A spontaneously seasonal hypercholesterolemic animal: plasma lipids and lipoproteins in the European badger (*Meles meles* L.)

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 The European badger has previously been shown to exhibit yearly cycles of locomotor activity, endocrine secretions, and body weight, as well as seasonal variations in plasma cholesterol. Over a period of 2 years, we have followed the plasma levels of free and esterified cholesterol, triglycerides and phospholipids, and of plasma lipoproteins (by means of polyacrylamide gel electrophoresis, agarose column chromatography and preparative and analytical ultracentrifugation). Some preliminary observations on the qualitative characteristics of the plasma apoproteins, obtained by application of electrophoretic techniques, are also described. Our results provide evidence for considerable synchronous and spontaneous variations of each of the plasma lipid components studied, all of them reaching a maximum in late autumn/early winter, then decreasing to a minimum in early spring. In some animals, the amplitude of observed variations was as large as 650% for total cholesterol, 420% for phospholipids and 180% for triglycerides. While the plasma concentration of very low density lipoproteins ($d < 1.006$ g/ml) remained at low or moderate levels, major changes in the lipoprotein spectrum occurred in the low density (1.006–1.063 g/ml) and high density (1.063–1.21 g/ml) lipoproteins, these two classes exhibiting marked heterogeneity. This led to an autumn/winter prominence of the 1.006–1.063 g/ml components and of those in the lower part of the high density range, with an enrichment in cholesterol in lipoproteins in the low density region. These phenomena occur simultaneously and/or immediately after the annual minimum of plasma thyroxine concentration in the species considered. In contrast, early spring patterns displayed more classical features with higher density lipoproteins predominating. Our findings thus suggest that the badger may provide a useful model for future experiments regarding the hormonal regulation of plasma lipid transport as well as the metabolism and physiopathological implications of some cholesterol-rich lipoproteins.—**Laplaud, P. M., L. Beaubatie, and D. Maurel.** A spontaneously seasonal hypercholesterolemic animal: plasma lipids and lipoproteins in the European badger (*Meles meles* L.). J. Lipid Res. 1980. **21:** 724–738.

Supplementary key words electrophoresis · ultracentrifugation · apoproteins · agarose column chromatography · cholesterol-rich lipoproteins

Studies on animal plasma lipoproteins are not only of proven interest in comparative biochemistry, but also provide models which may permit a better understanding of human physiopathological processes, such as atherosclerosis. A considerable variety of species have been more or less extensively studied and, in recent years, several research groups have presented their results, including comparative studies of a wide range of mammals, birds, reptiles, and fish (1–3).

However, relatively few authors have been concerned with the plasma lipids of animals, such as hibernators, or species exhibiting marked seasonal variations of weight or activity. Among these studies, those describing the distribution of plasma lipoproteins are rare; moreover, in such studies lipoproteins were often assayed at only one period of the year, generally with the use of electrophoretic methods which provide a rather simplified view of lipoprotein profiles.

The European badger (*Meles meles*) is a wild mammal which lives underground in an extensive branched system of burrows and offers a general locomotor activity exclusively nocturnal. While not a hibernator in temperate climates, this animal modulates its activity according to the season; the general activity

Abbreviations: VLDL, very low density lipoproteins, density < 1.006 g/ml; LDL, low density lipoproteins, density 1.006–1.063 g/ml, unless otherwise defined; IDL, intermediate density lipoproteins, a part of LDL with density 1.006–1.019 g/ml; HDL, high density lipoproteins, density 1.063–1.21 g/ml, subdivided into HDL_2, density 1.063–1.125 g/ml and HDL_3, density 1.125–1.21 g/ml; EDTA, ethylenediaminetetraacetic acid; S_f, flotation coefficient in a NaCl medium of density 1.063 g/ml; F, flotation coefficient in a NaCl/NaBr medium of density 1.21 g/ml; SDS, sodium dodecyl sulfate; TMU, tetramethylurea.

[1] Drs. Laplaud and Beaubatie.

[2] Dr. Laplaud.

[3] Dr. Maurel.

out of the burrow is less important in the winter. From a seasonal view point, too, the badger will show a clearly marked body weight cycle, becoming fatter during the cold months (4). Furthermore, Johansson (5) has shown that considerable variations occur in plasma cholesterol concentration in this species according to the period of the year. It thus seemed likely that the whole plasma transport system of lipids in the badger was submitted to largely varying loads during the year, and therefore it appeared of interest to perform a study of the qualitative and quantitative variations of the plasma lipids and lipoproteins over the period of a year in this species.

As part of our research program dealing with the plasma lipoproteins of some wild European mammals, which we consider as tools for comparative biochemical studies and as possible sources of findings regarding lipoprotein metabolism and the pathogenesis of atherosclerosis, we describe in this report our first series of investigations, performed during the period from March 1977 to March 1979.

MATERIALS AND METHODS

Animals

The adult male badgers used, bred in the Centre d'Etudes Biologiques des Animaux Sauvages, were approximately 2–6 years old; they were maintained under natural conditions of temperature and photoperiodism. The animals were offered a diet consisting primarily of 25–30 one-day-old chickens per day. The chickens were specially hatched on a standard farm. This resulted in a nutrient amount of approximately 600–700 calories, 60–75 g protein, and 35–40 g fat per animal per day. Occasionally, and exclusively in summer, this diet was supplemented with an ear of corn, thus increasing the daily food intake by approximately 200 calories and 40 g CHO, plus a small amount of vegetable protein and lipids. The food consumption of the animals appeared rather constant all along the year. As their body weights varied largely according to the season, the daily intake expressed as an amount/kg/day seems variable, but it should be borne in mind that the increase in body weight in the badger during the cold season results almost exclusively from an increase in adipose tissue.

