Br. J. Nutr. (1966), **20**, 571

The effect of an atherogenic diet on plasma lipid composition and aortic atherosis in two strains of New Zealand White rabbit

By J. H. MOORE* and D. L. WILLIAMS†

National Institute for Research in Dairying, Shinfield, Reading

(*Received* 16 *November* 1965—*Accepted* 23 *December* 1965)

1. Susceptibility to dietary induction of hypercholesterolaemia and aortic atherosis was compared in two groups of male New Zealand White rabbits. 2. Twelve rabbits were purchased from one breeding establishment (group 1) and twelve from another (group 2). On arrival at the laboratory six animals from each group were killed and the aortas were removed. Blood samples were taken from the remaining twelve animals and then they were given *ad lib.* for a period of 40 weeks an atherogenic diet containing 20 % butterfat. During this period food intake and body-weight were recorded. At the end of the period blood samples were taken from the animals and immediately afterwards they were killed and the aortas were removed. 3. No atheromatous lesions were found in the aortas of the rabbits in either group killed at the beginning of the experiment. There were no differences between the two groups of animals with respect to body-weight or concentration of cholesterol in the plasma at the beginning of the experiment, food intake during the experiment or body-weight at the end of the experiment. At the end of the experiment, the degree of aortic atherosis in the rabbits of group 1 was considerably greater than that in the rabbits of group 2. 4. At the end of the experiment the concentrations of total lipids, free cholesterol, esterified cholesterol and phospholipids in the plasma of the rabbits in group 1 were significantly higher than the corresponding concentrations of these lipid components in the plasma of the rabbits in group 2. The concentrations of palmitic, stearic and linoleic acids in the cholesterol esters and the concentration of palmitic acid in the unesterified fatty acids in the plasma of the rabbits in group 2 were significantly higher than the corresponding concentrations of these fatty acids in the plasma cholesterol esters and unesterified fatty acids in the rabbits of group 1. 5. It is concluded that these differences in response to the atherogenic diet were reflections of the differences in the susceptibilities to the dietary induction of hypercholesterolaemia and atherosis of the two differents strain of rabbit that had been established by the two commercial breeders. Such differences in susceptibility could readily explain certain discrepancies in the results of various research workers engaged in this field of investigation.

The work of Lambert, Miller, Olson & Frost (1958), Wigand (1959), Funch, Krogh & Dam (1960), Gottenbos & Thomasson (1961), Moore & Kon (1963) and Moore & Williams (1964*a*) has shown that rabbits develop hypercholesterolaemia and aortic atherosis when given purified or semi-purified diets with no added cholesterol, but containing certain of the more saturated 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. In spite of the general agreement on the qualitative aspects of the relationships between the type of dietary fat, blood lipid levels and cardiovascular degeneration in rabbits, there appear to be marked differences in the quantitative aspects of these relationships that have been reported from different laboratories. For instance, Funch *et al.* (1960) gave rabbits for 42 weeks a diet containing 20 % butterfat and observed that the mean concentration of cholesterol in the serum during the last

* Present address: Hannah Dairy Research Institute, Ayr.
† Present address: Department of Biochemistry, Johnston Laboratories, Liverpool 3.

20 weeks of the experiment was 658 mg/100 ml. In a similar experiment Moore & Williams (1964*a*) gave rabbits for 38 weeks a diet containing 20% butterfat, but found that the mean concentration of cholesterol in the plasma during the last 20 weeks of the experiment was only 140 mg/100 ml. It was also clear that the degree of aortic degeneration observed by Funch *et al.* (1960) in rabbits given the diet containing 20% butterfat was considerably greater than that observed by Moore & Williams (1964*a*) in rabbits given a similar diet for a similar period. Marked differences in the response to atherogenic diets of similar composition have been observed in separate experiments with rabbits carried out at different times in the same laboratory. Funch, Kristensen & Dam (1962) gave rabbits for 38 weeks a diet again containing 20% butterfat, but on this occasion found that the mean concentration of cholesterol in the serum during the last 20 weeks of the experiment was 270 mg/100 ml. In all three of these investigations (Funch *et al.* 1960; 1962; Moore & Williams, 1964*a*) the general experimental procedures and the compositions of the semi-purified basal diets, although not identical, were very similar, as were the ages and weights of the rabbits at the beginning of the experiments and the gains in weight during the experiments. However, Funch *et al.* (1960) used 'albino rabbits of the so-called country breed' in their experiments, whereas Funch *et al.* (1962) used 'albinos and hybrids' and Moore & Williams (1964*a*) used New Zealand White rabbits. It seemed reasonable to contend therefore that certain breeds or strains of rabbit might respond more readily than others to atherogenic diets. Support for this contention has recently been obtained in an investigation in which it has been possible to compare the effects of an atherogenic diet on two different strains of New Zealand White rabbit. The results of this investigation are now reported.

