Diet-induced and physiologically occurring hypercholesterolemias in the spontaneous hypothyroid European badger (*Meles meles* L.): a density gradient study of lipoprotein profile

P. Michel Laplaud, Laurence Beaubatie, and Daniel Maurel

Laboratoire de Biochimie médicale, Faculté de médecine et de pharmacie, 2 rue du Dr. Marcland, 87032 Limoges Cedex, France,[1] Laboratoire de Physiologie animale, U.E.R. des Sciences, 123 rue A. Thomas, 87060 Limoges Cedex, France,[2] and Centre d'études biologiques des animaux sauvages (CEBAS-CNRS), 79360 Beauvoir-sur-Niort, France[3]

Abstract As previously shown in this laboratory (Laplaud, P. M. et al. *J. Lipid Res.* 1980. **21:** 724–738), the European badger is, with regard to its plasma lipid transport system, an original and complex animal of great potential interest to lipoprotein research. In an effort to study the response of this animal to cholesterol feeding, we gave a diet supplemented with 1% cholesterol to six male badgers (group H) during the late fall period when spontaneous hypercholesterolemia and hypothyroidism occur. Six more male animals of similar age received the standard diet (group C) and were simultaneously used as controls. Plasma lipids were measured using enzymatic methodologies, while the use of a recently described density gradient ultracentrifugation technique allowed detailed examination of lipoprotein composition and polyacrylamide gel electrophoresis of lipoproteins and tetramethylurea-soluble apoproteins in the fractions. The results suggest the superimposition, in H badgers, of the spontaneous and diet-induced hypercholesterolemias, maximum levels being reached in December in both C and H groups. While the two groups were very similar at the beginning of the experiment, highly significant differences ($P < 0.01$) were subsequently observed between C and H animals in plasma cholesterol and phospholipid concentrations. Density gradient ultracentrifugation provided evidence for the following diet-induced changes in lipoprotein profile: *1*) a twofold increase in cholesteryl esters in particles of d < 1.006 g/ml; *2*) the occurrence of large amounts of supplementary cholesterol-rich low density lipoproteins, mainly in the 1.019–1.027 g/ml region; *3*) an increase in the 1.039–1.055 g/ml low density lipoproteins; and *4*) a change in the ratio of the concentrations of high density lipoproteins of d 1.065–1.100 g/ml and d 1.100–1.162 g/ml, to the benefit of the former. Electrophoresis of the density gradient fractions revealed marked heterogeneity, especially in the low density part of the spectrum. Electrophoresis of the low molecular weight, tetramethylurea-soluble apoproteins failed to show marked differences between C and H badgers. However, chromatographic determination of the proportion of apoB in the protein moiety of the two main low density components showed that *1*) it was consistently low, *2*) its contribution to the higher density fraction (d 1.039–1.046 g/ml) was unaffected by the hypercholesterolemic diet (being about 25% in both C and H animals), and *3*) its contribution to the lower density fraction (d 1.019–1.027 g/ml) decreased under the same nutritional conditions, representing about 20% in C as compared to about 10% in H badgers.—**Laplaud, P. M., Beaubatie, and D. Maurel.** Diet-induced and physiologically occurring hypercholesterolemias in the spontaneous hypothyroid European badger (*Meles meles* L.): a density gradient study of lipoprotein profile. *J. Lipid Res.* 1982. **23:** 782–794.

Supplementary key words polyacrylamide gel electrophoresis • heterogeneity of low density lipoproteins • cholesterol-rich lipoproteins • apoproteins

The use of animal models in atherosclerosis research has led in recent years to major advances in this field. Studies usually involve induction, by means of a high cholesterol diet, of hypercholesterolemia and of changes in the plasma lipoprotein pattern that in turn may be associated with the appearance of atherosclerotic lesions. Such work has been successfully conducted in many animal species, including nonhuman primates (1–7), rats (8), rabbits (9), dogs (10), swine (11, 12), guinea pigs (13, 14), etc., and several reviews have appeared of late which summarize studies in this field (15–18).

In a previous paper (19), we demonstrated that the European badger exhibits a spontaneous annual cycle of its plasma lipid components, with the occurrence of a marked hypercholesterolemia and hyperphospholipi-

Abbreviations: VLDL, very low density lipoproteins, d < 1.006 g/ml; LDL, low density lipoproteins, d 1.006–1.063 g/ml, unless otherwise defined; IDL, intermediate density lipoproteins, a part of LDL with d 1.006–1.019 g/ml; HDL, high density lipoproteins, d 1.063–1.21 g/ml (subdivided into HDL_2 d 1.063–1.125 g/ml and HDL_3 d 1.125–1.21 g/ml); EDTA, ethylene diamine tetraacetic acid; SDS, sodium dodecyl sulfate; TMU, tetramethylurea.

[1] P. M. Laplaud and L. Beaubatie.

[2] P. M. Laplaud.

[3] D. Maurel.

demia during the late fall and beginning of winter. This phenomenon occurs simultaneously with a redistribution of plasma cholesterol between the respective lipoprotein density classes, essentially to the benefit of the 1.006–1.063 g/ml components, and during the weeks following an abrupt decrease in plasma thyroxine concentration, as demonstrated by Maurel and Boissin (20).

It is well-known that hypothyroidism results in an increased sensitivity to atherosclerotic processes, and some authors have consequently used surgically thyroidectomized animals for their experiments (10). However, the badger, despite repeated seasonal hypercholesterolemias, is not known to exhibit early cardiovascular disease. In view of our continuing interest in this animal, which appears likely to be an original and useful model in lipoprotein research, it was of importance to determine its response to an hypercholesterolemic diet. We have therefore taken advantage of that part of the year when spontaneous hypercholesterolemia and hypothyroidism occur concomitantly in this species, in order to superimpose a diet-induced hypercholesterolemia in six male badgers. Their plasma lipids and lipoproteins were subsequently analyzed on repeated occasions for up to 11 weeks and were compared to those of a control group, while plasma thyroxine was similarly assayed in all animals.

MATERIALS AND METHODS

Animals and diets

The adult male badgers used, bred in the Centre d'Etudes Biologiques des Animaux Sauvages, were approximately 2–6 years old. They were kept individually in 6 m^2 parks under natural conditions of light, temperature, and rainfall. None of the animals exhibited pathological manifestations during the course of the present study. The 12 badgers used were divided into two equal groups of 6 animals. One group after denoted as group C (control), received the standard diet, consisting of a commercial food for dogs (Canina, Duquesne-Purina) that contained (proportions by weight of the major constituents) protein, 20%; animal fat, 6%; carbohydrate, 5%; vitamin A, 15000 IU/kg; and vitamin D_3, 1500 IU/kg. Water was provided ad libitum. The other group, denoted as group H, received the same diet supplemented with 1% crystalline cholesterol (CH-USP, Sigma). Inasmuch as each animal received and ate about 800 g of food per day, the supplement amounted to 8 g of cholesterol/animal per day in group H.

Collection and treatment of blood

For each successive series of manipulations, blood samples were taken from animals that had been fasted overnight (approximately 18 hr). Blood was collected on EDTA (final concentration 1 mM), at approximately 11:00 AM, by puncture of the radial vein. Plasma was then separated by low speed centrifugation and brought to the laboratory on ice.

Chemical analysis

Total and free cholesterol were measured by enzymatic methods using commercially available reagent kits (Boehringer Mannheim; total cholesterol, ref. 187313; unesterified cholesterol, ref. 124087, omitting cholesterol-esterase in the reaction medium). The cholesteryl ester concentration was taken as (total cholesterol concentration–unesterified cholesterol concentration) × 1.68. Plasma triglyceride concentrations were equally measured by enzymic methodology obtainable as a commercial kit (Boehringer Mannheim, ref. 126039) that determines the total glycerol content of the sample. As no technique allowing separate determination for plasma free glycerol was available, the term "triglycerides" herein designates the plasma content in triglycerides plus free glycerol, and thus constitutes on overestimate. Phospholipids were determined by means of the "Phospholipids B-Test" (Wako Chemicals, Osaka, Japan), which liberates choline using phospholipase D. The choline was then estimated by colorimetry (reaction with phenol and 4-amino antipyrine) and specific measurement of the choline content of all choline-containing phospholipids was thus obtained.

All the techniques were first checked for linearity over the entire range of values observed, and were under continuous monitoring by use of several control sera. All the measurements were performed on a semi-automated Clinicon Ultrolab analytical apparatus (LKB Instruments, Bromma, Sweden).