Collection and treatment of blood

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

Chemical analysis

All of the following assays were performed on a semi-automated LKB Ultrolab analytical apparatus (LKB Instruments, Bromma, Sweden). 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); cholesteryl ester concentration was taken as (total cholesterol concentration–unesterified cholesterol concentration) × 1.68. Triglycerides were estimated by an enzymatic method (commercial kit, Roche Diagnostica ref. 1070) that determines the total glycerol content of the sample. Plasma free glycerol was not determined. The term "triglycerides" herein designates the plasma content of triglycerides plus free glycerol, and thus constitutes an overestimate. Phospholipids were measured by a conventional technique (6) involving mineralization in a perchloric and nitric acid medium, and addition of ammonium molybdate and vanadate as color reagent; absorption was subsequently measured at 405 nm. All the determinations were made in duplicate. The precision of the assays was determined and expressed as the technical error, defined as $\sqrt{\Sigma d^2/2N}$ where d is the difference between duplicate estimations, and N the number of duplicates. The values obtained, when related to the mean of all the assays of a specific lipid component, led to the following values of the coefficients of variation: total cholesterol, ±1.07%; unesterified cholesterol, ±1.17%; triglycerides, ±2.52%; phospholipids, ±1.92%. Protein measurements were made by the method of Lowry et al. (7), using bovine serum albumin as standard.

Gradient gel electrophoresis of plasma lipoproteins

Polyacrylamide gel electrophoresis in a three-step gradient was performed according to the method of Fruchart (8), 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. The gels were scanned with a Densicomp 445 densitometer (Clifford Instruments, Natick, MA). 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 lipoproteins by this procedure.

Column chromatography

Chromatography was carried out according to the method of Rudel et al. (9) after lipoproteins were isolated from a plasma pool by ultracentrifugation. For this purpose, 2 ml of plasma was mixed in each tube with 4 ml NaBr (4.929 molal), thus attaining a density at 26°C of 1.225 g/ml. Centrifugation was then carried out in a MSE Superspeed 65 ultracentrifuge, (MSE, Crawley, England) using an aluminum fixed-angle rotor, (capacity 8 × 14 ml), for 36 hr at 40,000 rpm (100,000 *g*-avg) and 17°C. After completion of the run, the background salt density of the top 1 ml had decreased to about 1.21 g/ml and the floating lipoproteins were removed by aspiration. As estimated by measurements of cholesterol concentration in whole plasma and in the top fraction thus obtained, the yield of the procedure was consistently found to be more than 92%.

The lipoprotein concentrate (15–25 mg lipoprotein cholesterol) was then applied to a chromatographic column of Bio Gel A-5 m, (200–400 mesh) and 6% (w/v) agarose (Bio Rad Laboratories, Richmond, CA), packed in a 12 × 1000 mm glass column, eluted at 4°C with a solution containing 0.15 M NaCl, EDTA, (0.4 g/l), sodium azide (0.1 g/l), and merthiolate (1 mg/l), at pH 7.0, at a rate of about 6 ml/hr. The separation was monitored at 280 nm with a LKB Uvicord II detector and recorder. Fractions were collected in a LKB 17,000 fraction collector maintained at 4°C.

Fractions resulting from the chromatographic separation were correlated with electrophoretic bands, using the same electrophoretic system described above. Experiments conducted on human lipoproteins were consistent with the findings of Rudel et al. (9), especially with regard to the resolution obtained between LDL and HDL.

Ultracentrifugal methods

In each of the manipulations 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 precision refractometry (Abbe type refractometer, model G, Carl Zeiss Iena, D. D. R., thermostated to ±0.05°C) and later by means of a DMA 46 calculating precision density meter (Anton Paar KG, Graz, Austria).

Preparative ultracentrifugation. Sequential preparative ultracentrifugation, in the same rotor and centrifuge as described above (see Column chromatography), was performed according to established procedures (10).

In cases in which small amounts of chylomicrons were present, plasma samples were first layered with NaCl (0.196 molal), and centrifuged for 30 min at 26,000 *g*-avg, according to Hatch and Lees (11). After completion of the run, the infranatant was aspirated and used for further isolation of the lipoproteins. Polyacrylamide gel electrophoresis of the top fraction consistently showed no band in the VLDL position.

Prior to further chemical or electrophoretic analysis, all lipoprotein fractions were dialyzed in Spectrapor tubing, (Spectrum Medical Industries, Los Angeles, CA, exclusion limit about 3500), for 3 × 12 hr at 4°C against 0.196 molal NaCl, with magnetic stirring of the dialysate. It should be noted that the use of dialysis allowed application of the assay for plasma triglycerides to the determination of the glyceride content of the ultracentrifugally-prepared lipoproteins. The efficiency of the dialysis procedure was checked in the following manner: solutions of glycerol (10–40 mg/100 ml) were dialyzed in the same conditions as described above; the absence of glycerol in the retentate was then verified by means of our procedure used for total glycerol quantitation.

Chemical analysis of the fractions was performed using the same techniques described above for measurement of the plasma lipids. Here again, all the determinations were made in duplicate. As determined by means of the technical error (see Chemical analysis), the respective coefficients of variations, calculated for all the measurements of each lipoprotein component, were: ±0.88% for total cholesterol, ±0.91% for unesterified cholesterol, ±1.88% for triglycerides, ±1.75% for phospholipids and ±2.34% for protein.

Analytical ultracentrifugation. Lipoproteins were first isolated by preparative ultracentrifugation. The conditions of Ewing, Freeman, and Lindgren (12) were used for VLDL and LDL; i.e., 3 ml of plasma was mixed with 3 ml of 3.398 molal NaCl, to give a background salt solution of d_{26} = 1.065 g/ml before the run (100,000 *g*-avg, 20 hr, 17°C); this decreased to d_{26} = 1.063 g/ml in the lipoprotein-containing top 1 ml.

The lipoproteins with hydrated density less than 1.21 g/ml were obtained by centrifugation in medium of density 1.21 g/ml under the experimental conditions described previously for the preparation of lipoproteins for column chromatography. In each type of manipulation, the second 1 ml of each tube was used for the monitoring of density.