EXPERIMENTAL

Rabbits, diets and experimental procedure. Twenty-four male New Zealand White rabbits were obtained at the age of 6 months and at the same time from two local breeders, twelve from one breeder (group 1) and twelve from the other (group 2). The general appearance of the rabbits in group 1 was similar to but not identical with that of the animals in group 2. It was, in fact, a relatively simple matter to distinguish the rabbits in group 1 by their characteristically heavier facial features. On arrival at the laboratory six of the animals from each group were killed by a blow on the head. As rapidly as possible thereafter the aorta, extending to the point of division into the two common iliac arteries, was removed from each rabbit. The remaining twelve animals were housed in individual cages and were given a commercial rabbit diet for a short period until they became accustomed to their surroundings. The commercial diet was then gradually replaced by the experimental diet during a 4-week period, by which time all the rabbits were readily consuming the experimental diet. The experimental diet was then given to the animals for a period of 40 weeks (experimental period). Throughout the whole experiment the rabbits were given food and water *ad lib.*

The percentage composition of the experimental diet was: butterfat 20, wheat starch (Starch Products Ltd, Slough, Bucks) 16·3, sucrose 10, casein (Lactic acid casein; Glaxo Research Ltd, Greenford, Middx) 25·0, Cellophane Spangles (British

Cellophane Ltd, Bridgwater, Somerset) 19·0, methyl cellulose (Celacol M 450; J. M Steel and Co Ltd, London, WC2) 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. The methods of preparing the butterfat and pelleting the diet have been given by Moore & Williams (1964*a*), as have the compositions of the salt and vitamin mixtures.

The rabbits were weighed at the beginning of the experimental period and at fortnightly intervals thereafter. At the beginning of the experimental period a small sample of blood was taken from the marginal ear vein of each rabbit for the determination of plasma cholesterol levels. A record was made of the total amount of diet consumed by the two groups of rabbits during the experiment. At the end of the experiment, large samples of blood were taken from the marginal ear veins of the rabbits and immediately afterwards the animals were killed and the aortas were removed as described above.

Treatment of tissues and methods of analysis. After the removal of adventitious fatty tissues, the aortas were fixed and stained by the methods described previously (Moore & Williams, 1964*a*). The degree of atheromatous degeneration of the intimal surface of each aorta was then assessed by the technique of Moore & Williams (1964*a*), account being taken of the area covered and the intensity of the Sudan IV staining of the atheromatous lesions. Each aorta was compared with the 'standard' atheromatous aorta referred to previously (Moore & Williams, 1964*a*).

The concentrations of total cholesterol in the small samples of plasma were determined by a combination of the methods of Abell, Levy, Brodie & Kendall (1952) and Brown (1959) as described previously (Moore & Williams, 1964*a*). The lipids were extracted from the large plasma samples by the method of Nelson & Freeman (1959) and the total lipid contents of the extracts were determined gravimetrically. The lipids were fractionated on columns of silicic acid by the procedures described in detail by Moore & Doran (1962) and Moore & Williams (1964*b*). The various fractions obtained from the columns of silicic acid were analysed for glyceride glycerol, cholesterol, phosphorus and unesterified fatty acids by the methods used previously (Moore, 1962; Moore & Doran, 1962; Moore & Williams, 1963). Weights of triglycerides and unesterified fatty acids were calculated as triolein and oleic acid respectively, and weights of phospholipid were obtained by multiplying the phosphorus values by 25 (Wittcoff, 1951). The compositions of the fatty acids present in the various plasma lipids were determined by gas–liquid chromatography (Moore & Williams, 1963, 1964*c*).