Gradient gel electrophoresis of plasma lipoproteins and of lipoprotein fractions

Polyacrylamide gel electrophoresis in a three-step gradient was performed according to the method of Fruchart (21), in which pre-staining of lipoproteins with diformazan of nitro blue tetrazolium (Sigma) was followed by migration in glass tubes, (75 mm × 7 mm), in Tris-glycine buffer, pH 8.3, at 4°C. In view of the lack of precise information regarding the possible differential binding of nitro blue tetrazolium to the different lipid components of the lipoproteins, no attempt was made to quantify the various components.

Application of this technique to the electrophoretic examination of the fractions obtained by density gradient ultracentrifugation provided evidence for considerable heterogeneity, with several distinct bands in the LDL region (see Results). In order to ascertain whether this phenomenon was artifactual, we subsequently performed

the following procedures. *1*) Inasmuch as heterogeneity was first observed on density gradient fractions originating from pools of plasma from six animals, samples from individual animals were electrophoresed. *2*) In order to eliminate any possible influence of the type of gel used, comparative electrophoreses were conducted on polyacrylamide gradient gel slabs, 2 to 16% monomer concentration (PAA 2/16, Pharmacia, Uppsala, Sweden). *3*) In some experiments, pre-staining of lipoprotein fractions was replaced by conventional staining, after the electrophoretic migration, with 1% Coomassie Brilliant Blue R (Sigma).

In all three types of manipulations, similar heterogeneity was observed. Furthermore, recombination of the density gradient fractions corresponding to the 1.015–1.065 g/ml class, followed by examination of the resultant mixture under our usual electrophoretic conditions, gave a pattern which resembled the more diffuse one obtained with the total 1.006–1.063 g/ml density class isolated by sequential preparative ultracentrifugation (see below) from the same samples. Finally, it is to be noted that the electrophoretic heterogeneity typical of badger LDL was not observed in several samples from healthy humans examined under identical conditions.

Ultracentrifugal methods

In each of the procedures described below, all the NaCl and/or NaBr solutions used for adjustment of densities, as well as for dialysis of the lipoprotein fractions, contained EDTA (0.4 g/l), sodium azide, (0.1 g/l), and merthiolate, (1 mg/l). Monitoring of the actual background densities was performed by means of a DMA 46 calculating precision density meter (Anton Paar KG, Graz, Austria), at 17°C.

Sequential preparative ultracentrifugation. Plasma lipoproteins were isolated in the classical density intervals (i.e., d < 1.006 g/ml, d 1.006–1.063 g/ml, and d 1.063–1.21 g/ml), according to established procedures (22). This was performed in a MSE Prepspin 50 ultracentrifuge (MSE, Crawley, England), using an aluminum fixed-angle rotor (capacity 8 × 14 ml), at 17°C.

Density gradient centrifugation. Gradients were constructed essentially as described by Chapman et al. (23) except that NaCl-NaBr solutions were used in place of NaCl-KBr solutions. Discontinuous five-step gradients were thus obtained, with successive densities (from top to bottom of tube) of 1.006 g/ml (3 ml), 1.019 g/ml (2.5 ml), 1.063 g/ml (2 ml), 1.21 g/ml (3 ml of serum adjusted to this density with solid KBr), and 1.24 g/ml (2 ml). Control gradients were also constructed, using 3 ml of NaCl-NaBr solution of d 1.21 g/ml instead of the adjusted serum sample.

The gradients were then placed in a MSE 6 × 14 ml titanium swing-out rotor and centrifuged for 48 hr at 40,000 rpm (196 000 *g*-avg.) at 17°C. No braking was employed at the end of the run. The serum-containing gradients were then divided into successive 0.5-ml fractions by stepwise aspiration with a micropipette; the background density of each fraction was determined by reference to the density profile obtained from control gradients.

Prior to further chemical or electrophoretical analysis, all lipoprotein fractions obtained either by sequential or density gradient ultracentrifugation were dialyzed in Spectrapor tubing, (Spectrum Medical Industries, Los Angeles, CA, USA, exclusion limit approx. 3,500), for 3 × 12 hr at 4°C against 0.196 molal NaCl, with magnetic stirring of the dialysate. As previously shown (19), the use of dialysis allowed application of the assay for plasma total glycerol to the determination of the glyceride content of the ultracentrifugally-prepared lipoproteins. Chemical analysis of the fractions was performed using the same techniques described above for measurement of the plasma lipids, while the method of Lowry et al. (24) was employed for the assay of protein concentrations; all determinations were in duplicate.

Estimation of the apoprotein B-like content of fractions obtained by density gradient ultracentrifugation

Our data suggested that the two different subfractions in the LDL density range, i.e., the 1.019–1.027 g/ml and 1.039–1.046 g/ml intervals, should be considered separately (see Results). We therefore estimated the relative proportions of the apoB-like protein and of the lower molecular weight peptides in each of these density intervals by taking advantage of the adaptation by Chapman and Goldstein (25) of the chromatographic technique of Herbert et al. (26). For this purpose, samples of native lipoproteins of d 1.019–1.027 g/ml or d 1.039–1.046 g/ml, containing 2–10 mg protein, were delipidated by extraction with ethanol–diethyl ether 3:1 (v/v); the apoprotein residue was then dried under N_2 and dissolved in 0.01 M sodium phosphate buffer containing 1% (w/v) SDS and 1% β-mercaptoethanol, pH 8.0. Apo-LDL was subsequently fractionated by gel filtration chromatography on a Sephadex G-200 column exactly as described by Chapman and Goldstein (25), using an LKB Uvicord II apparatus. Under these experimental conditions, the recovery of protein from the column was greater than 85%, and the pattern of the apoproteins, belonging to either of the two density intervals concerned, consisted of two well-resolved and symmetrical peaks. The apoprotein content of each of these two peaks was assayed both by SDS-polyacrylamide gel electrophoresis according to Weber and Osborn (27) (about 100 μg of protein were layered on each gel), and by the technique used for the determination of the pattern of TMU-sol-

uble peptides (see below, "Electrophoresis of apolipoproteins"). The first peak, which eluted in the void volume, was shown to contain exclusively a protein with molecular weight typical of apoB (about 250,000). The second peak was found to be exclusively representative of lower molecular weight, TMU-soluble apoproteins. The ratio of the areas of these two peaks, as evaluated on the recorder, was therefore considered as a measure of the respective proportions of apoB and of the other apoproteins in the lipoprotein fractions.

Electrophoresis of apolipoproteins

The TMU-soluble apoprotein components of the lipoprotein fractions isolated by density gradient ultracentrifugation were electrophoresed according to the modification by Kane (28) of the procedure of Davies (29). After completion of the electrophoretic runs, the gels were fixed in 10% trichloroacetic acid, stained with 1% Coomassie Brilliant Blue R (Sigma), and destained in 10% trichloroacetic acid. For the reasons discussed by Chapman, Mills, and Ledford (30), no attempt was made to quantify each band of apoprotein.

Plasma thyroxine assay

Total plasma thyroxine was assayed by the isotopic competition technique described by Vigouroux (31). This technique is the same as that used previously for examination of the seasonal variations of plasma thyroxine in the badger (20).

Statistical methods

In view of the small number of animals in each group, the statistical significance of variations in plasma lipid levels was assayed by means of the nonparametric test of Mann and Whitney (32). For this purpose, individual sets of data, (total and unesterified cholesterol, triglycerides, and phospholipids) from each of the two groups of animals and corresponding to each successive series of blood samples, were compared both with the preceding and following ones in the same group and with the same data obtained at the same date in the other group.

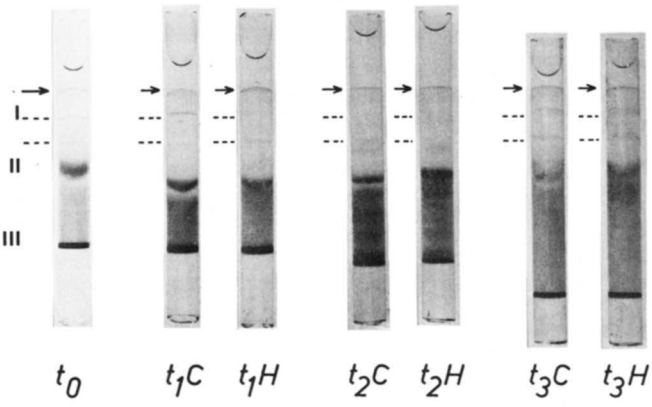

Fig. 1. Polyacrylamide gel electrophoresis of whole plasma lipoproteins. Direction of migration: top to bottom. Arrows indicate the position of layers; upper and lower dotted lines correspond to the limits between 1st and 2nd, and 2nd and 3rd gels, respectively. From left to right: t_0, profile representative of all of the twelve animals in October (i.e., immediately before the induction of alimentary hypercholesterolemia); the t_1C and t_1H profiles are typical of the control group and of the group receiving the hypercholesterolemic diet, respectively, in November; the t_2C and t_2H profiles are typical of the same animals in December; the t_3C and t_3H profiles are typical of the same animals in January.