Analytical runs were then carried out on a MSE Centriscan 75 analytical ultracentrifuge, operating in the refractometric mode at 550 nm. A pair of 10

mm single-sector cells was used, one of the cells was filled with the lipoprotein solution to be studied and the second one was filled with the NaCl or NaBr solution corresponding to the background density of the sample. Conditions employed in the runs were 52,000 rpm (196,000 *g*-avg) and 26°C; the up-to-speed time was about 13 min. A wedge-window cell was used for checking the reproducibility of the settings of the optical system, at a constant selected knife-edge angle of 50°.

For the low-density runs (i.e., d = 1.063 g/ml) scans were taken at 9, 16, and 40 min, respectively, after the beginning of the run, thus allowing analysis of VLDL of S_f 100–400, VLDL of S_f 20–100 and LDL of S_f 0–20. In the high-density run, scans were taken at 30, 40, 50, and 60 min. Immediately after each scan, the knife-edge of the optical system was withdrawn from the light beam pathway and another scan taken, thus giving the position of the corresponding baseline.

Determination of the plasma lipoprotein concentrations from analytical scans involved calibration of the machine by spinning solutions of bovine serum albumin (Sigma) in NaCl (0.15 M) at concentrations from 6 to 10 mg/ml under the same conditions as outlined above, and measurement by numerical integration of areas inscribed between the curves and baselines. Reproducibility was assayed on three series of five manipulations and consistently found to be greater than 98.5%.

The proportionality between the areas and concentrations of bovine serum albumin and of the different classes of lipoproteins was then obtained by using the following values for specific refractive increment: 0.00189 Δn/g/100 ml for albumin, 0.00154 Δn/g/100 ml for VLDL and LDL, and 0.00149 Δn/g/100 ml for HDL. These values are those proposed by Lindgren, Freeman, and Ewing (13) for human serum lipoproteins. Areas were standardized by correcting for knife-edge angle influence and radial dilution; flotation coefficients were corrected for the viscosity and density of the medium, and for concentration-dependence using a K value of $0.89.10^{-4}$ $(mg/100\ ml)^{-1}$.

Electrophoresis of apolipoproteins

After sequential preparative ultracentrifugation and dialysis as outlined above, the protein components of badger plasma lipoproteins were studied in two electrophoretic systems. After delipidation with ethanol–diethyl ether 3:1 (v/v) essentially as described by Chapman, Mills, and Ledford (14), the molecular weights of the apoproteins were estimated by electrophoresis in SDS-polyacrylamide gel according to Weber and Osborn (15). TMU-soluble apoproteins were electrophoresed according to the modification of Kane (16) of the procedure of Davis (17). In both cases, gels were subsequently stained with Coomassie Brilliant Blue. For the reasons extensively discussed by Chapman et al. (14), no attempt was made to quantify each band of apoprotein as isolated by these manipulations.

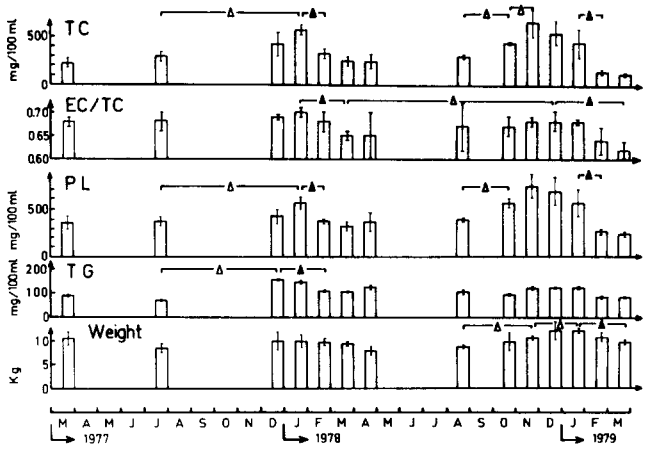

Fig. 1. Graphic representation of seasonal variations in badger body weight and plasma lipids during the 2 years of experiments. Values are means ± SD from a series of four animals. Δ indicates a significant increase, ▲ a significant decrease according to the statistical test of Mann and Whitney (18). TC, total cholesterol; EC/TC, concentration ratio of esterified cholesterol to total cholesterol; PL, phospholipids; TG, triglycerides.

Statistical methods

As a consequence of the small number (i.e., four) of animals in each sampling, the statistical significance ($P < 0.05$) of the results regarding the seasonal variations of the plasma lipids was assayed by means of the non-parametric test of Mann and Whitney (18). For this purpose, each set of data obtained for total and unesterified cholesterol, triglycerides, and phospholipids from each successive series of blood samplings was compared with the preceding and following ones, using the above-mentioned test.

RESULTS

Plasma lipids

Fig. 1 provides evidence for considerable seasonal variation in the lipid components assayed, all of them exhibiting yearly cycles with maxima at the end of autumn or the beginning of winter, minima occurring in early spring. Differences between mean values (expressed in mg/100 ml) as observed at the maximum and minimum of lipidemia are, during the first and second year of our work, respectively, about 264%

TABLE 1. Examples of the chronologic evolution of body weight and plasma lipids in individual animals

Identification	Variable Assayed	Date 1 26.01.78	Date 2 23.03.78	R 1/2	Date 3 29.11.78	Date 4 28.03.79	R 3/4
Badger 18	BW	10.2	10.0		11.7	10.2	
	TC	599	241	2.49	492	129	3.81
	EC/TC	0.70	0.63		0.69	0.61	
	TG	145	99	1.46	142	72	1.97
	PL	525	317	1.66	645	235	2.74
Badger 28	BW	8.5	8.9				
	TC	490	236	2.08			
	EC/TC	0.71	0.65				
	TG	90	85	1.06			
	PL	491	298	1.65			
Badger 52	BW				10.2	10.5	
	TC				788	121	6.51
	EC/TC				0.67	0.64	
	TG				141	78	1.81
	PL				861	205	4.20

BW, body weights; TC, total cholesterol; EC/TC, ratio of esterified cholesterol/total cholesterol; TG, triglycerides; PL, phospholipids; R 1/2, ratio of the concentration value of a particular component at date 1 to the same component at date 2; R 3/4, same ratio, between dates 3 and 4.

