The Metabolic Modification of Low-Density Lipoproteins in Normal and Hypercholesterolaemic Guinea Pigs

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(Received 27 February 1978)

1. Low-density lipoproteins were isolated by ultracentrifugation from the serum of guinea pigs that were fed either on a normal diet, or on a diet supplemented with corn oil and cholesterol. 2. After labelling with tracer amounts of radioactive iodine, these lipoproteins were injected into the bloodstream of guinea pigs that were fed either on the normal or on the supplemented diet. 3. In all cases, the density of the labelled lipoproteins was increased by exposure for 24–48 h to the metabolic processes of the guinea pig. 4. The final density reached by lipoproteins isolated from fat-fed guinea pigs was less than that reached by lipoproteins from normal animals. 5. Fat-fed guinea pigs were unable to increase the density of either normal lipoproteins, or those from fat-fed guinea pigs, to the same extent as animals fed on the normal diet. 6. It is concluded that the lipid-rich diet brings about a modification of lipoprotein metabolism in the guinea pig, which plays an important part in determining the nature of the low-density lipoprotein that is present in the plasma.

The apolipoproteins of plasma LD lipoproteins have been shown to have their origin in the VLD lipoproteins by studies *in vivo* in the rat (Faergeman *et al.*, 1975), rabbit (Rodriguez *et al.*, 1976) and man (Bilheimer *et al.*, 1972; Phair *et al.*, 1975; Sigurdsson *et al.*, 1975). In addition, the conversion of VLD into LD lipoproteins by rat 'post-heparin plasma' has been demonstrated *in vitro* by Eisenberg & Rachmilewitz (1975). These observations suggest that LD lipoproteins are formed from VLD lipoproteins by the removal of lipid, which results in an increase in the density of the lipoprotein particle. The evidence for this has been reviewed in detail by Eisenberg & Levy (1975).

An unusual feature of the guinea pig, by comparison with other mammals, is the relatively high density of its LD lipoprotein (Mills, 1976). When guinea pigs are fed for a short time on a diet rich in fat and cholesterol, these lipoproteins are almost completely replaced by LD lipoproteins of different composition and lower density (Mills *et al.*, 1972*a*). Earlier work (McTaggart, 1974; Mills & McTaggart, 1974) has shown that feeding this lipid-rich diet brings about

the formation of VLD lipoproteins of abnormal composition, and decreases the rate at which the LD lipoproteins are catabolized. However, it was not clear from these earlier experiments whether the normal and abnormal LD lipoproteins are metabolically interconvertible nor, if so, whether the lipid-rich diet could alter the process. The present paper describes an investigation of this question.

Experimental

Materials

The reagents used for the radioiodination of LD lipoproteins were obtained from the sources quoted by Weech *et al.* (1978). Cholesterol of biochemical quality was purchased from BDH Chemicals (Poole, Dorset, U.K.), analytical grade sucrose from Hopkin and Williams (Chadwell Heath, Essex, U.K.), and bovine plasma albumin from Sigma Chemical Co. (Kingston on Thames, Surrey, U.K.). A Pyrex filter holder for micro-analysis (type XX10) was obtained from Millipore (U.K.) Ltd. London NW10 7SP, U.K. All other reagents and materials were as detailed by Chapman *et al.* (1975) and by Chapman & Mills (1977).

Animals and diet

Male guinea pigs (approx. 750 g) were obtained either from the closed breeding colony of the Courtauld Institute, or from Grayston Guinea Pigs, Little Lions Farm, Ashley Heath, Ringwood, Hants.,

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Abbreviations used: LD lipoprotein, low-density lipoprotein of density as defined in the text; LR-LD lipoprotein, low-density lipoprotein isolated from the plasma of guinea pigs fed on a diet supplemented with corn oil and cholesterol (density defined in the text); VLD lipoprotein, very-low-density lipoprotein of density less than 1.007 g/ml.

U.K. They were fed *ad libitum* on rabbit pellets (Spillers Ltd., London E16 1AX, U.K.), supplemented with hay and cabbage. The experimental diet consisted of rabbit pellets to which 1.6% cholesterol and 15% of corn oil by weight were added (Mills *et al.*, 1972a.) This diet was given for 6–8 days, during which it was freely available but was not supplemented with hay or cabbage.