RESULTS

In the interest of brevity, the different dates of experiments will be referred to as follows: t_0, October 22, 1980 (series of sampling immediately before the choles-

TABLE 1. Successive variations in badger plasma lipids and body weights during the experiment

Variable Assayed	Group of Animals	Dates of Sampling[a] 22.10.80	12.11.80	03.12.80	07.01.81
Total cholesterol, mg/dl	C	255 ± 24[b]	372 ± 25††	416 ± 36†	350 ± 44††
	H	244 ± 47	570** ± 106††	634** ± 95	548** ± 86†
Esterified cholesterol / Total cholesterol	C	0.65 ± 0.01	0.68 ± 0.01††	0.68 ± 0.01	0.66 ± 0.01††
	H	0.66 ± 0.01	0.68 ± 0.01††	0.67 ± 0.01	0.65 ± 0.01††
Phospholipids, mg/dl	C	382 ± 24	445 ± 19††	532 ± 42††	482 ± 46
	H	377 ± 51	604** ± 76††	729** ± 82†	684** ± 106
Triglycerides, mg/dl	C	86 ± 6	118 ± 10††	137 ± 12†	107 ± 22†
	H	91 ± 16	143* ± 23††	158 ± 30	128 ± 13††
Body weight, kg	C	14.1 ± 1.4	14.7 ± 1.4	15.4 ± 1.4	16.0 ± 1.4
	H	14.0 ± 1.6	14.5 ± 1.7	15.3 ± 1.8	16.9* ± 2.0††

[a] t_0, t_1, t_2, and t_3, respectively.
[b] Mean ± SD; n = 6.
* and ** indicate a significant difference, respectively, at the 5% and 1% levels, between the C and the H groups at the same date. † and †† indicate a significant difference, respectively, at the 5% and 1% levels, between two successive series of samplings in the same group.

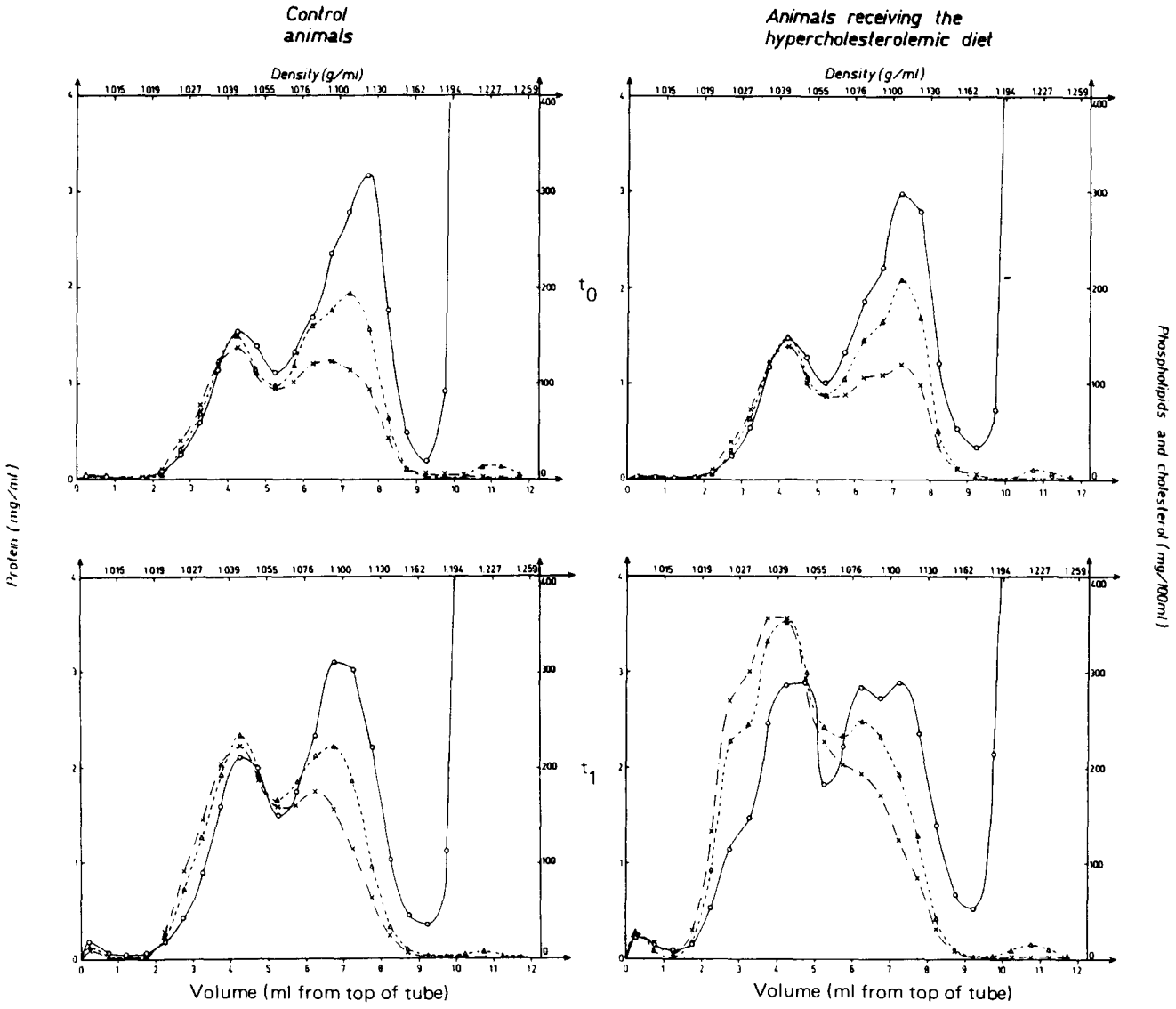

Fig. 2. Badger plasma lipoprotein profiles, as assayed by density gradient ultracentrifugation at the different stages of the experiment. Each diagram is representative of the results obtained when examining one pool made up from equal volumes of plasma from the six animals in each group. See text for experimental conditions. Left column: control animals; right column: animals submitted to the hypercholesterolemic diet. t_0, t_1, t_2, and t_3 are, respectively, related to October, November, December, and January samples. In all eight diagrams, — O — refers to protein determinations, – • – × – • – to cholesterol, and – – Δ – – to phospholipids. In addition, in the two lower diagrams, – – □ – – refers to triglyceride distribution.

terol-containing diet was given to group H animals); t_1, November 12, 1980; t_2, December 3, 1980; and t_3, January 7, 1981.