Body weights are expressed in kg; lipid concentrations are expressed in mg/100 ml.

(580–220) and 457% (640–140) for total cholesterol, 229% (160–70) and 144% (130–90) for triglycerides, and 160% (560–350) and 317% (760–240) for phospholipids.

Statistical analysis of the data obtained in 1977–1978 by means of the Mann-Whitney test shows that a significant increase occurred in all of these lipid components between July 1977 and January 1978, (or December 1977 when values for triglycerides are considered), the corresponding significant decrease taking place during the period from January to February 1978; this diminution continued until April. Thus, in less than a month, cholesterol decreased by more than 40%, phospholipids by more than 30% and triglycerides by about 25%. The same phenomenon was observed during the second year of our study, when the maxima occurred earlier but reached even higher values. As in 1978, the beginning of 1979 saw the same rapid and marked decrease, the mean plasma cholesterol value in February being 64% less than that observed 1 month earlier.

It is however noticeable that triglycerides, while being equally subject to large variations, always remained at moderate levels, leading to nonsignificant changes during the 1978–1979 cycle. On the other hand, consideration of successive mean values for the esterified cholesterol/total cholesterol ratio revealed a small lowering (of the order of 0.03 to 0.05%), in March–April 1978 as well as in February–March 1979, as compared to the corresponding values observed during the two winters.

Finally, it is necessary to call attention to the fact that the body weights of our animals increased in winter, but not to such an extent as one might have expected.

Turning to individual features, although circumstances did not permit the use of the same animals during the 2 years of this study, some of them have been investigated on several successive occasions, thus allowing individual comparisons of the chronologic modifications of their plasma lipid levels. It then becomes apparent that individual variations may sometimes be more striking than one might conclude from examination of the mean values of the series, the largest observed differences concerning total cholesterol, followed by phospholipids, and, to a much lesser degree, by triglycerides. **Table 1** provides some examples of such individual variations, showing that, in some cases, huge differences occur. In badger No. 52, a 651% variation in the total cholesterol level was observed over a 120-day time interval, without loss of body weight.

Electrophoresis of plasma lipoproteins

Typical results are shown in **Fig. 2,** which reveals wide differences in electrophoretic profile at different periods of the year. The electrophoretic spectrum of badger plasma lipoproteins consisted of three main bands. Band 1 had the same migration characteristics as human VLDL and was undetectable in most samples except in autumn/winter. Band 2 appeared to migrate farther than human LDL, although this feature was most noticeable in spring; its intensity varied considerably, being quite faint in spring, then increasing to reach a maximum in winter, when it sometimes became the dominant component. Band 3

exhibited the same electrophoretic behavior as human HDL, and remained intense, irrespective of season, in all the samples assayed. The distance and aspect of the region between band 2 and band 3 altered according to the period of the year. While a shoulder was always present immediately preceding band 3, the upper part of the interval was usually completely clear in early spring. However, small amounts of material appeared in this region during summer and increased during the following autumn to lead to a complete continuity in this part of the electrophoretic distribution. Furthermore, in most animals, several (at least three), distinct supplementary bands were found superimposed on this background, as is evident on Fig. 2D. These additional bands have been shown to persist in individual animals for various periods of time during the decrease of plasma lipids at the end of winter; however, they had completely disappeared in March in all of the badgers examined.

In addition, it is noteworthy that a small amount of chylomicrons was present in the serum of a few animals, regardless of the season. As early as 1959, Tayeau et al. (19) examined the serum lipoproteins of some badgers by paper electrophoresis, samples being presumably obtained in early spring. These authors observed the presence of two bands; one corresponded to the β-lipoproteins and was fainter and migrated farther than its human counterpart, while the α-lipoproteins were much more intense than in man. Thus these results appear quite consistent with our own observations in the same season.

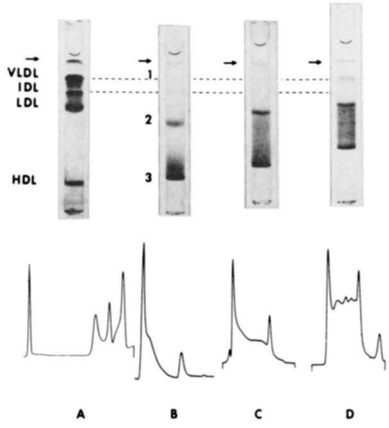

Fig. 2. Polyacrylamide gel electrophoresis of whole plasma lipoproteins after pre-staining with nitro blue tetrazolium. Top, photographs of the electrophoreses; arrows indicate the position of layers; upper and lower dotted lines correspond to the limits between 1st and 2nd, and 2nd and 3rd gels, respectively. Bottom, corresponding densitometric scannings. Direction of migration: photographs, top to bottom; scans, right to left. A, human plasma lipoproteins, from a donor exhibiting hypertriglyceridemia with intermediate density lipoproteins. B, C, D, badger plasma lipoproteins; B, spring profile; C, summer profile; D, late autumn/winter profile. Duration of the migration in sample D has been shortened in order to permit better visualization of bands intermediary between LDL and HDL. Longer migration resulted in a small widening of these bands which, although still slightly visible, became difficult to distinguish from the highly and continuously stained background.