Methods

Blood samples. Samples were obtained under diethyl ether anaesthesia, by heart puncture, after overnight starvation. When the blood was to be used as a source of lipoproteins for radioiodination, it was delivered into glass tubes and left to clot at about 20°C for 4h before the serum was isolated by centrifugation at about 1000g for 20min at 7°C.

When blood was taken from animals that had received tracer doses of radioactively labelled lipoprotein, samples (1.8ml) were delivered into glass tubes containing about 2mg of EDTA. The first sample was taken about 4min after the labelled compounds had been injected (this will be referred to as the zero-time sample), and further samples were then taken over a period of up to 72h. The plasma from these samples was recovered by centrifugation as before.

Isolation of lipoproteins. The different lipoprotein fractions used in the study were isolated by sequential ultracentrifugation. The lower limiting density was set at 1.018–1.019g/ml (except where stated), to exclude VLD lipoproteins. The upper limiting density was made 1.063g/ml when LD lipoproteins were isolated from fat-fed animals, but was 1.100g/ml when the denser LD lipoproteins from normal animals were required. In some experiments only the lowest density fraction of the LD lipoproteins was used, and in these, the upper density limits were adjusted to 1.045 and 1.063g/ml for abnormal and normal LD lipoproteins respectively.

All ultracentrifugations were carried out in the Spinco 30.2 rotor at 29000 rev./min (74312g_{av.}) for 18h at 11°C. The serum or plasma was first adjusted to the lower limiting density by the addition of an appropriate volume of NaCl solution. After centrifugation, the supernatant lipoprotein layer was discarded and the density of the remainder raised to the required upper limit by the addition of the calculated amount of NaBr solution. After ultracentrifugation as before, the lipoprotein layer was recovered by aspiration and washed once by centrifugation at the higher limiting density.

The salt solutions used to make these adjustments ranged in density up to 1.286g/ml, the values being chosen to give easily measurable volumes at each stage. Each solution contained 1mM-EDTA, 0.01% (w/v) NaN₃, and was adjusted to pH7.0 with solid

NaHCO₃. The preparation of these solutions and the adjustment of the serum were carried out at 20°C. Densities were measured by pycnometry.

Radioiodination of lipoproteins. Labelling of the isolated lipoproteins with radioactive iodine was performed by the chloramine-T procedure as described by Weech *et al.* (1978). After iodination, the lipoproteins were washed by ultracentrifugation at their upper density limit as described above, and were then dialysed against 0.9% (w/v) NaCl for 30min. For some experiments, LD lipoproteins from guinea pigs that had been fed on the lipid-rich diet were labelled with ¹³¹I, and normal guinea-pig LD lipoproteins were labelled with ¹²⁵I. The two preparations were then washed ultracentrifugally, mixed, and dialysed against 0.9% NaCl. In all these experiments, the labelled lipoproteins were administered to guinea pigs in doses of 0.3–1.8mg of apolipoprotein (1–2ml) by intra-cardiac injection while the animals were under diethyl ether anaesthesia.

Density-gradient ultracentrifugation. Samples of serum taken from the experimental animals were analysed by density-gradient ultracentrifugation. The gradients were made from the following solutions: (1) phosphate buffer containing 25mM-Na₂HPO₄, 0.1M-NaCl, 1mM-EDTA and 0.01% (w/v) NaN₃, adjusted to pH7.5 with HCl; (2) 'light solution': approx. 1.0% (w/v) sucrose in phosphate buffer, to give a final density of 1.0093 ± 0.0005g/ml at 20°C; (3) 'heavy solution': approx. 20.5% (w/v) sucrose in phosphate buffer, to a final density of 1.0803 ± 0.0005g/ml at 20°C.

The density gradients were made at room temperature (18–22°C) by one of the following two methods. (A) This method was used in early experiments. Sucrose (0.132g) was dissolved in 0.5ml of plasma or serum in the bottom of a 8.89cm × 1.429cm (3.5in × 0.563in) Spinco polyallomer centrifuge tube. A linear gradient was then layered over this solution by means of an MSE gradient maker, which was filled with 5ml of both the 'light' and the 'heavy' solutions. Finally, 1ml of the 'light' solution was layered on top of the gradient. After centrifugation, this gradient ranged in density from 1.010 to 1.095g/ml, and was linear between 1.016 and 1.063g/ml.