Plasma lipids

Plasma lipid levels at the four dates are summarized in **Table 1**. From the data it is evident that at the beginning of the experiment the two groups of badgers were strictly comparable with respect to all the variables assayed and especially to cholesterol concentration (mean 255 mg/dl and 244 mg/dl for groups C and H, respectively). In the C group, the seasonal hyperlipidemia (19) occurred as usual, with a maximum at t_2. Significant differences in cholesterol and phospholipids between the H and C animals were observed as early as the second series of samplings. Indeed, at t_1 (i.e., only 18 days after introduction of the cholesterol-containing diet), the H group exhibited mean values as high as 570 mg cholesterol/dl and 604 mg phospholipid/dl (372 mg/dl and 445 mg/dl, respectively, in the C group). The two sets of animals simultaneously reached maximum hyperlipidemia at t_2, 634 mg/dl for plasma cholesterol and 729 mg/dl for phospholipids in the H group and, respectively, 416 mg/dl and 532 mg/dl in the C group. The last series of samplings showed that plasma lipid concentrations had started to decrease in all animals; this

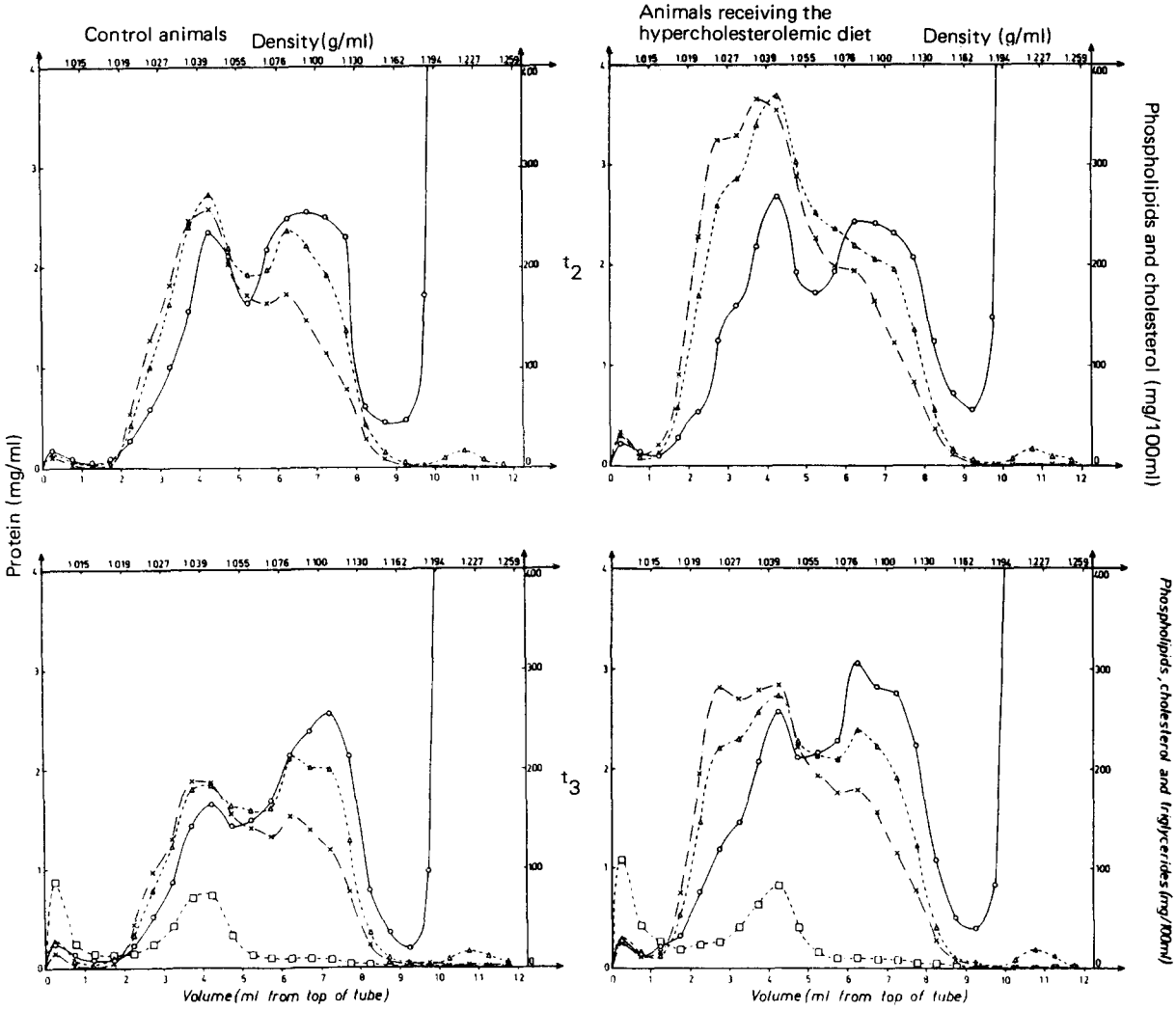

Fig. 2. (*Continued*)

phenomenon was most pronounced in the case of cholesterol. Indeed, even in the H animals, the lowering of plasma cholesterol was statistically significant; the mean value in this group was 548 mg/dl (group C, 350 mg/dl). The ratio of esterified to total cholesterol remained comparable in the two groups and paralleled the degree of hyperlipidemia, with a maximum (0.68) at t_1 and t_2, followed by a significant decrease at t_3. Finally, body weights in the C and H animals were similar except at the date of the final samplings, when badgers receiving the cholesterol-containing diet exhibited statistically significant elevations; the weights of these animals were also significantly different from those of the C group.

Electrophoresis of plasma lipoproteins

A striking feature of the electrophoretic patterns (**Fig. 1**) was the high degree of similarity in plasma samples from all animals before induction of the hypercholesterolemia, as well as in plasma from the individual animals within each group in the subsequent examinations. With the exception of faint bands of VLDL present in some plasmas, all the samples from each group were essentially identical at each time point.

The evolution of the common electrophoretic characteristics noted in patterns from our animals can be summarized as follows. First, no chylomicrons were ever observed in any sample, while VLDL (band I), if present, was reduced to minimal amounts in some C or H plasmas. Band II, which has previously been shown (19) to represent the main LDL component, typically migrated far into the lower gel and, in each series, was of similar intensity in all animals, as judged by visual examination. On the other hand, a difference between the two groups of animals, which appeared as soon as t_1 and remained until t_3, was the presence in C animals of a short non-stainable interval immediately below the LDL band while, at the same dates, complete LDL-HDL continuity was noted in the H animals. At t_2, this LDL-

HDL interval exhibited three or four supplementary bands in both groups. Finally, the band migrating as human HDL (band III), although strongly stained in all samples assayed, was clearly decreased in the t_2 experiments.

Density gradient fractionation of plasma lipoproteins

Chemical analysis. The profiles of protein, cholesterol, and phospholipid concentrations in the successive 0.5-ml density gradient fractions in C and H animals are summarized in **Fig. 2**; the distribution of triglycerides was also examined in the last series of samples (at t_3). As is evident from the figure, the profiles noted at t_0 in the two groups of badgers were very similar, both qualitatively and quantitatively. With regard to the C group, the phenomenon already described by this laboratory (19), and defined as a redistribution of cholesterol between the LDL and HDL density classes, is again evident. Simple calculations show that, at t_0, plasma cholesterol is distributed in approximately equal amounts between the 1.015–1.065 g/ml and the 1.065–1.162 g/ml intervals. At maximum hyperlipidemia (t_2), these proportions changed to 67.5% and 32.5%, respectively, while an inverse evolution began at t_3 (62.5% and 37.5%, respectively).

However, the principal feature of Fig. 2 is the difference between the C and H groups as a result of the cholesterol-containing diet. It seems that the principal difference consisted of the progressive appearance, beginning as soon as t_1, of supplementary lipoproteins of major quantitative importance in the d 1.019–1.027 g/ml interval. The proportion of plasma cholesterol transported by these lipoproteins increased gradually and, at t_3, represented an amount similar to that in the other and denser LDL component (ca. d 1.039–1.046 g/ml). As judged by our measurements, the respective proportions of cholesterol, phospholipids, and protein in these cholesterol-induced lipoproteins did not alter appreciably from t_1 to t_3. On the other hand, the proportions were modified in the d 1.039–1.046 g/ml LDL subfraction, in which the cholesterol/protein ratio changed from about 0.95 at t_0 to 1.25 at t_1, 1.30 at t_2, and subsequently decreased to 1.10 at t_3. In the C group, a minor enrichment in cholesterol in the same density interval also occurred. Furthermore, the spontaneous appearance, in these latter animals, of small amounts of LDL of lower density with composition similar to that induced by the diet in the H group, was suggested by the appearance of a slight shoulder in the cholesterol profile in the t_3 sample.

Major changes were also observed in the higher density part of the spectrum. However, consideration of the distribution of cholesterol revealed a progressive shift towards HDL of lower density in both the C and H groups; consequently, the profiles in the 1.065–1.162 g/ml region are quite similar in the t_2 samples. However, a return to the t_0 pattern began in the C animals at t_3, the protein peak being clearly located in the d > 1.100 g/ml section. At the same date, this peak was situated in the 1.076–1.087 g/ml fraction in H badgers.

Examination of the cumulative percentage distribution of cholesterol vs. density showed that, in each series of C or H samples, the S-shaped curves shifted towards the lower densities from t_0 to t_2, with an opposite tendency evident at t_3. At t_0, the curves for the C and H groups were strictly superimposable while at t_1, t_2, and t_3, the H curve was shifted more than the C curve. From these tracings, the density of the mid-distribution of cholesterol in each pool may be determined; our results show that this point is reduced more in the H group (t_0: 1.060 g/ml, t_2: 1.037 g/ml) than in C animals (t_0: 1.060 g/ml, t_2: 1.046 g/ml). At t_3, this value was either unchanged or began to move in the opposite direction.