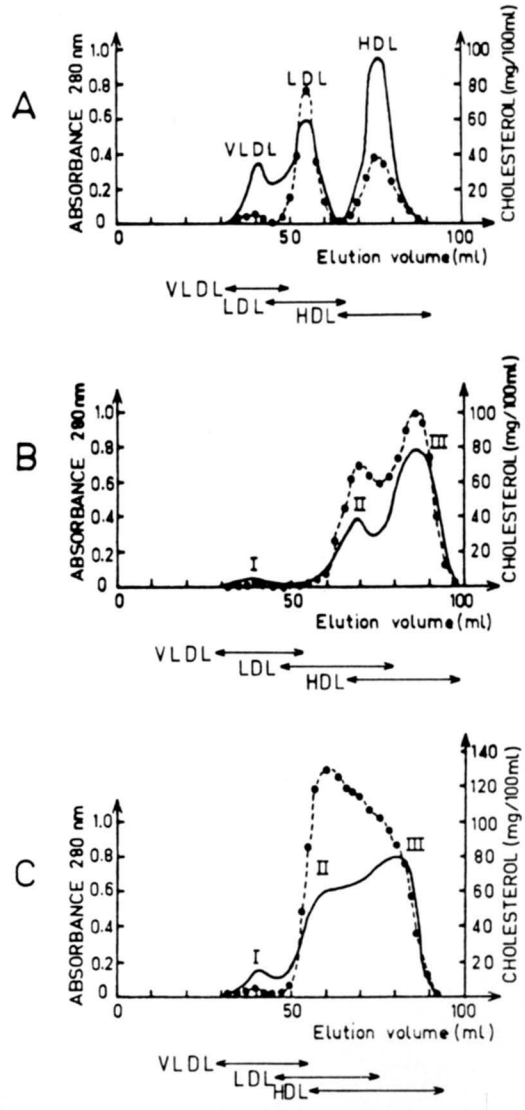

Fig. 3. Typical results from agarose column chromatography of human and badger plasma lipoproteins, the latter isolated from pools of four animals. See text for experimental conditions. Continuous lines, protein absorbance curves at 280 nm; dotted lines, distribution of cholesterol in the fractions. A, human pattern; B, badger pattern, spring; C, badger pattern, autumn. Below each pattern are indicated the regions where the bands with VLDL, LDL, and HDL electrophoretic mobilities were observed when examining the fractions from the corresponding chromatographic experiments.

Column chromatography

As in the electrophoretic patterns, considerable modifications in chromatographic lipoprotein profile occurred during the year. **Fig. 3** shows two typical

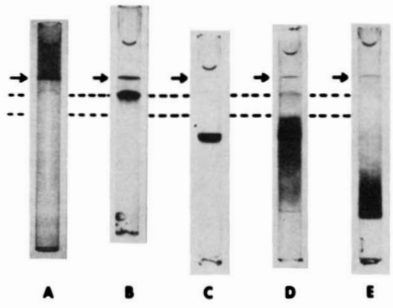

Fig. 4. Polyacrylamide gel electrophoresis of badger plasma lipoproteins, separated by preparative ultracentrifugation; conditions as described in text. Direction of migration, top to bottom. Arrows indicate position of layers; upper and lower dotted lines correspond to limits between 1st and 2nd, and 2nd and 3rd gels, respectively. A, chylomicrons; B, density < 1.006 g/ml, concentrated 16 times; C, density 1.006–1.063 g/ml, spring; D, density 1.006–1.063 g/ml, late autumn; E, density 1.063–1.21 g/ml.

examples of the tracings observed at the minimum (spring) and maximum (late autumn) of lipidemia and compares them with a normal human profile determined under the same experimental conditions. With regard to the protein absorbance curves, a consistently small amount of component I, possessing the same elution characteristics as human VLDL, was detected. Component II appeared as a peak of moderate intensity in spring and became progressively modified during summer and autumn, at which time it was seen as a large shoulder. Its elution volume exhibited marked changes according to the date of sampling and decreased by more than 12% between spring and autumn, thus indicating a higher molecular weight. Component III, evidently corresponding to HDL was detected throughout the year as a peak of high intensity. Even in spring, a complete resolution between components II and III was never attained, and the autumn/winter patterns consistently appeared as a massive continuum. As shown in Fig. 3, correlation between the material eluted from the column and electrophoretic bands provided evidence of a large chromatographic overlap between lipoproteins exhibiting LDL and HDL mobilities in the badger. Furthermore, this overlap was greater in the autumn/winter samples, almost exclusively to the benefit of HDL-like migrating lipoproteins. At this date, electrophoresis of the fractions collected between components II and III showed the existence of intermediary lipoprotein material. This appeared as a strongly colored background progressing towards the typical position of HDL as fractions nearer from component III were examined. Assay of cholesterol concentrations in the fractions revealed the seasonal redistribution of this lipid throughout the whole lipoprotein spectrum. In spring, most of the cholesterol appeared in the higher density lipoproteins, but it is evident from the figure that the situation was completely reversed in winter when the prominent cholesterol carriers were in both the low density region and in the lower part of the high-density range; furthermore, at least two small shoulders were evident on the curve between components II and III.

Electrophoresis of the major density-classes of lipoproteins

Examples of the electrophoretic characteristics of these fractions are shown in **Fig. 4.** Irrespective of season, chylomicrons and VLDL exhibited a behavior comparable to that of their counterparts in man. However it was necessary to present the different appearances of the 1.006–1.063 g/ml fraction according to the period of the year. Actually, in many badgers, spring LDL appeared as a single band like human LDL, while in autumn/winter it appeared as a spectrum of lipoproteins with components exhibiting lower and greater mobility than the major band, some of them attaining the HDL position where a band was detected as early as summer, becoming more intense as the cold season was reached. On the other hand, the 1.063–1.21 g/ml fraction was of almost constant appearance during the entire year, appearing as a unique, strong major band followed by a trail consistently more intense than that observed in man.

Owing to the volume of samples available and to limited ultracentrifugal facilities, it was not feasible to determine precisely the lower density limit of lipoproteins with HDL mobility in the 1.006–1.063 g/ml class. Considering the few experiments that have been performed this limit seemed, in spring, very different according to individual animals, with values ranging from 1.045–1.050 g/ml to more than 1.063 g/ml, while they could be less than 1.035 g/ml in autumn.