(B) In this method, 0.132g of sucrose was dissolved in 0.5ml of plasma, as before, but in a cellulose nitrate tube (3.5in × 0.563in). A linear gradient was layered over this by means of the MSE gradient maker and an increased load of 6ml of each solution. This gradient covered the range 1.015 to 1.100g/ml and was linear from 1.016 to 1.076g/ml. Adjacent fractions in the linear part of the gradient differed in density by 0.003g/ml, and the 95% confidence interval in this region was from 0.001 to 0.0018g/ml.

The loaded tubes were centrifuged at 39000 rev./min in a Spinco SW41 rotor at 5°C for 18.6h (including 0.33 of the acceleration and deceleration times). The

gradients were fractionated by puncturing the base of the tube with a 21-gauge hypodermic needle connected to a polyvinyl chloride tube (15 cm long $\times$ 1.0 mm bore), and collecting fractions of approx. 0.4 ml in glass tubes (9.75 mm $\times$ 50 mm). One tube, to which of 0.1 M-NaCl was added instead of serum, was reserved for a determination of the shape of the gradient by refractometry. An Abbe refractometer was used, which allowed the refractive index to be determined to ± 0.00002 , which was equivalent to ± 0.00009 g/ml at the centre of the gradient.

When the gradients were loaded with lipoprotein, the fractions were treated in one of the two following ways. (1) For this 0.1 ml of 30% bovine plasma albumin was added to each fraction, followed by 0.1 ml of 50% trichloroacetic acid. After mixing on a Whirlimixer, the tube was heated for 3–4 min in a water bath at 90°C. The precipitate was centrifuged down (2200g for 10 min) and the supernatant aspirated off. The precipitate was then washed by centrifugation, once with 1 ml of acetone, and twice with 1 ml of ethanol/ether (3:1, v/v).

(2) For this 0.4 ml of tetramethylurea (Kane, 1973) was added to each tube, mixed and left for 15 min. The suspension was then transferred with a Pasteur pipette to a Millipore micro-analysis filter holder which was fitted with a Whatman GF/C glass filter paper (Reichl *et al.*, 1977). The tube was afterwards washed successively with 1 ml of 4.2 M-tetramethylurea and 1 ml of 0.1 M-NaCl, which were also transferred to the filter. The residue on the filter was washed under gentle suction, with 10 ml of 0.1 M-NaCl, followed by 5 ml of chloroform/methanol (2:1, v/v).

The radioactivity of the precipitates isolated by both these methods was determined either on Wallac Decem GTL or on Beckman Biogamma II direct-

absorption gamma radiation counters. Under the conditions used, the radioactivity of the least-active fractions was approximately 10 times the background rate.

Results

Fig. 1 summarizes the result of a typical experiment in which a mixture of radioactively labelled LD lipoproteins from a control guinea pig, and from a fat-fed animal, were injected into a third guinea pig fed on the normal diet. Fig. 1(a) shows how the original mixture (zero-time sample) was resolved by centrifugation on a type A gradient. The peaks of radioactivity occurred at densities of 1.056 and 1.041 g/ml, which are consistent with earlier observations on the hydrated density of LD lipoproteins from normal and fat-fed guinea pigs (Mills *et al.*, 1972a). When a plasma sample, obtained about 48 h later, was centrifuged on a similar gradient, the radioactivity was distributed as shown in Fig. 1(b). Both peaks reached equilibrium at the same point, which corresponded to a density of 1.068 g/ml. Not only therefore had both labelled lipoproteins apparently increased in density, but the difference between them had diminished.

When the same mixture of labelled lipoproteins was administered to a guinea pig that had been fed for 5 days on a diet supplemented with cholesterol and corn oil, a much smaller change in density was produced. After 48 h, the equilibrium density of the abnormal LD lipoprotein had increased from 1.042 to 1.050 g/ml, whereas that of the normal LD lipoprotein was almost unchanged at 1.058 g/ml.