The successive shifts described for cholesterol were, qualitatively, equally evident upon examination of the density distributions of phospholipids or lipoprotein protein. On a quantitative basis, the difference between the C and H groups at t_3 was similar for total cholesterol, cholesteryl esters, and phospholipids, for which 50% of total plasma content was reached at a density that was about 0.012 g/ml lower in the H than in the C animals. This difference was smaller for protein (about 0.006 g/ml), free cholesterol (about 0.004 g/ml), and triglycerides (about 0.002 g/ml).

In these t_3 experiments, measurement of the free and esterified cholesterol and triglycerides allowed us to determine the detailed composition of plasma lipoproteins in each density fraction. From results presented in **Table 2**, it may first be noted that, in the top fraction (ca. d < 1.013 g/ml), the major difference lay in the twofold increase in the relative proportion of cholesteryl esters + free cholesterol in the H animals when compared with the C group; at the same time, the esterified/total cholesterol ratio increased from 0.08 in C to 0.47 in H. Analysis of authentic VLDL isolated by conventional preparative ultracentrifugation from the same samples led to similar results. Secondly, the chemical composition of the lipoproteins isolated in the 1.017–1.032 g/ml fractions appeared to differ markedly between the two groups; this was mostly attributable to an enrichment in cholesteryl esters (on the order of 5–7%) in the H animals, together with a concomitant decrease in glyceride content.

Finally, the esterified/total cholesterol ratio in the higher density part of the spectrum decreased successively in increments of 5–13% in successive fractions within the 1.087–1.130 g/ml interval in H animals as compared to that in the C group.

Using an appropriate concentration factor, it was also possible to calculate the approximate plasma concentra-

TABLE 2. Chemical composition of some fractions of plasma lipoproteins isolated by density gradient ultracentrifugation and particularly representative of the general trends noted in the lipoprotein spectrum in our animals

Fraction (ml, from top of tube)	Density limits (g/ml)	Group of Animals	Cholesteryl Esters	Free Cholesterol	Triglycerides	Phospholipids	Protein
0–0.5	<1.013	C	1.3	8.9	58.4	19.5	11.9
		H	11.6	7.7	54.7	15.5	10.5
2.0–2.5	1.019–1.024	C	31.8	13.6	11.4	25.8	15.7
		H	39.1	14.9	4.5	26.6	14.9
4.0–4.5	1.039–1.046	C	27.6	10.6	10.7	27.0	24.1
		H	29.7	10.7	8.3	25.4	25.8
6.0–6.5	1.076–1.087	C	28.7	5.8	1.3	32.0	32.0
		H	27.5	5.9	1.2	29.5	35.8
7.5–8.0	1.115–1.130	C	22.7	3.0	1.1	27.6	45.6
		H	19.9	5.2	1.1	24.9	48.9

Values, expressed as percentages by weight of total lipoprotein, are those obtained from the pools of plasmas isolated at t_3 (i.e., at the end of the experiment) from control (C) animals and badgers receiving the cholesterol-containing diet (H). See text for experimental conditions.

tions of the lipoproteins present in each fraction. Thus, in these t_3 samples, the concentration of lipoproteins with $d < 1.015$ g/ml, which may be considered as an approximate measurement of VLDL, reached about 40 mg/dl and 50 mg/dl in C and H animals, respectively. The 1.015–1.065 g/ml class accounted for approximately 620 mg/dl (C badgers) and 1050 mg/dl (H); among these lipoproteins, those of d 1.015–1.033 g/ml represented about 28% in the C group and more than 40% in the H group. Concentrations of the higher components (d 1.065–1.162 g/ml) were about 570 mg/dl (C) and 630 mg/dl (H).

Electrophoresis of lipoproteins. As shown in **Fig. 3**, polyacrylamide gel electrophoresis of the fractions revealed an unusual heterogeneity of the lipoprotein spectrum in the badger, especially in the LDL region. This contrasted strongly with the much simpler profile seen in normal humans using the same analytical conditions.

At t_0, the electrophoretic appearance of the pools representative of the C and H animals were so similar that we have only presented the C pattern. The general trend during the following months may be described both in C and H badgers as a progression of detectable lipoprotein material towards the lower densities. Lipoproteins with $d < 1.013$ g/ml, undetectable at t_0, were evident as two bands in the two groups in the t_3 samples. The prominent band migrated as typical human and badger VLDL, while the second, which was more intensely stained in the H sample, migrated as human IDL. Electrophoresis, under the same conditions, of VLDL prepared from the same samples by conventional preparative ultracentrifugation again led to very similar results, and especially with regard to the presence of the band with IDL mobility in H animals.

It was difficult to relate components migrating in the upper and middle part of the lower gel to precise lipoprotein species. The following points are of note: starting from very similar profiles at t_0, the 1.027–1.055 g/ml interval remained comparable in the two groups until the t_2 maximum of hyperlipidemia, with at least four different bands discernible in each group. At this later date, an extension of detectable lipoprotein material towards the lower densities was noted, especially in the H badgers where it reached the 1.017–1.019 g/ml fraction, containing VLDL and IDL-like migrating components. At t_3, in each of the two groups, the density distribution of detectable lipoprotein remained similar to that noted in the corresponding t_2 sample. However, in both sets of animals, the bands representative of LDL-like migrating lipoproteins were less prominent, particularly in the 1.039–1.055 g/ml fractions. At the same time, the relative importance of the different components in the 1.027–1.055 g/ml interval was distinct in the C and H badgers.

Lipoprotein material with mobility intermediary between LDL and HDL positions was present in all samples examined and its intensity was greater at the later dates. These lipoproteins presented as a spectrum, with the distance of migration of the leading component increasing with density. Typical HDL-like migrating material was present in the 1.039–1.046 g/ml fraction in the t_0 samples, but at densities greater than 1.055 g/ml in subsequent samples from the two groups of animals. These latter lipoproteins appeared to reach their maximal intensity of staining over a density interval as wide as 1.065–1.146 g/ml at t_0, but only over the 1.076–1.130 g/ml region at t_3.

Estimation of the apoprotein B-like content of subfractions of LDL. Using the chromatographic technique presented in the Materials and Methods section, estimation of apoB was conducted on two different subfractions, each obtained by density gradient ultracentrifugation of the two pools of plasmas originating, respectively, from C and H animals at t_3. These two subfractions