Statistical analysis. From a preliminary examination of the results it was evident that the standard deviation between the animals in each group tended to be proportional to the group mean. Consequently, all observational values were transformed to logarithms in order to reduce heterogeneity among the within-group variances. The *t* tests summarized in Tables 2–6 refer to differences between the mean logarithmic values of each group. These mean logarithmic values are presented in Tables 2–6 as their antilogarithms which are the geometric means for each group. The arithmetic means of the untransformed values are also given in Tables 2–6.

RESULTS

There was no evidence of any atheromatous degeneration in the aortas of the six rabbits from each group that were killed on arrival at the laboratory. The results given in Table 1 show that the weights of the animals at the beginning of the experimental period and the gains in weight during the experiment were very similar for both groups of rabbits. There were no differences between the two groups of rabbits either in the

Table 1. *Weights of rabbits at the beginning and end of the experiment, daily dry-matter intakes during the experiment, concentrations of cholesterol in the plasma at the beginning of the experiment and degrees of atherosis in the aortas at the end of the experiment*

	Group 1	Group 2
Weight of rabbits at beginning of expt* (kg)	2·60 ± 0·14	2·55 ± 0·11
Weight of rabbits at end of expt* (kg)	3·48 ± 0·13	3·44 ± 0·13
Dry-matter intake (g/rabbit)	92	93
Plasma cholesterol level* (mg/100 ml)	48·1 ± 4·1	46·5 ± 5·0
Degree of atherosis*†	55·0 ± 7·6	13·3 ± 5·7

* Mean values with their standard errors.
† Arbitrary scale (see Moore & Williams, 1964*a*).

Table 2. *Concentrations (mg/100 ml) of lipid components in the plasma of two strains of rabbits given a 20% butterfat diet*

	Group 1 (*a*)	Group 2 (*b*)	*a* − *b*	Standard error of *a* − *b* (10 df), log units
Total lipid:				
Arithmetic mean	1070	402	668	—
Geometric mean	923	374	549	—
Mean log	2·96	2·57	0·39**	± 0·12
Free cholesterol:				
Arithmetic mean	87·8	28·9	58·9	—
Geometric mean	75·7	24·7	51·0	—
Mean log	1·88	1·39	0·49**	± 0·15
Esterified cholesterol:				
Arithmetic mean	249	62·0	187	—
Geometric mean	205	52·4	153	—
Mean log	2·31	1·72	0·59**	± 0·17
Triglycerides:				
Arithmetic mean	104	45·4	58·6	—
Geometric mean	84·1	42·9	41·2	—
Mean log	1·93	1·63	0·30	± 0·14
Phospholipids:				
Arithmetic mean	443	207	236	—
Geometric mean	378	196	182	—
Mean log	2·58	2·29	0·29*	± 0·12
Unesterified fatty acids:				
Arithmetic mean	17·0	10·5	6·5	—
Geometric mean	15·0	9·6	5·4	—
Mean log	1·18	0·98	0·20	± 0·14

* $P < 0.05$. ** $P < 0.01$.

amount of food consumed during the experiment or in the concentration of cholesterol in the plasma at the beginning of the experiment (Table 1). At the end of the experiment, however, the degree of atheromatous degeneration of the aortas in the rabbits of group 1 was much greater than that in the rabbits of group 2 (Table 1). In the aortas of the rabbits in group 1, the proportion of the total intimal surface area covered by

Table 3. *Fatty acid compositions (weight percentages) of the cholesterol esters in the plasma of two strains of rabbits given a 20% butterfat diet*

