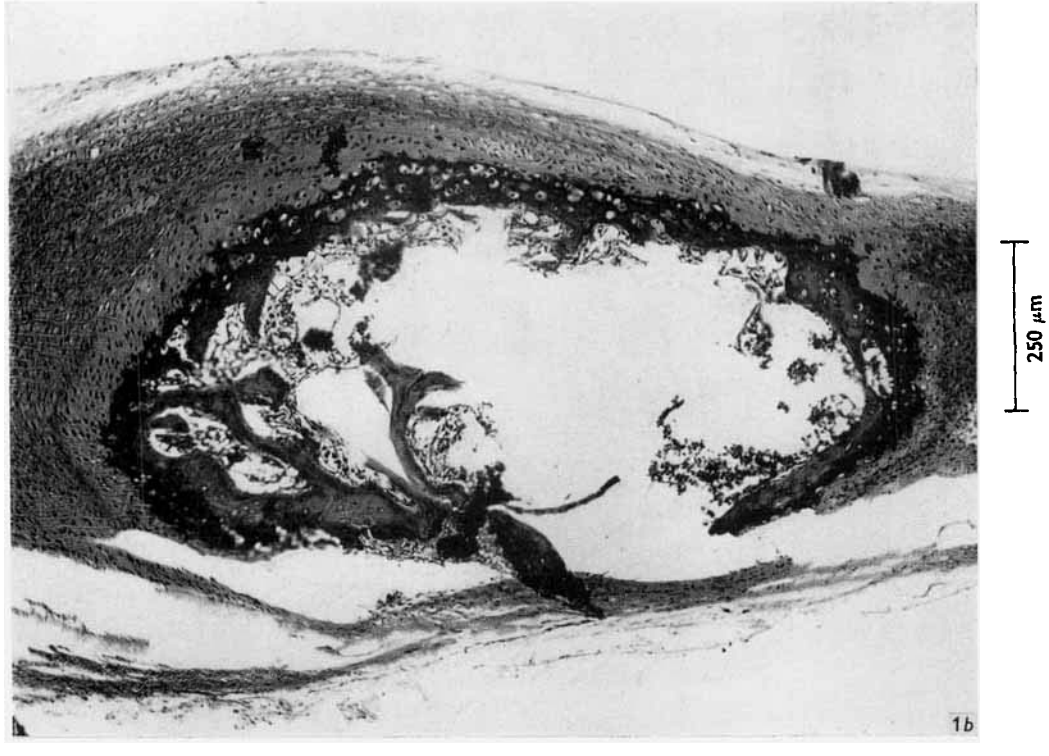

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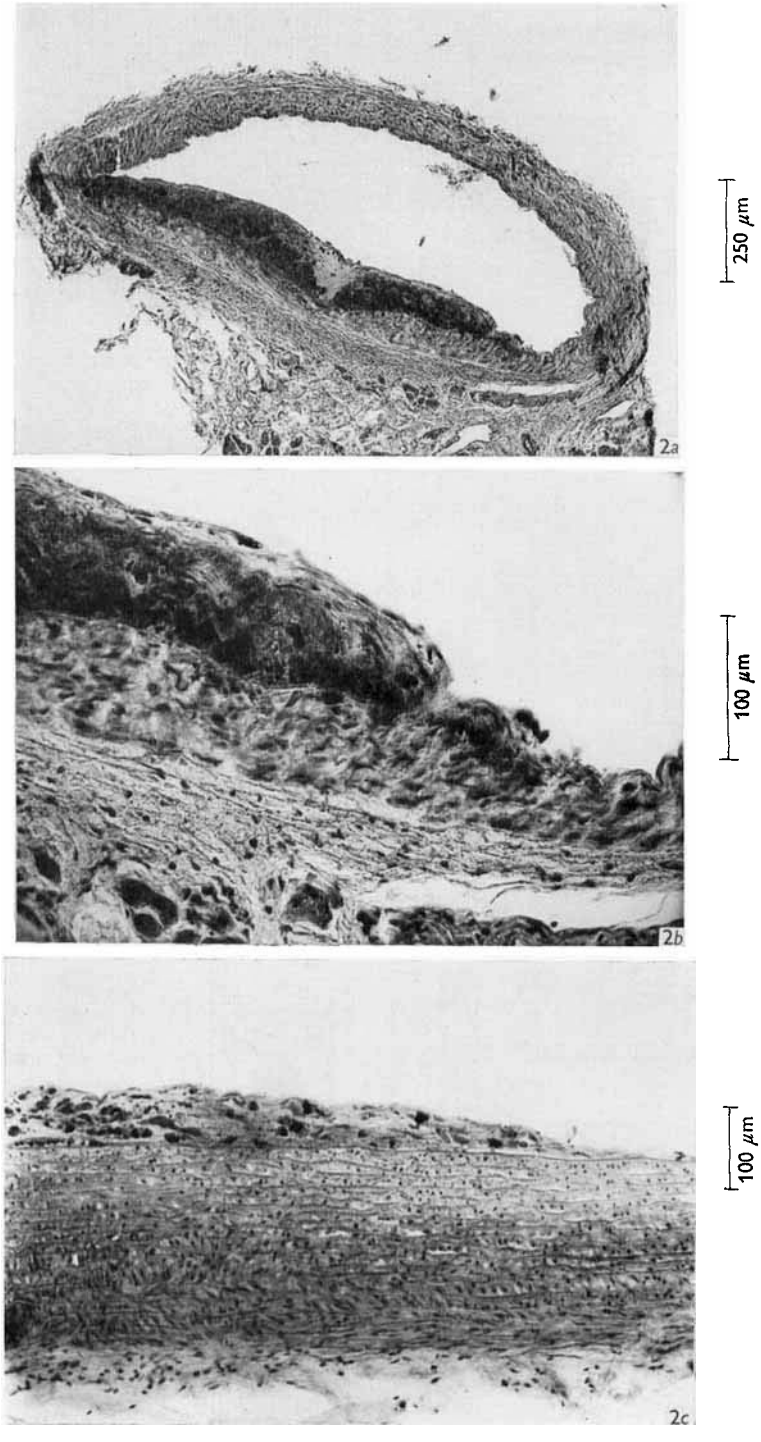

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EXPLANATION OF PLATES

Plate 1

Photomicrographs of transverse sections cut on a freezing microtome of the aortic arch of a rabbit given the 20% butterfat diet (Expt 1, group 3).

(*a*) Earlier stages of sclerotic degeneration in the media. Haematoxylin and eosin.

(*b*) More advanced stage of medial sclerosis. Weigert and van Gieson's stain.

Plate 2

Photomicrographs of sections cut on a freezing microtome of tissues of a rabbit given the high-starch diet (Expt 1, group 5). Haematoxylin, eosin and Sudan IV.

(*a*) Transverse section of a large coronary artery showing an atheromatous plaque projecting into the lumen.

(*b*) Detail of section (*a*). The dense lipid accumulation between the internal elastic membrane and the layer of endothelial cells is clearly evident.

(*c*) Longitudinal section of the aorta showing a typical atheromatous plaque.

Printed in Great Britain

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