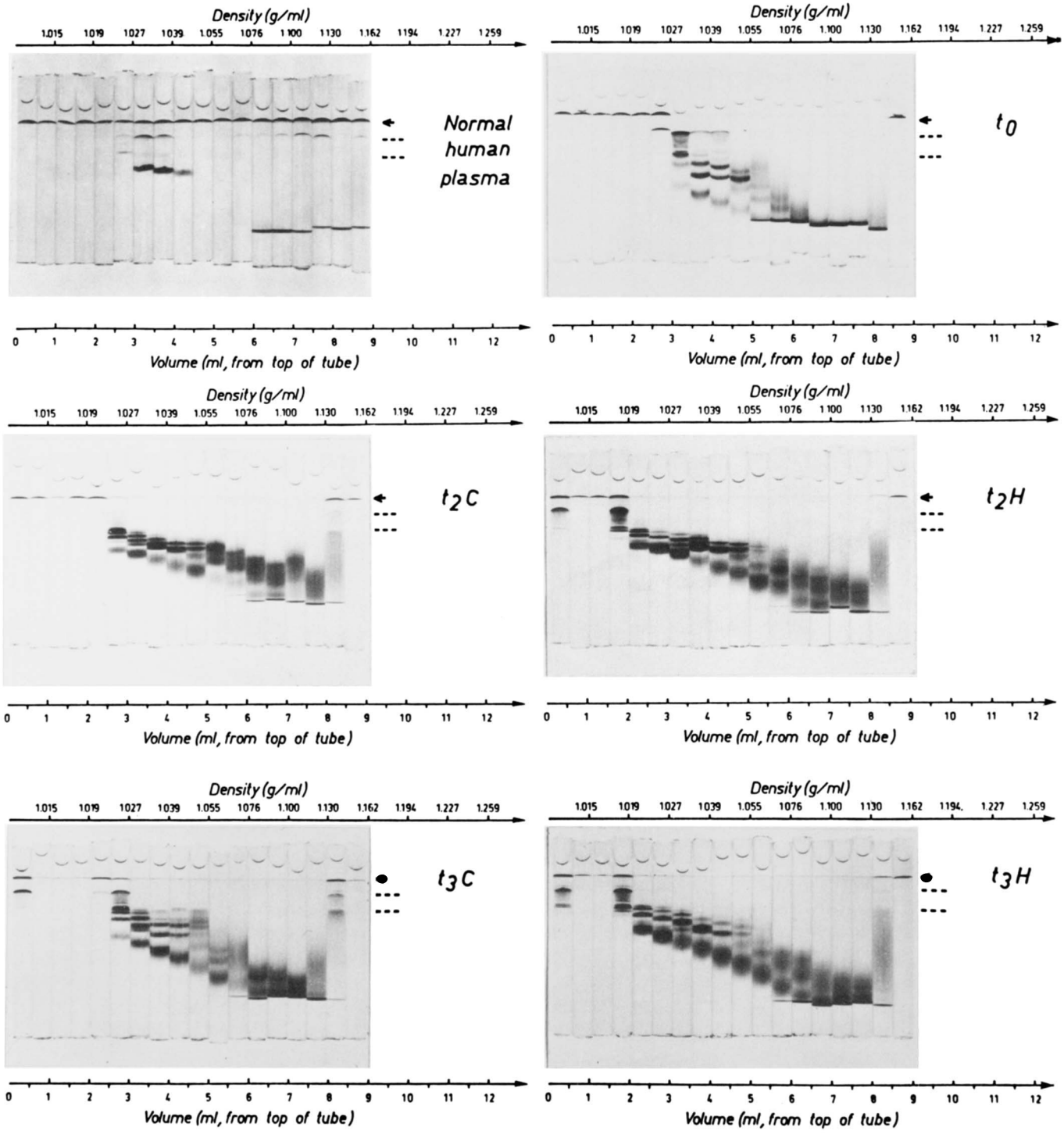

Fig. 3. Polyacrylamide gel electrophoresis of the fractions obtained by density gradient ultracentrifugation from the same pools described in the legend to Fig. 2. For comparative purposes, a typical profile obtained when assaying a normal human plasma is also shown. On each photograph, the direction of migration is from top to bottom; arrows indicate the position of layers; upper and lower dotted lines correspond to limits between 1st and 2nd, and 2nd and 3rd gels, respectively. The t_0 profile is representative of the two pools of badgers in October (i.e., before the induction of the hypercholesterolemic diet). The t_2C and t_2H profiles are of pools from the control animals and badgers having received the hypercholesterolemic diet, respectively, in December; the t_3C and t_3H profiles are typical of the same animals in January.

corresponded, respectively, to the density intervals 1.019–1.027 and 1.039–1.046 g/ml, and represented the two major LDL components. The proportion of apoB in the protein moiety was, in C and H animals, respectively, 18.8% and 10.2% in the 1.019–1.027 g/ml region, and 24.2% and 23.3% in the 1.039–1.046 g/ml interval.

Electrophoresis of TMU-soluble apoproteins. **Fig. 4** shows typical patterns of the TMU-soluble apoprotein components of lipoproteins of different densities. The results are generally consistent with those presented in a previous paper (19). As concluded from data obtained by chemical analyses or lipoprotein electrophoresis, the C and H pools were very similar at t_0, except that a band with migration characteristics similar to that of human apoprotein D was observed only in pool C; this component was not apparent in subsequent samples from either C or H animals. On the other hand, the following peptides were noted: band I, a component migrating similarly to human apoprotein C-I; band II, possibly related to apoprotein A-IV as described by Swaney, Reese, and Eder (33); band III, migrating as apoprotein E; band IV, exhibiting an electrophoretic behavior comparable to that of apoprotein A-I; and bands V, VI, VII, and VIII, which may represent counterparts to apoproteins C-II and different forms of C-III. Band I was present only in trace amounts and was typical of the middle region of the HDL density spectrum. Band II, always present in low concentration, was most evident in the 1.100–1.162 g/ml interval while band III appeared both in low and high density regions. Band IV was the prominent component in all the fractions; however, its relative contribution was obviously decreased in the 1.032–1.055 g/ml region, most probably to the benefit of apoprotein B. Finally, among the bands related to equivalents to apoprotein C-II and different forms of apoprotein C-III, those possibly representative of C-III_0 and C-III_1 were the most intensely stained throughout the density spectrum.

When looking at the results from the t_1, t_2, and t_3 experiments, very few qualitative or semi-quantitative modifications seem to have been induced either by the spontaneous or alimentary hypercholesterolemias. The C profile at t_3, as well as those obtained at t_1 and t_2, was very similar to that in the first series of samplings, with the possible exception of a decrease in the concentration of the component responsible for band III in the low density region. The increased amount of lower density lipoprotein material allowed us to examine the d < 1.013 g/ml top fraction, as well as that with d 1.019–1.023 g/ml; this latter fraction exhibited a profile similar to that in the adjacent one (d 1.023–1.027 g/ml). The top 0.5 ml was characterized by the prominence of the faster-migrating apoprotein C-like components, together with a non-negligible amount of an apoA-I-like peptide.

No marked differences could be discerned between the C and H profiles at the end of the diet experiment, except that the supplementary increase in lipoproteins with d 1.017–1.019 g/ml in the H animals allowed us to examine this fraction; results were again very comparable with those obtained in the adjacent sample of higher density. As for lipoprotein electrophoresis, lipoproteins

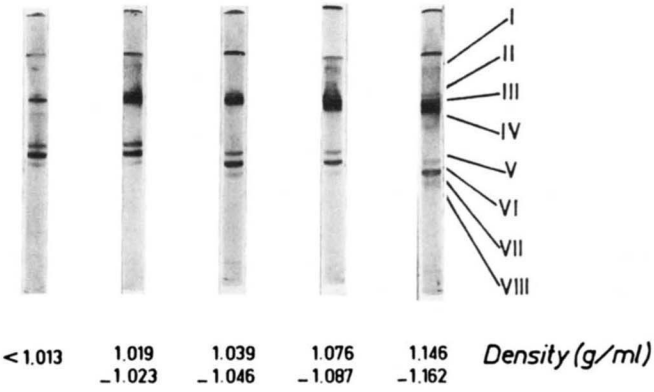

Fig. 4. TMU-soluble apoproteins in some representative fractions obtained by density gradient ultracentrifugation from the pool of plasmas obtained from H animals at t_3. As detailed in the text, all profiles noted at the successive dates of experiment, irrespective of the group of animals concerned, were qualitatively and semi-quantitatively very similar with respect to their TMU-soluble apoprotein content in all fractions examined. For this reason, only one example is shown. Approximately 70 μg of total protein was applied to each gel. After completion of electrophoresis, gels were fixed in 10% trichloroacetic acid, stained with 0.5% Coomassie brilliant blue and destained for about 48 hr in 10% trichloroacetic acid.

with d < 1.006 g/ml (VLDL) from the same animals were examined for their content of TMU-soluble apoproteins; results were entirely consistent with those in the d < 1.013 g/ml density gradient fraction.

Plasma thyroxine concentrations

The evolution of plasma thyroxine levels was very similar in the two groups of badgers during our experiments (**Fig. 5**), recorded values comparing well with those presented previously (20). Although not presented, values determined in February showed an almost twofold increase, an observation equally consistent with the published data.

DISCUSSION

The interest of the present study is twofold. Thus, the use of density gradient ultracentrifugation has provided interesting supplementary information on the spontaneous changes occurring during seasonal hyperlipidemia in badger plasma lipoproteins, and has revealed the modifications induced by a hypercholesterolemic diet in this animal during late fall.