	Group 1 (*a*)	Group 2 (*b*)	*a* − *b*	Standard error of *a* − *b* (10 df), log units
Myristic acid:				
Arithmetic mean	0·9	1·3	−0·4	—
Geometric mean	0·9	1·2	−0·3	—
Mean log	−0·033	0·078	−0·11	±0·060
Palmitic acid:				
Arithmetic mean	18·1	21·8	−3·7	—
Geometric mean	18·0	21·5	−3·5	—
Mean log	1·25	1·33	−0·080*	±0·036
Palmitoleic acid:				
Arithmetic mean	3·5	3·4	0·1	—
Geometric mean	3·5	3·4	0·1	—
Mean log	0·54	0·53	0·013	±0·038
Stearic acid:				
Arithmetic mean	5·9	8·2	−2·3	—
Geometric mean	5·8	7·9	−2·1	—
Mean log	0·76	0·89	−0·14*	±0·059
Oleic acid:				
Arithmetic mean	59·9	49·4	10·5	—
Geometric mean	59·8	48·3	11·5	—
Mean log	1·78	1·68	0·093	±0·043
Linoleic acid:				
Arithmetic mean	5·6	10·4	−4·8	—
Geometric mean	5·2	9·2	−4·0	—
Mean log	0·72	0·97	−0·25*	±0·068

* $P < 0·05$.

atheromatous lesions was considerably greater than in the aortas of the animals in group 2, but the most pronounced difference between the two groups of aortas was observed in the thickness or depth of the lesions. The thickness of the lesions of the aortas in group 1 was two to three times greater than that of the lesions of the aortas in group 2.

The concentrations of the various lipid components in the plasma of the two groups of rabbits are given in Table 2. It is clear from these results and those given in Table 1 that the butterfat diet exerted a more marked hypercholesterolaemic effect in the rabbits of group 1 than in those of group 2. Thus, in group 1 the total cholesterol level in the plasma had risen during the experimental period from 48 to 337 mg/100 ml but in group 2 the corresponding rise in the level of plasma total cholesterol was from 46 to only 91 mg/100 ml. The concentrations of total lipid, free cholesterol and esterified cholesterol in the plasma of the animals in group 1 were significantly higher ($P < 0·01$) than the corresponding concentrations of these lipids in the plasma of the animals in

Table 4. *Fatty acid compositions (weight percentages) of the triglycerides in the plasma of two strains of rabbits given a 20% butterfat diet*

	Group 1 (*a*)	Group 2 (*b*)	*a*−*b*	Standard error of *a*−*b* (10 df), log units
Myristic acid:				
Arithmetic mean	5·2	5·0	0·2	—
Geometric mean	5·1	4·3	0·8	—
Mean log	0·71	0·63	0·077	±0·130
Palmitic acid:				
Arithmetic mean	34·7	36·2	−1·5	—
Geometric mean	34·4	36·1	−1·7	—
Mean log	1·54	1·56	−0·022	±0·034
Palmitoleic acid:				
Arithmetic mean	2·8	2·5	0·3	—
Geometric mean	2·7	2·5	0·2	—
Mean log	0·43	0·40	0·026	±0·051
Stearic acid:				
Arithmetic mean	9·7	10·6	−0·9	—
Geometric mean	9·3	10·3	−1·0	—
Mean log	0·97	1·01	−0·040	±0·078
Oleic acid:				
Arithmetic mean	35·3	31·6	3·7	—
Geometric mean	34·6	31·2	3·4	—
Mean log	1·54	1·49	0·045	±0·051
Linoleic acid:				
Arithmetic mean	4·3	6·1	−1·8	—
Geometric mean	4·1	5·9	−1·8	—
Mean log	0·61	0·77	−0·164	±0·079

Table 5. *Fatty acid compositions (weight percentages) of the phospholipids in the plasma of two strains of rabbits given a 20% butterfat diet*

	Group 1 (*a*)	Group 2 (*b*)	*a*−*b*	Standard error of *a*−*b* (10 df), log units
Palmitic acid:				
Arithmetic mean	25·2	25·5	−0·3	—
Geometric mean	25·1	25·5	−0·4	—
Mean log	1·40	1·41	−0·006	±0·018
Palmitoleic acid:				
Arithmetic mean	0·9	0·7	0·2	—
Geometric mean	0·9	0·7	0·2	—
Mean log	−0·06	−0·15	0·087	±0·048
Stearic acid:				
Arithmetic mean	16·4	17·8	−1·4	—
Geometric mean	16·3	17·8	−1·5	—
Mean log	1·21	1·25	−0·037	±0·019
Oleic acid:				
Arithmetic mean	30·9	28·0	2·9	—
Geometric mean	30·8	27·9	2·9	—
Mean log	1·49	1·45	0·044	±0·020
Linoleic acid:				
Arithmetic mean	18·5	20·2	−1·7	—
Geometric mean	18·4	20·0	−1·6	—
Mean log	1·27	1·30	−0·037	±0·028
Arachidonic acid:				
Arithmetic mean	3·2	2·7	0·5	—
Geometric mean	3·1	2·6	0·5	—
Mean log	0·49	0·42	0·079	±0·060