Analytical ultracentrifugation

Analytical ultracentrifugal scans, taken at a density of 1.21 g/ml, provide evidence for a continuous lipoprotein distribution between the HDL and LDL density classes, although the general appearance of the whole spectrum changed considerably according to the season. In spring, the higher-density HDL largely predominated, a small amount of material extending to the LDL in most animals. It should be noticed however that in a few cases (as shown in **Fig. 5B**), a short discontinuity appeared between the two density classes. This phenomenon was not detected at any other period of the year, at which times the relative importance of both lower-density

HDL and of LDL was substantially larger (Fig. 5C and D).

Whatever the date, the denser portion of the HDL spectrum exhibited heterogeneity, two components being regularly observed. In all animals examined, the quantitatively more important component was always slower-migrating, with an F value of about 2.5–3.0. A faster component (F 5.0–5.8), though consistently present, varied in concentration in individual animals, ranging from a simple shoulder to a peak of size comparable with that of F 2.5–3.0. As early as July in most animals, the F 7–20 region contained a substantial amount of material which subsequently subdivided into several incompletely-separated components with flotation coefficients of about F 10.0–11.0, 13.0–13.5, and 16.0–16.5. It should be noticed that this latter flotation coefficient may correspond to a component of hydrated density

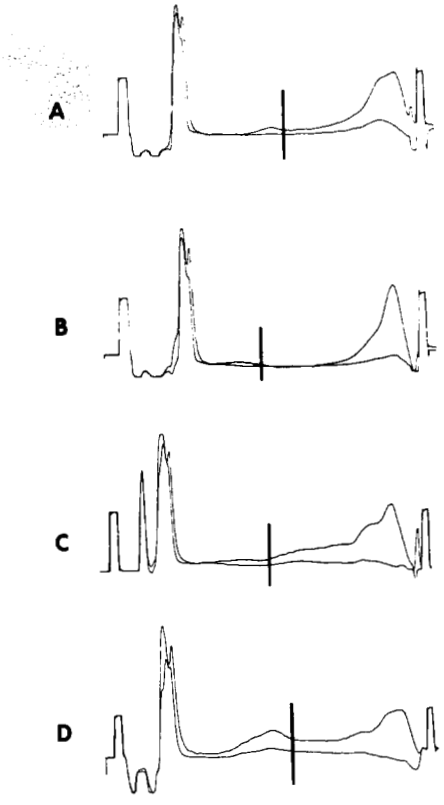

Fig. 5. Analytical ultracentrifugation of plasma lipoproteins at a density of 1.21 g/ml. Experimental conditions were 52,000 rpm, 26°C, refractometric mode at 550 nm; scans were taken 40 min after beginning of the run. Direction of flotation, right to left. Vertical lines indicate position (determined as that of F = 20 at the time of scanning) of lipoproteins with hydrated density 1.063 g/ml. Top to bottom: A and B, two examples of profiles observed in early spring; C, typical profile in summer; D, typical profile in late autumn. No important differences regarding the shape of the HDL distribution could be observed on scans taken at later times (up to 60 min). F rates of the different HDL components were as quoted in the text.

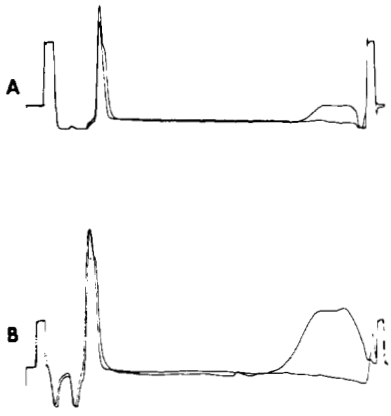

Fig. 6. Analytical ultracentrifugation of plasma lipoproteins at a density of 1.063 g/ml. Experimental conditions were 52,000 rpm, 26°C, refractometric mode at 550 nm; scans were taken 40 min after beginning of run. Direction of flotation, right to left. Figure provides typical examples of LDL patterns in early spring (A) and in late autumn (B). S_f rates of the two major components were respectively 2.5 (spring) and 4.1 (late autumn) for the slower-migrating and 4.2 (spring) and 7.2 (late autumn) for the faster.

less than 1.063 g/ml, i.e., 1.04–1.06 g/ml as pointed out by Puppione et al. (20).

The LDL distribution as assayed at 1.063 g/ml (**Fig. 6**) equally showed qualitative and quantitative modifications, both with regard to individual animals and to the period of year. The only common feature shared by all samples was an heterogeneity with at least two components, whose relative proportions varied widely in a manner that we were unable to correlate with another individual or seasonal characteristic. Although the ultracentrifugal conditions were rigorously adhered to, some variability in the S_f values was observed, paralleling the date of sampling. In brief, the S_f of the two components increased with the amount of 1.006–1.063 g/ml lipoproteins (and thus from spring to winter), from 2.5 to 4.0 for the slower-migrating and from 4.2 to near 8.0 for the faster one. Considering the extension of the LDL distribution towards the lower densities, similar seasonal modifications were evident. While in early spring, (with the exception of two animals in March 1979), this distribution was generally interrupted at about S_f 9.0, the following period up to the next winter gave rise to a small quantity of S_f 12–20 lipoproteins (IDL). The amounts of these IDL varied according to individual animals, but never accounted for more than 7% of the total 1.006–1.063 g/ml lipoproteins in late spring or summer, or for more than 10% of the same material in autumn.