The former changes are in general agreement with our previous results (19), the simultaneous maxima of plasma cholesterol, phospholipids, and triglycerides occurring at t_2. However, from a quantitative viewpoint, cholesterol and phospholipids, although still largely predominating over triglycerides, attained more moderate levels than those noted in some past years. This may be accounted for by variations which tend to occur between successive years, and possibly by the influence of environmental factors; alternatively, it may be considered

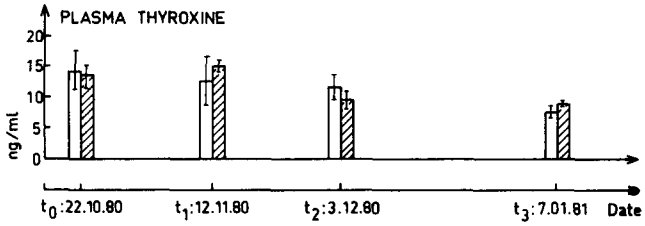

Fig. 5. Plasma thyroxine concentrations, determined at the different dates of sampling. Values are means ± SD from groups of six badgers. Open areas: control animals; hatched areas: animals receiving the hypercholesterolemic diet.

that, owing to the suddenness and amplitude of plasma lipid variations in the badger during winter, the precise date of the maximum in lipid levels was either before or after the 3rd of December, the latter being the actual date of sampling for this month.

Density gradient centrifugation provided a more precise view of the detailed chemical composition of the different parts of the lipoprotein spectrum than did conventional preparative methods. For example, data obtained at t_3 from C animals showed that the lipid composition of the lipoproteins isolated from the successive 0.5-ml fractions over the 1.019–1.046 g/ml interval was relatively constant, while the upper part of the conventional LDL density class (i.e., 1.046–1.065 g/ml) exhibited a net diminution in its glyceride content.

Electrophoresis of the various subfractions provided evidence for a rather unusual complexity that was especially apparent in the low density region. Multiple bands migrating in the region typical of LDL were consistently noted; the tests described in the Materials and Methods section, considered together with the representative normal human profile, make it unlikely that this phenomenon is artifactual. The presence of large amounts of lipoprotein material with electrophoretic mobility intermediary between the typical positions of LDL and HDL is also consistent with our previous data. Finally, it must be kept in mind that our electrophoretic technique is unable to resolve HDL_2 from HDL_3 in human plasma and therefore the single band observed in the badger fractions migrating as HDL is likely to represent the sum of different components.

With particular regard to the heterogeneity noted in the LDL region, it must be kept in mind that at least a part of this phenomenon may be related to the seasonally low levels of plasma thyroxine noted in our two groups of animals. Indeed, it has long been known that, in man, hypothyroidism is associated both with hypercholesterolemia and with increased concentrations of LDL. Recently, Ballantyne et al. (34) have studied the plasma concentration and chemical composition of three subfractions of LDL, with S_f 10.4–20, 5.7–12, and 3.5–6.5, in primary hypothyroid patients. These authors showed that, in such patients, the elevation in plasma LDL concentration was essentially accounted for by an increase in lighter lipoproteins (S_f 10.4–20) and, to a lesser degree, in S_f 5.7–12 components. This resulted in the concomitant presence of large amounts of the three different subfractions.

However, the main purpose of this study was to examine the influence of a hypercholesterolemic diet on plasma lipoprotein patterns. It is classical to compare atherosclerosis-resistant animals, such as the dog (10) and rat (8), in which most of plasma cholesterol is normally transported in HDL, and atherosclerosis-susceptible species such as the swine (11) and the *Erythrocebus patas* monkey (3), in which LDL is the predominant cholesterol carrier. According to season, the European badger belongs alternatively to each of these categories with regard to its plasma lipoprotein pattern. The present study was conducted during the late fall period of the year, when plasma cholesterol reaches values as high as 500–600 mg/dl, while LDL and LDL-cholesterol are elevated and while plasma thyroxine levels are low (about two to three times lower than in late summer (20)). On the other hand, at the beginning of the year, plasma thyroxine and testosterone increase once more (35), while plasma lipid levels decrease; at the same time, the ratio of the concentrations of VLDL + LDL/HDL reverse, largely as a result of a decrease in the former lipoproteins (19). All these metabolic events combine to make the end-of-winter badger quite distinct from its autumnal status. It thus seemed logical to terminate the dietary experiment in January in order to draw conclusions from data obtained in animals under given metabolic conditions.

Comparison of the plasma lipid concentrations in C and H animals suggested that the seasonal and the diet-induced hypercholesterolemias may superimpose during the experiment. The elevations in cholesterol and phospholipids occurred simultaneously in the two groups, although they were more marked in animals in the H group. The quasi-identity of the two groups at t_0, considered together with the subsequent highly significant differences ($P < 0.01$) consistently observed in plasma cholesterol and phospholipid concentrations between these same groups as early as t_1, favor the argument that the amount of cholesterol in the diet of the H group was sufficient to induce a nonphysiological hypercholesterolemia. Of particular interest was the significant ($P < 0.05$) decrease seen in plasma cholesterol in the H group at t_3, despite the continuation of the hypercholesterolemic diet until that date. This finding was equally consistent with the suggestion that the H badgers continued to follow their natural annual cycle of plasma lipids even though submitted to nonphysiological nutritional conditions.

The lower density portion of the plasma lipoprotein pattern, i.e., the d < 1.013 g/ml fraction obtained from

density gradient experiments, was closely related to conventional VLDL as judged by electrophoresis and lipid composition. These lipoproteins remained of minor quantitative importance in the two sets of animals, even during the t_2 maximum of hyperlipidemia. Only at t_3 was the amount of these components slightly increased, but this occurred in an almost similar manner in the C and H animals. On the other hand, the hypercholesterolemic diet resulted in a twofold increase in the cholesterol content (as cholesteryl ester) in these VLDL. It is of note that the chemical composition of these lipoproteins was somewhat different from that observed earlier (19). This is especially true in respect to the esterified/total cholesterol ratio which was lower in the C animals in the present study than in previous measurements; the reason for this discrepancy is unknown. Another noticeable feature of H badgers was the presence of d < 1.013 g/ml, and even d < 1.006 g/ml, material migrating in the position of IDL. Abnormal lipoproteins with similar electrophoretic behavior were observed with the same technique in sera from patients with type III hyperlipoproteinemia; thus, their equivalent in the cholesterol-fed badger could be related to the β-VLDL observed in certain animal species when a similar type of diet is administrated (18). However, the TMU-soluble apoproteins in the d < 1.013 g/ml fraction in the two sets of animals resembled each other and, in addition to counterparts to the C apoproteins, contained a prominent component migrating like apoprotein A-I rather than as apoprotein E, the latter being typically observed in β-VLDL (18).

The main difference between C and H animals is undoubtedly in the progressive increase in diet-induced low density, cholesterol-rich lipoproteins in the d 1.019–1.027 g/ml region; although of slightly higher density, these components may be correlated with the IDL whose increase is a consistent feature in cholesterol-fed animals (18). Similarly consistent with findings in other species was the increase in H (as compared to C) animals of as much as 45% in LDL of d 1.039–1.046 g/ml at t_3.

Particular reference could be made at this stage to the report of Rudel, Shah, and Greene (7), regarding the changes induced by cholesterol feeding in the plasma lipoprotein spectrum of the Rhesus monkey (*Macaca mulatta*). In this latter species, such a dietary experiment resulted in a considerable increase in the plasma concentration of the d < 1.006 g/ml and d 1.006–1.063 g/ml lipoprotein material. The former density range was heterogeneous, as it exhibited three different subfractions that migrated in the β and pre-β regions on agarose electrophoresis. All of them were cholesteryl ester-rich, while one was of particular interest as it contained no detectable apoprotein E. The d 1.006–1.063 g/ml interval equally included different cholesteryl ester-rich lipoprotein components, these belonging to two successive fractions on agarose chromatography. Both fractions were greatly increase by cholesterol feeding, that corresponding to LDL containing as much as 80% of total plasma cholesterol. However, an important difference between these monkeys and the badger is that, in this latter species, the apoB content of the whole LDL density class appeared low (primarily to the benefit of an apoA-I-like component), and furthermore decreased in LDL of lower density in cholesterol-fed animals. The precise significance of these observations remains to be assessed.

A fourth characteristic observed in most mammals fed diets with a high cholesterol content is the occurrence of HDL_c, a spectrum of cholesterol- and apoprotein E-rich lipoproteins which extends from the HDL density interval to lower density regions (36). This situation contrasts with that in the badger in which the electrophoretic equivalent (band III) of apoE remained of little quantitative importance irrespective of diet. At the same time, band IV, which corresponds to a protein akin to human apoprotein A-I,[4] was present throughout the density spectrum in both C and H animals.

The fifth characteristic of cholesterol-fed animals is the tendency to reduction in concentrations of their HDL of higher density, a phenomenon that may be related to the formation of HDL_c. Such a diminution was evident in our animals since the ratio of concentration of d 1.065–1.100 g/ml to d 1.100–1.162 g/ml components at t_3 was about 1.4 in C animals as compared to 1.9 in the H group.