group 2 (Table 2). With respect to the concentrations of triglycerides and unesterified fatty acids, the differences between the two groups of rabbits did not achieve the 5% level of significance, but the plasma phospholipid level in group 1 was significantly higher than that in group 2 ($P < 0.05$).

Table 6. *Compositions (weight percentages) of the unesterified fatty acids in the plasma of two strains of rabbits given a 20% butterfat diet*

	Group 1 (*a*)	Group 2 (*b*)	*a*−*b*	Standard error of *a*−*b* (10 df), log units
Myristic acid:				
Arithmetic mean	6·0	5·8	0·2	—
Geometric mean	5·9	5·8	0·1	—
Mean log	0·77	0·76	0·009	±0·038
Palmitic acid:				
Arithmetic mean	33·6	39·2	−5·6	—
Geometric mean	33·4	39·0	−5·6	—
Mean log	1·52	1·59	−0·067*	±0·027
Palmitoleic acid:				
Arithmetic mean	2·3	2·0	0·3	—
Geometric mean	2·2	1·9	0·3	—
Mean log	0·34	0·29	0·049	±0·076
Stearic acid:				
Arithmetic mean	15·5	16·5	−1·0	—
Geometric mean	15·4	16·4	−1·0	—
Mean log	1·19	1·22	−0·029	±0·028
Oleic acid:				
Arithmetic mean	22·3	19·5	2·8	—
Geometric mean	21·2	19·3	1·9	—
Mean log	1·33	1·29	0·041	±0·067
Linoleic acid:				
Arithmetic mean	2·1	4·1	−2·0	—
Geometric mean	2·0	3·3	−1·3	—
Mean log	0·29	0·51	−0·219	±0·138

* $P < 0.05$.

In addition to the differences in the absolute concentrations of the various lipid components in the plasma, it should be noted that there were also differences in the composition of the total plasma lipids in the two groups of animals. Total cholesterol constituted 32% of the total plasma lipids in group 1 but only 23% in group 2. On the other hand, phospholipid accounted for 52% of the total plasma lipids in group 2 but for only 41% in group 1. The ratios, phospholipid to total cholesterol in the plasma of the rabbits in groups 1 and 2 were 1·3 and 2·3 respectively, and of the total plasma cholesterol, 74% occurred in the esterified form in group 1 compared with 68% in group 2.

The fatty acid compositions of the cholesterol esters, triglycerides, phospholipids and unesterified fatty acids in the plasma of the two groups of rabbits are given in Tables 3, 4, 5 and 6 respectively. The concentrations of palmitic, stearic and linoleic acids in the plasma cholesterol esters of the rabbits in group 2 were significantly higher ($P < 0.05$) than the corresponding concentrations of these acids in the plasma cholesterol esters in group 1 (Table 3). The lower concentrations of palmitic, stearic and

linoleic acids in the plasma cholesterol esters in group 1 appeared to be counterbalanced to some extent by a somewhat higher concentration of oleic acid, but the difference between the concentrations of oleic acid in the cholesterol esters of groups 1 and 2 did not achieve the 5% level of significance (Table 3). There were no significant differences between the two groups of rabbits with respect to the fatty acid compositions of the plasma triglycerides and phospholipids (Tables 4 and 5) but the concentration of palmitic acid in the plasma unesterified fatty acids of the rabbits in group 2 was significantly higher ($P < 0.05$) than the concentration of this acid in the plasma unesterified fatty acids in group 1.