This experiment was repeated in three more guinea pigs, with results essentially the same as those just

Fig. 1. Density distribution of radioactively labelled LD lipoproteins after circulation in the plasma of a guinea pig (a) after 4 min and (b) after 48 h 11 min

A mixture of normal guinea-pig LD lipoprotein labelled with ^{125}I (open histogram) and LR-LD lipoprotein labelled with ^{131}I (shaded histogram) was injected into the heart of a second normal guinea pig. Samples of plasma were taken at intervals of approx. 24 h and centrifuged on a sucrose density gradient as described in the text. The radioactivity in the fractions obtained from the gradients was expressed as a percentage of the total recovered.

Fig. 2. Density distribution of radioactively labelled LR-LD lipoprotein compared with that of endogenous LR-LD lipoprotein in a fat-fed guinea pig (a) after 4 min and (b) after 72 h 52 min

LD lipoproteins of density 1.019–1.045 g/ml were prepared from the serum of a guinea pig fed for 8 days on a diet enriched with fat and cholesterol. After iodination with ^{125}I , these lipoproteins were injected into the heart of another guinea pig fed on the same diet for 7 days. Samples of plasma were taken and centrifuged on a sucrose density gradient as described in the text. The protein in each fraction from the gradient was precipitated with tetramethylurea and measured by the method of Reichl *et al.* (1977). The distribution of apolipoprotein is shown by the shaded histogram and that of radioactivity by the open histogram. Radioactivity is expressed as a percentage of the total recovered from the gradient.

described. Several gradient centrifugations were performed at various times up to 48 h during each experiment. After this time, the remaining radioactivity was generally too weak for accurate measurement. In each case, the density of the labelled lipoproteins increased progressively until a constant value was reached between 24 and 48 h after the start of the experiment (cf. Fig. 3). It appeared therefore that the guinea-pig LD lipoproteins had been subjected to a metabolic process that resulted in their becoming more dense. Moreover, in guinea pigs that had a diet-induced hypercholesterolaemia, the process was much slower than in normal animals.

To determine the relationship between the changing pattern of the labelled lipoproteins and the steady-state pattern of the endogenous lipoproteins of the recipient guinea pig, a second series of experiments was done, of which that shown in Fig. 2 is typical. In this case, LD lipoproteins of density 1.019–1.045 g/ml were prepared from guinea pigs fed on the cholesterol/fat diet for 8 days. This density range was chosen to isolate the lipoproteins that formed the lowest-density half of the LD-lipoprotein fraction in these animals. After being labelled with ^{125}I , a part of the product was injected into the heart of another guinea pig fed on the same diet for 7 days. Samples of plasma were taken at intervals of about 24 h and centrifuged on type B gradients. The distribution of radioactively labelled apolipoproteins was then determined by the tetramethylurea procedure. The amount of precipitated apolipoprotein in each fraction was also determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Fig. 2 shows that,

at the start of the experiment, the peak of the radioactivity (shown by the open histogram) was at density 1.040 g/ml, whereas the peak of the endogenous lipoprotein was at density 1.044 g/ml (shaded histogram). There was only 19.7% of the labelled apolipoprotein between $\rho = 1.044$ g/ml and $\rho = 1.060$ g/ml, whereas 57% of the endogenous apolipoprotein lay between these limits. After 73 h, the distribution of apolipoprotein precipitable by tetramethylurea was essentially unchanged (peak density 1.043 g/ml), but 80% of the radioactivity present at that time was by then between density 1.044 and 1.060 g/ml (peak at 1.050 g/ml). The labelled material had thus moved to the part of the gradient occupied by the most dense endogenous LD lipoproteins. This movement of the radioactivity through the endogenous-lipoprotein distribution was essentially complete after 48 h, the two patterns being almost superimposable after 24 h (protein peak, $\rho = 1.045$ g/ml; peak of radioactivity, $\rho = 1.047$ g/ml).