Considering the relative amounts of the different lipoprotein species, three different phenomena may be noted (**Fig. 7**). *1*) Irrespective of their hydrated

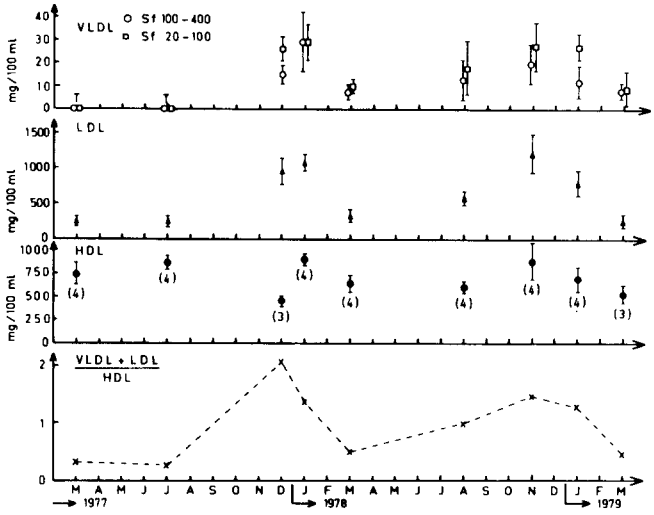

Fig. 7. Seasonal modifications in the concentrations of plasma lipoproteins as assayed in the analytical ultracentrifuge (see text for experimental conditions). For the three lipoprotein classes, values are means ± SD from individual measurements on fractions from the number of animals quoted in brackets in the part of the figure concerning HDL. Letter T (traces) for the first two series of VLDL experiments implies that mean concentrations were lower than 5 mg/100 ml and impossible to measure accurately. The lower curve is a representation of the successive values of the concentration ratio VLDL + LDL/HDL as determined from these analyses.

density sub-class, the VLDL were generally maintained at a very low level, except in autumn/winter when moderate levels were reached (about 50 mg/100 ml). *2*) In the 1.006–1.063 g/ml class, more considerable seasonal modifications occurred, with a four- or five-fold change in the mean value, from less than 250 mg/100 ml in early spring to more than 1200 mg/100 ml in late autumn; while the increase in concentration occurred in a progressive way, the consecutive decrease was both rapid and considerable in the two years. As was previously noted when examining plasma lipids, successive determinations in the same animals confirmed that these changes really occurred in individuals. *3*) Seasonal differences seemed more moderate in the HDL density class, the amount of these lipoproteins being maintained on all occasions at what appears as a very high level when compared to man. Recorded mean values ranged from about 450 mg/100 ml to near 900 mg/100 ml. Two aspects are noteworthy at this stage. First, seasonal HDL variations occurred less regularly than in LDL; large differences are evident between the mean values observed at the same dates in successive years, for example July–August in 1977 and 1978, or March in 1977, 1978, and 1979. Second, if one tentatively subdivides the HDL class on the basis of flotation coefficient it becomes evident that the lower density HDL, with flotation coefficients greater than about 7.0, are responsible for the largest part of the chronologic variations observed, their relative percentage among total HDL ranging from about 15% in March to more than 40% in winter.

The lower part of Fig. 7 shows the different values of the ratio of the concentrations of VLDL + LDL/HDL at different dates. Successive inversions of this ratio are evident according to the season; the sum of VLDL + LDL largely predominating during the cold months, while HDL becomes the prominent species at the beginning of spring.

Chemical composition of plasma lipoproteins

The respective percentages of the different components have been determined on several occasions, using pools from the plasmas of four animals in the same series (**Table 2**). In order to permit ready comparison between the different series of measurements (and since quantitative protein determination was not performed in the early analyses), all the values quoted below are percentages of total lipids in the various lipoproteins, unless otherwise stated.

The prominent feature of the VLDL was the high content of triglycerides (more than 70% of their lipids), compared with currently available data in man (21, 22). Relative levels of phospholipids (about 10%) and of both free and esterified cholesterol were consequently low. Comparison of the VLDL composition as determined in August and December 1978 showed that a minor enrichment in cholesteryl esters occurred between these two dates. However, the ratio of esterified/total cholesterol remained rather constant, near 60%. The protein moiety accounted for about 10–11% of the total lipoprotein.

Determination of the lipid composition of LDL on five separate occasions revealed an apparently regular cyclic redistribution between triglycerides and cholesterol in this density class. Most of the quantitative differences noted regarding cholesterol concern cholesteryl esters, whose relative level varied from more than 40 or even 45% during the winter maxima of hypercholesterolemia to less than 18% in March 1979. Corresponding variations affected the proportions of triglycerides, which were present as minor components in January 1978 (4.2%) and December 1978 (8.9%), while in early spring 1979 their relative contribution increased considerably to about 40%. As seen in Table 2, we have also determined two additional values. First, the ratio (% triglycerides/% cholesteryl esters), which exhibited successive large variations with a maximum in March 1979, while two minima were noticed during the two winters. In

TABLE 2. Chemical composition of badger plasma lipoproteins[a]

VLDL (d < 1.006 g/ml)	August 1978		December 1978	
% Protein		9.9		10.8
% Unesterified cholesterol	4.4	4.0	5.7	5.1
% Cholesteryl esters	10.1	9.1	13.6	12.1
% Phospholipids	10.2	9.2	9.6	8.6
% Triglycerides	75.3	67.8	71.1	63.4
Esterified cholesterol / Total cholesterol	0.58		0.59	

LDL (d 1.006 – 1.063 g/ml)	July 1977		January 1978		August 1978		December 1978		March 1979	
% Protein	ND[b]		ND[b]		26.2		19.5		27.8	
% Unesterified cholesterol	12.6		15.0		12.9	9.5	14.5	11.7	12.5	9.0
% Cholesteryl esters	31.8		46.8		28.9	21.3	41.4	33.3	17.9	12.9
% Phospholipids	39.0		34.0		36.7	27.1	35.2	28.3	28.4	20.5
% Triglycerides	16.6		4.2		21.5	15.9	8.9	7.2	41.2	29.8
Esterified cholesterol / Total cholesterol	0.60		0.65		0.57		0.63		0.46	
(% cholesteryl esters + % triglycerides)	48.4		51.0		50.4		50.3		59.1	
% Triglycerides / % Cholesteryl esters	0.52		0.09		0.74		0.21		2.3	

HDL (d 1.063 – 1.21 g/ml)	January 1978		August 1978		December 1978		March 1979	
% Protein	ND[b]		40.1		36.8		44.8	
% Unesterified cholesterol	7.1		7.7	4.6	8.3	5.2	5.6	3.1
% Cholesteryl esters	41.9		40.6	24.3	43.5	27.5	41.5	22.9
% Phospholipids	50.2		50.9	30.5	45.7	28.9	46.0	25.4
% Triglycerides	0.8		0.8	0.5	2.5	1.6	6.9	3.8
Esterified cholesterol / Total cholesterol	0.78		0.76		0.76		0.81	

[a] Lipoproteins were ultracentrifugally prepared (see text for experimental conditions) from pools of plasma from four animals; the samples were free of chylomicrons as judged by visual examination and polyacrylamide gel electrophoresis.