DISCUSSION

The results of this investigation show clearly that the two groups of rabbits differed markedly in their response to the atherogenic diet. It seems almost certain that this difference in response was a reflection of the difference in the susceptibility to the dietary induction of hypercholesterolaemia and atherosis of the two different strains of New Zealand White rabbit that had been established by the two commercial breeders. As far as we were able to judge, the housing, nutrition and management of the breeding does and young rabbits were very similar at both breeding establishments. It should be mentioned, however, that different commercial rabbit diets were used at the two establishments, but analyses in our laboratory of samples of these two diets revealed little difference between them with respect to their content of the individual fatty acids listed in Tables 3–6 and crude protein, crude fibre, ash and nitrogen-free extract. When different strains of the same breed of rabbit differ so noticeably in their susceptibility to the dietary induction of hypercholesterolaemia and atherosis it is not surprising that certain discrepancies have appeared in the results of various research workers engaged in this field of investigation.

The greater degree of aortic atherosis in the rabbits of group 1 was associated with a much greater degree of hypercholesterolaemia, and at the end of the experiment the concentration of total cholesterol in the plasma of the animals in group 1 was nearly four times that in the plasma of the animals in group 2. Although it may be mere coincidence, it should perhaps be noted that the degree of aortic atherosis in the rabbits of group 1 was also about four times greater than that in the rabbits of group 2. Nevertheless, the butterfat diet did not appear to be without effect on the concentration of cholesterol in the plasma of the rabbits of group 2. Moore & Williams (1964*a*) have shown that when rabbits, 6 months old at the beginning of the experiment, were given a commercial diet for 9 months there was no development of atheromatous lesions in the aorta, and the concentration of cholesterol in the plasma did not change appreciably during the experiment from a level of about 48 mg/100 ml. At the end of the experiment now reported, the concentration of cholesterol in the plasma of the rabbits in group 2 (Table 2) was almost twice this level. Attention should be drawn to the fact that the concentration of linoleic acid in the plasma cholesterol esters in group 2 was about twice the concentration of this acid in the plasma cholesterol esters in group 1 (Table 3). Moore & Williams (1965) have presented evidence indicating that the extent to which the plasma cholesterol is esterified with linoleic acid may be

an important factor in controlling the concentration of cholesterol in the plasma of the rabbit.

At present it is difficult to explain why these two strains of New Zealand White rabbits differed so markedly in their response to the atherogenic diet containing 20% butterfat. Since it has been firmly established that the liver is the major source of the lipids circulating in the plasma (Haft, Roheim, White & Eder, 1962; Havel, Felts & Van Duyne, 1962; Roheim, Haft, Gidez, White & Eder, 1963; Marsh, 1963), it is possible that the butterfat diet induced a higher rate of lipoprotein synthesis in the livers of the rabbits in group 1 than it did in the livers of the rabbits in group 2. Comparative studies on the rates of cholesterol synthesis and the utilization of linoleic acid for cholesterol ester synthesis in the livers of these two strains of rabbits under different dietary regimes might yield rewarding results. It is also possible that there were differences between the two groups of rabbits with respect to the facility with which endogenous cholesterol was eliminated from the body. Such differences would be evident from an investigation into the excretion of sterols and bile acids in the faeces of the two strains of rabbits when they were given the butterfat diet. The more extensive atheromatous degeneration of the aortas in the rabbits of group 1 could have been due to a greater derangement of metabolism within the arterial tissues of this group of animals. On the other hand, this higher degree of aortic atherosis might simply have been a reflection of the grossly elevated levels of plasma lipids in the rabbits of group 1.