When the same labelled LD lipoproteins that were used in this experiment were injected into a normal guinea pig, a similar movement of the peak of radioactivity relative to the endogenous lipoprotein took place. In this case, however, the modal density of the endogenous LD lipoprotein was 1.058 g/ml. During its circulation in the bloodstream the labelled LD lipoprotein, which started at $\rho = 1.040$ g/ml, became progressively more dense until it became superimposed on the peak of endogenous lipoprotein after 48 h. No further change in density appeared to take place after this time. These experiments confirmed the initial observation that the metabolism

Fig. 3. Mean increase in modal density of radioactively labelled LD lipoproteins during circulation in the plasma of guinea pigs fed on different diets

The standard deviation of the mean density at any point is represented by the vertical height of the shaded curves. The upper curve shows the change in peak density of labelled normal LD lipoprotein (LDL); the lower curve refers to LR-LD lipoprotein (LR-LDL). (a) Recipient guinea pig fed on a normal diet; (b) recipient guinea pig fed on a diet supplemented with corn oil and cholesterol.

of LD lipoproteins in guinea pigs with a diet-induced hypercholesterolaemia differed from that in normal guinea pigs.

A total of 11 experiments were performed in these two series, the results of which are summarized in Fig. 3, in which the mean peak density of the LD lipoproteins is plotted against the time of circulation in the guinea pig. The vertical height of the shaded area indicates the standard deviation of the peak density. In every case, the labelled LD lipoproteins were transformed by normal guinea pigs into substances of higher density (Fig. 3a). After 48 h, normal LD lipoproteins had increased from a mean density of 1.056 ± 0.0025 g/ml to a mean of 1.071 ± 0.0025 g/ml, and the mean density of LR-LD lipoproteins had risen from 1.041 ± 0.0013 g/ml to 1.065 ± 0.0026 g/ml. At the end of the experiments therefore the average difference in density between the two LD-lipoprotein preparations had narrowed from 0.015 to 0.006 g/ml. Despite this convergence, the two species of lipoprotein still differed significantly in density ($P < 0.02$).

In the fat-fed guinea pig, these effects were always much smaller (Fig. 3b). After 48 h, there was no detectable change in the mean density of the labelled normal LD lipoproteins (1.056 ± 0.0028 g/ml). By contrast, the density of LR-LD lipoproteins increased slowly to a mean of 1.0485 ± 0.0015 g/ml. Although this increment was much smaller than that produced in the normal guinea pigs, the final product differed significantly ($P < 0.001$) in density from the starting material.

In a third set of six experiments, which were carried out as described for the second series, labelled human LD lipoproteins of density 1.026–1.056 g/ml

were substituted for lipoproteins from guinea pigs. When the recipient animals were fed on the normal diet, the mean density of the human LD lipoproteins increased from 1.044 ± 0.0014 g/ml to 1.056 ± 0.0032 g/ml after 24 h. Little change then took place, the mean density being 1.057 ± 0.0038 g/ml after approx. 48 h. Although there was thus some modification of the human lipoproteins, the mean increment was only 0.0128 ± 0.0034 g/ml, whereas the increment for the guinea-pig LR-LD lipoproteins was 0.0245 ± 0.0023 g/ml. The difference in density (δ) between the human and the guinea-pig lipoproteins was therefore bigger at the end of the experiment ($\delta = 0.008$ g/ml; $P < 0.02$) than it was at the start ($\delta = 0.003$ g/ml; $P < 0.01$). This restricted ability to metabolize human LD lipoproteins was also observed when the recipient animals had been fed on the lipid-rich diet. Under these conditions, there was no change in the density of the human lipoproteins, whereas the LD lipoproteins from fat-fed guinea pigs increased in density by an average of 0.008 g/ml in 48 h.

Discussion

The progressive conversion of VLD lipoproteins into substances of higher density has been known since the work of Pierce (1954) in rabbits, and has since been shown to occur in other species (Faergeman *et al.*, 1975; Rodriguez *et al.*, 1976; Bilheimer *et al.*, 1972). The mechanism that brings about these changes is not clearly understood, but is thought to involve the removal of lipid from the lipoprotein particle by the action of enzymes in the plasma (Eisenberg & Levy, 1975). However, although it is thought that LD lipoproteins may be the final

Fig. 4. Transfer of labelled lipoprotein across three density boundaries during circulation in the plasma of a normal guinea pig

Each line shown refers to one of three consecutive density fractions obtained from a sucrose-density-gradient centrifugation performed on plasma obtained at three different times after injection of labelled normal LD lipoprotein. Each point represents the absolute amount of radioactivity measured (counts/180 s). The density of the fractions was as follows: ●, $\rho = 1.054$ g/ml; ▲, $\rho = 1.057$ g/ml; ■, $\rho = 1.060$ g/ml.

product of this process (Eisenberg & Levy, 1975), there has hitherto been little evidence to show that the LD lipoproteins themselves are subject to a similar progressive degradation. Our experiments have now shown that this may be an important feature of the metabolism of LD lipoproteins in guinea pigs.