From the present study, several intriguing questions arise regarding a number of phenomena, such as the unusual heterogeneity of LDL as well as the ubiquitous presence of apoprotein A-I and consequently the metabolism of chylomicrons and chylomicron remnants. A study of the mechanisms by which the reverse plasma transport of large amounts of cholesterol may occur without apparent need for large concentrations of apoprotein E-rich lipoproteins will be of similar interest. Further studies dealing with those problems are currently in progress in our laboratory.

Dr. M. J. Chapman is gratefully acknowledged for helpful criticism. The authors wish to thank Mr. J. C. Fage and Mr. J. P. Rambaut for their excellent technical assistance.

Manuscript received 16 June 1981 and in revised form 1 February 1982.

REFERENCES

1. Blaton, V., and H. Peeters. 1976. The nonhuman primates as models for studying human atherosclerosis: studies on the chimpanzee, the baboon and the rhesus macacus. *Adv. Exp. Med. Biol.* **67:** 33–64.
2. Lee, J. A., and M. D. Morris. 1976. The effect of cholesterol feeding on primate serum lipoproteins. I. Low den-

[4] Laplaud, P. M. Unpublished data.

sity lipoprotein characterization from rhesus monkeys with high serum cholesterol. *Biochem. Med.* **16:** 116–126.
3. Mahley, R. W., K. H. Weisgraber, and T. Innerarity. 1976. Atherogenic hyperlipoproteinemia induced by cholesterol feeding in the Patas monkey. *Biochemistry.* **15:** 2979–2985.
4. Srinivasan, S. R., C. C. Smith, B. Radhakrishnamurthy, R. H. Wolf, and G. S. Berenson. 1976. Phylogenetic variability of serum lipids and lipoproteins in nonhuman primates fed diets with different contents of dietary cholesterol. *Adv. Exp. Med. Biol.* **67:** 65–75.
5. Nelson, C. A., and M. D. Morris. 1977. Effects of cholesterol feeding on primate serum lipoproteins. II. Low density lipoprotein characterization from rhesus monkeys with a moderate rise in serum cholesterol. *Biochem. Med.* **17:** 320–332.
6. Rudel, L. L., L. L. Pitts, and C. A. Nelson. 1977. Characterization of plasma low density lipoproteins of nonhuman primates fed dietary cholesterol. *J. Lipid Res.* **18:** 211–222.
7. Rudel, L. L., R. Shah, and D. G. Greene. 1979. Study of the atherogenic dyslipoproteinemia induced by dietary cholesterol in rhesus monkeys (*Macaca mulatta*). *J. Lipid Res.* **20:** 55–65.
8. Mahley, R. W., and K. S. Holcombe. 1977. Alterations of the plasma lipoproteins and apoproteins following cholesterol feeding in the rat. *J. Lipid Res.* **18:** 314–324.
9. Shore, B., and V. Shore. 1976. Rabbits as a model for the study of hyperlipoproteinemia and atherosclerosis. *Adv. Exp. Med. Biol.* **67:** 123–141.
10. Mahley, R. W., K. H. Weisgraber, and T. Innerarity. 1974. Canine lipoproteins and atherosclerosis. II. Characterization of the plasma lipoproteins associated with atherogenic and nonatherogenic hyperlipidemia. *Circ. Res.* **35:** 722–733.
11. Mahley, R. W., K. H. Weisgraber, T. Innerarity, H. B. Brewer, Jr., and G. Assmann. 1975. Swine lipoproteins and atherosclerosis. Changes in the plasma lipoproteins and apoproteins induced by cholesterol feeding. *Biochemistry.* **14:** 2817–2823.
12. Tall, A. R., D. Atkinson, D. M. Small, and R. W. Mahley. 1977. Characterization of the lipoproteins of atherosclerotic swine. *J. Biol. Chem.* **252:** 7288–7293.
13. Puppione, D. L., C. Sardet, W. Yamanaka, R. Ostwald, and A. V. Nichols. 1971. Plasma lipoproteins of cholesterol-fed guinea pigs. *Biochim. Biophys. Acta.* **231:** 295–301.
14. Chapman, M. J., and G. L. Mills. 1977. Characterization of the serum lipoproteins and their apoproteins in hypercholesterolemic guinea pigs. *Biochem. J.* **167:** 9–21.
15. Clarkson, T. B. 1972. Animal models of atherosclerosis. *Adv. Vet. Sci. Comp. Med.* **16:** 151–173.
16. Kritchevsky, D. 1975. Animal models for atherosclerosis research. In Hypolipidemic Agents. D. Kritchevsky, editor. Handbook of Experimental Pharmacology, Springer, New York. **41:** 215–227.
17. Mills, G. L. 1976. Lipoproteins in animals. *In* Handbuch der Inneren Medizin, Band VII/4, Fettstoffwechsel. G. Schettler, H. Greten, G. Schlierf, and D. Seidel editors. Springer Verlag, Berlin. 173–195.
18. Mahley, R. W. 1978. Alterations in plasma lipoproteins induced by cholesterol feeding in animals including man. *In* Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, A. M. Gotto, Jr. and J. A. Ontko, editors. American Physiological Society, Bethesda, MD. 181–197.
19. Laplaud, P. M., L. Beaubatie, and D. Maurel. 1980. A spontaneously seasonal hypercholesterolemic animal: plasma lipids and lipoproteins in the European badger (*Meles meles* L.). *J. Lipid Res.* **21:** 724–738.
20. Maurel, D., and J. Boissin. 1979. Seasonal variations of thyroid activity in the adult male badger (*Meles meles* L.). *Gen. Comp. Endocrinol.* **38:** 207–214.
21. Fruchart, J. C. 1976. Exploration biochimique des lipides et des lipoprotéines. *In* Lipides et lipoprotéines. G. Sezille, J. C. Fruchart, J. Jaillard, P. Dewailly, editors. Crouan et Roques, Lille, France. 92–95.
22. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
23. Chapman, M. J., S. Goldstein, D. Lagrange, and P. M. Laplaud. 1981. A density gradient ultracentrifugal procedure for the isolation of the major lipoprotein classes from human serum. *J. Lipid Res.* **22:** 339–358.
24. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
25. Chapman, M. J., and S. Goldstein. 1976. Comparison of the serum low density lipoprotein and of its apoprotein in the pig, rhesus monkey and baboon with that in man. *Atherosclerosis.* **25:** 267–291.
26. Herbert, P. N., R. S. Shulman, R. I. Levy, and D. S. Fredrikson. 1973. Fractionation of the C-apoproteins from human plasma very low density lipoproteins. *J. Biol. Chem.* **248:** 4941–4946.
27. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4411.
28. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
29. Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **121:** 404–427.
30. Chapman, M. J., G. L. Mills, and J. H. Ledford. 1975. The distribution and partial characterization of the serum apolipoproteins in the guinea pig. *Biochem. J.* **149:** 423–436.
31. Vigouroux, E. 1972. Radiodosage ultrasensible de la thyroxine par la méthode de compétition. *C.R. Acad. Sci. Paris.* **275 D:** 579–582.
32. Mann, H. B., and D. R. Whitney. 1947. On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Stat.* **18:** 50–60.
33. Swaney, J. B., H. Reese, and H. A. Eder. 1974. Polypeptide composition of rat high density lipoprotein: characterization by SDS-gel electrophoresis. *Biochem. Biophys. Res. Commun.* **59:** 513–519.
34. Ballantyne, F. C., A. A. Epenetos, M. Caslake, S. Forsythe, and D. Ballantyne. 1979. The composition of low density lipoprotein and very low density lipoprotein subfractions in primary hypothyroidism and the effect of hormone replacement therapy. *Clin. Sci.* **57:** 83–88.
35. Maurel, D., J. Joffre, and J. Boissin. 1977. Cycle annuel de la testostéronémie et de la thyroxinémie chez le Blaireau européen (*Meles meles* L.). *C.R. Acad. Sci. Paris.* **284 D:** 1577–1580.
36. Mahley, R. W., K. H. Weisgraber, T. P. Bersot. and T. L. Innerarity. 1978. Effects of cholesterol feeding on human and animal high density lipoproteins. *In* High Density Lipoproteins and Atherosclerosis. A. M. Gotto, Jr., N. E. Miller and M. F. Oliver, editors. Elsevier, Amsterdam. 149–176.