As far as we are aware there is no other information in the literature on the comparative susceptibility to atherosclerosis of different breeds or strains of rabbits, but pronounced differences have been reported in the incidence of atheromatous lesions in the aorta and coronary arteries of various breeds of pigeons. Clarkson, Prichard, Netsky & Lofland (1959) found that intimal lesions covered about 10% of the total surface area of the thoracic aorta in three breeds of pigeons (Autosexing Kings, Silver Kings and White Carneaux), whereas the thoracic aortas of two other breeds (Racing Homers and Show Racers) were almost devoid of such lesions. All birds had been given a commercial pigeon diet. Contrary to our observations with the two strains of rabbits, Lofland & Clarkson (1959, 1960) found that the occurrence of atheromatous plaques in the aortas of the susceptible breeds of pigeons was associated neither with elevated levels of total lipids, free cholesterol, esterified cholesterol, phospholipids and β-lipoproteins in the serum nor with changes in the pattern of β-lipoproteins in the serum. These findings suggested that in these breeds of pigeons, the difference in susceptibility to atheromatous degeneration of the aorta is associated solely with some difference in the structure or metabolism of the arterial tissues. In fact, from experiments with cultures of aortic cells that had been established from the intimal tissues of White Carneau (susceptible) and Show Racer (resistant) pigeons, Smith, Strout, Dunlop & Smith (1965) have concluded that there is a metabolic defect in the synthesis of arachidonic acid by the aortic cells of the White Carneau birds.

The authors gratefully acknowledge the encouragement and advice given by Professor S. K. Kon and thank Mr D. R. Westgarth for advice on the statistical

analysis and presentation of the results, and Miss J. Carrinci, Mr E. V. Ruby and Mr G. Quelch for skilled technical assistance. This study was supported by a grant from the Butter Information Council.

REFERENCES

Abell, L. L., Levy, B. B., Brodie, B. B. & Kendall, F. E. (1952). *J. biol. Chem.* **195**, 357.
Brown, W. D. (1959). *Aust. J. exp. Biol. med. Sci.* **37**, 523.
Clarkson, T. B., Prichard, R. W., Netsky, M. G. & Lofland, H. B. (1959). *Archs. Path.* **68**, 143.
Funch, J. P., Kristensen, G. & Dam, H. (1962). *Br. J. Nutr.* **16**, 497.
Funch, J. P., Krogh, B. & Dam. H. (1960). *Br. J. Nutr.* **14**, 355.
Gottenbos, J. J. & Thomasson, H. J. (1961). *Colloques int. Cent. natn. Rech. scient.* no. 99, p. 221.
Haft, D. E., Roheim, P. S., White, A. & Eder, H. A. (1962). *J. clin. Invest.* **41**, 842.
Havel, R. J., Felts, J. M. & Van Duyne, C. M. (1962). *J. Lipid Res.* **3**, 297.
Lambert, G. F., Miller, J. P., Olsen, R. T. & Frost, D. V. (1958). *Proc. Soc. exp. Biol. Med.* **97**, 544.
Lofland, H. B. & Clarkson, T. B. (1959). *Circulation Res.* **7**, 234.
Lofland, H. B. & Clarkson, T. B. (1960). *Proc. Soc. exp. Biol. Med.* **103**, 238.
Marsh, J. B. (1963). *J. biol. Chem.* **238**, 1752.
Moore, J. H. (1962). *J. Dairy Res.* **29**, 141.
Moore, J. H. & Doran, B. M. (1962). *Biochem. J.* **84**, 506.
Moore, J. H. & Kon, S. K. (1963). *Chemy Ind.* p. 165.
Moore, J. H. & Williams, D. L. (1963). *Can. J. Biochem. Physiol.* **41**, 1821.
Moore, J. H. & Williams, D. L. (1964*a*). *Br. J. Nutr.* **18**, 253.
Moore, J. H. & Williams, D. L. (1964*b*). *Br. J. Nutr.* **18**, 431.
Moore, J. H. & Williams, D. L. (1964*c*). *Biochim. biophys. Acta* **84**, 41.
Moore, J. H. & Williams, D. L. (1965). *Br. J. Nutr.* **19**, 407.
Nelson, G. J. & Freeman, N. K. (1959). *J. biol. Chem.* **234**, 1375.
Roheim, P. S., Haft, D. E., Gidez, L. I., White, A. & Eder, H. A. (1963). *J. clin. Invest.* **42**, 1277.
Smith, S. C., Strout, R. G., Dunlop, W. R. & Smith, E. C. (1965). *J. Atheroscler. Res.* **5**, 379.
Wigand, G. (1959). *Acta med. scand.* **166**, Suppl. no. 351.
Wittcoff, H. (1951). *The Phosphatides*, p. 171. New York: Reinhold Publishing Corp.

Printed in Great Britain