The experiments in which the apolipoprotein was precipitated by tetramethylurea showed that the increase in mean density of the labelled LD lipoprotein while it was circulating in the guinea pig was real, and was not an artifact arising from the selective removal of small labelled peptides from the less-dense fraction of the LD lipoproteins. However, the effect could be explained if the LD lipoproteins of lowest density were more rapidly catabolized than those of higher density. This would bring about an apparent redistribution of lipoprotein towards the higher densities, without any net transfer. In some experiments, a distribution of radioactivity, which was skewed towards the low densities at the start, became skewed in the opposite sense after 48 h. This suggests that a small differential catabolic effect may exist. However, the observation summarized in Fig. 4 shows that it was not the principal cause of the increase in density of the LD lipoproteins. Fig. 4 shows that the absolute amount of radioactivity in the three consecutive fractions between densities

1.048 and 1.053 g/ml at first increased and then decreased as the peak of labelled lipoprotein passed through them during the experiment. Since these fractions were to the dense side of the peak at the start of the experiment, a transfer of labelled lipoprotein towards the high-density fractions must have taken place. For the purposes of discussion we shall refer to this process as 'transformation' of the lipoproteins.

It is noteworthy that this transformation always proceeded in the direction of increasing density. Thus the LR-LD lipoproteins are not formed as a result of their abnormally long exposure to metabolic processes in the fat-fed guinea pig (McTaggart, 1974; Mills & McTaggart, 1974). Moreover, since the LR-LD lipoproteins have an abnormal composition (Mills *et al.*, 1972a), it is unlikely that the products of their transformation are the same as those that are produced from normal LD lipoproteins. It is now evident that, although the lipid-rich diet is known to modify lipoprotein synthesis in guinea pigs (McTaggart, 1974; Chapman *et al.*, 1973), the partial inhibition of the transformation reaction also plays an important part in determining the nature of the LD lipoprotein that accumulates in their plasma. The nature of the transformation process is unknown, but it seems likely to be essentially similar to that which brings about the progressive increase in density of the very-low-density lipoproteins (Eisenberg & Levy, 1975). It has been claimed that lipoproteins of density as high as 1.019 g/ml are derived from VLD lipoproteins in this way (Nichols *et al.*, 1968; Eisenberg *et al.*, 1973). It may also be significant that Adams & Schumaker (1969) observed the formation of new LD lipoproteins of unusually high density (1.038 g/ml compared with a normal value of 1.028 g/ml) in men who had received an intravenous injection of heparin. However, the complexity of any transformation process in man may be inferred from the observations that neither human 'post-heparin plasma', nor the lipoprotein lipase which is activated by apolipoprotein C-I, had any significant effect on human LD lipoproteins *in vitro* (Lindgren *et al.*, 1955; Adams & Schumaker, 1969; Lee *et al.*, 1976).

The work of McTaggart (1974) showed that LR-LD lipoproteins were always metabolized more slowly than normal LD lipoproteins, no matter on which diet the guinea pigs were fed. This difference in behaviour is also evident in our experiments; even after 72 h the modal density of the LR-LD lipoproteins never quite reached that attained by the normal compounds. The difference in composition of the normal and LR-LD lipoproteins (Mills *et al.*, 1972a) offers a plausible explanation of this effect. However, human LD lipoproteins, which are very similar in composition to the guinea-pig LR-LD lipoproteins (Lee & Alaupovic, 1970), were transformed to a much smaller extent. It therefore appears that the process is

influenced by other structural factors which are at present unknown.

Although there is some evidence (Weech *et al.*, 1978) that the catabolic rate for guinea-pig LD lipoproteins is dependent on the size of the plasma lipoprotein pool, there is at present nothing to show how this could affect the transformation reaction. The almost complete suppression of the transformation of normal LD lipoproteins in the fat-fed guinea pig suggests that the reaction does not merely suffer a partial inhibition, but is changed in a more complex way. This would imply that transformation is a process involving several steps, or enzymes, which are affected differently when the animal is made hypercholesterolaemic.

Diet is known to be one of the factors that influence the concentration of human plasma lipoproteins, and may therefore make an important contribution to the risk of arterial disease by inducing a hyperlipoproteinemia. Moreover, it has been suggested that differences in the composition of human LD lipoproteins from people of different social status may be the result of different standards of nutrition (Mills *et al.*, 1972b). The prompt and easily identified response of the guinea pig to changes in its diet suggests that it may be a useful model in which to study this aspect of lipoprotein metabolism.

This work has been performed with the support of the Medical Research Council. The material presented forms a part of a thesis presented for the degree of Doctor of Philosophy of the University of London by P. K. W.

References

- Adams, G. H. & Schumaker, V. N. (1969) *Ann. N.Y. Acad. Sci.* **164**, 130–146
- Bilheimer, D. W., Eisenberg, S. & Levy, R. I. (1972) *Biochim. Biophys. Acta* **260**, 212–221
- Chapman, M. J. & Mills, G. L. (1977) *Biochem. J.* **167**, 9–21
- Chapman, M. J., Mills, G. L. & Taylaur, C. E. (1973) *Biochem. J.* **131**, 177–185
- Chapman, M. J., Mills, G. L. & Ledford, J. H. (1975) *Biochem. J.* **149**, 423–436
- Eisenberg, S. & Levy, R. I. (1975) *Adv. Lipid Res.* **13**, 1–89
- Eisenberg, S. & Rachmilewitz, D. (1975) *J. Lipid Res.* **16**, 341–351
- Eisenberg, S., Bilheimer, D. W., Levy, R. I. & Lindgren, F. (1973) *Biochim. Biophys. Acta* **326**, 361–377
- Faergeman, O., Sata, T., Kane, J. P. & Havel, R. J. (1975) *J. Clin. Invest.* **56**, 1396–1403
- Kane, J. P. (1973) *Anal. Biochem.* **53**, 350–364
- Lee, D. M. & Alaupovic, P. (1970) *Biochemistry* **9**, 2244–2252
- Lee, D. M., Ganesan, D. & Bass, H. B. (1976) *FEBS Lett.* **64**, 163–168
- Lindgren, F. T., Nichols, A. V. & Freeman, N. K. (1955) *J. Phys. Chem.* **59**, 930–938
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- McTaggart, F. (1974) Ph.D. Thesis, University of London
- Mills, G. L. (1976) in *Handbuch der inneren Medizin* (Schettler, G., Greten, H., Schlierf, G. & Seidel, D., eds.), vol. 7, part 4, pp. 173–195, Springer-Verlag, Berlin
- Mills, G. L. & McTaggart, F. (1974) in *Atherosclerosis III* (Schettler, G. & Weizel, A., eds.), pp. 398–401, Springer-Verlag, Berlin
- Mills, G. L., Chapman, M. J. & McTaggart, F. (1972a) *Biochim. Biophys. Acta* **260**, 401–412
- Mills, G. L., Chong, Y. H. & Taylaur, C. E. (1972b) *Clin. Chim. Acta* **42**, 273–283
- Nichols, A. V., Strisower, E. H., Lingren, F. T., Adamson, G. L. & Coggiola, E. L. (1968) *Clin. Chim. Acta* **20**, 277–283
- Phair, R. D., Hammond, M. G., Bowden, J. A., Fried, M., Fisher, W. R. & Berman, M. (1975) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 2263–2270
- Pierce, F. T. (1954) *Metabolism* **3**, 142–153
- Reichl, D., Myant, N. B., Pflug, J. J. & Rudra, D. N. (1977) *Clin. Sci. Mol. Med.* **53**, 221–226
- Rodriguez, J. L., Catapano, A., Ghiselli, G. L. & Sirtori, C. R. (1976) *Atherosclerosis* **23**, 85–96
- Sigurdsson, G., Nicoll, A. & Lewis, B. (1975) *J. Clin. Invest.* **56**, 1481–1490
- Weech, P. K., McTaggart, F. & Mills, (1978) *Biochem. J.* **169**, 687–695