[b] Not determined.

Each value is the mean of two determinations. For each series of measurements, values in the first column correspond to percentages of total lipids, and in the second column to percentages of the whole lipoprotein molecule.

contrast, the sum (% cholesteryl esters + % triglycerides) remained relatively constant at about 50%, except in March 1979.

In addition, smaller modifications in the proportion of phospholipids were noted, their relative contribution remaining approximately constant between summer and winter, varying only from 35 to 39%, but being reduced to less than 30% in March 1979. Study of the esterified/total cholesterol ratio showed some seasonal variation which paralleled those observed in the same ratio applied to plasma cholesterol; values ranged from 0.63–0.65 at the beginning of winter to 0.46 in March 1979. The proportion of protein in LDL varied somewhat, a minimum being noted in December 1978 (19.5%) while samples from March 1979 exhibited a higher value (27.8%).

The 1.063–1.21 g/ml density class showed less seasonal variation than the LDL. However, our data suggested that enrichment in cholesterol could occur during the cold months. This phenomenon is more evident upon examination of the percentage composition of the whole lipoprotein, values for cholesteryl esters ranging from 27.5% in December 1978 to 22.9% in March 1979. In contrast, protein content appeared maximal in early spring (about 45%) and decreased in winter (value for December 1978, 36.8%), when the relative amount of the lower-density HDL was at its annual maximum; an intermediate value (40.1%) was observed in summer. Glycerides were maintained at a low level (except in March 1979 (6.9%)), while phospholipids usually ranged from 46 to 51% of the total lipids. The ratio of esterified cholesterol to total cholesterol remained rather constant (76–81%).

Molecular weight determination of apoprotein components

Fig. 8 shows representative electrophoretic patterns in SDS polyacrylamide gels of the apoproteins present in the LDL and HDL density classes in the badger,

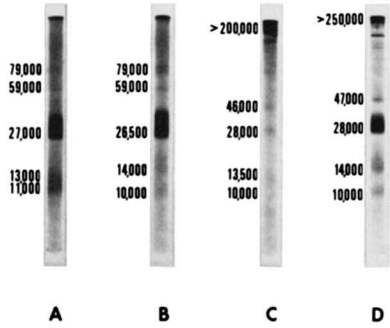

Fig. 8. SDS polyacrylamide gel electrophoresis (7.5% monomer concentration) of plasma lipoprotein apoproteins. Samples (ca. 70 μg total protein) are: A, human HDL; B, badger HDL; C, human LDL; D, badger LDL. Electrophoresis was performed and molecular weight was estimated according to the procedure of Weber and Osborn (15). Gels were fixed in 20% sulfosalicylic acid, stained with 0.5% Coomassie brilliant blue and destained for 72 hours or more in 10% trichloroacetic acid.

and compares them with the corresponding human apoproteins. With regard to the peptides present in the HDL class, one can note that the prominent component in both species was in the molecular weight range 24,000–28,000, a size typical of apoprotein A-I. On the basis of a simple visual examination, it appears that this peptide may constitute a higher proportion of apoHDL in the badger than in man. In the lower molecular weight range, the badger profile differed from man in that the 17,000–19,000 band, corresponding to human apoprotein A-II, could not be detected, even with the large quantities of material (about 200 μg protein) used in some of our electrophoreses. Other minor components were noticeable in the 10,000–14,000 range. The higher molecular weights were represented in the two species by a minor band at about 79,000. No protein of higher molecular weight was observed in this class in man, while in the badger a very small amount of apoprotein B-like material (molecular weight about 250,000) was noticed on some occasions, and especially in winter samples.

The apoprotein profile in the 1.006–1.063 g/ml interval appeared completely different in badger and in man. In man, the only component essentially detectable had a molecular weight typical of apoprotein B (250,000 or more). Such a protein was also present at high concentrations in the badger, but the peptide of molecular weight 25,000–28,000 was also observed in large amounts. Moreover, the band of molecular weight 46,000–47,000, occasionally present as a trace in human samples, was a major component in the badger and could be tentatively related, solely on the basis of its molecular weight, to apoprotein A-IV, as described by Swaney, Reese, and Eder (23) and Weisgraber, Bersot, and Mahley (24). Finally, it is of note that, in the badger, LDL and HDL apoprotein profiles were unaffected by the presence or absence of reducing agent.

Distribution of the soluble apoproteins

Examples of patterns of TMU-soluble apoproteins in the LDL and HDL density classes are provided in **Fig. 9,** and are compared with corresponding human profiles. The number of peptide species was greater in man than in the badger, and one can note the prominence of a peptide in the badger of electrophoretic mobility similar to human apoprotein A-I (band III). The major difference between the two species lay in the absence of an apoprotein A-II-equivalent in the badger. Also absent was an equivalent to the peptide observed in human samples and exhibiting a mobility equivalent to that of apoprotein D as isolated by McConathy and Alaupovic (25). On the other hand, examination of gels from top to bottom shows that there were bands present with migration characteristics similar to human apoprotein C-I (band I), to apoprotein E which appears to be present at higher concentrations than in man (band II), to apoprotein C-II (band IV), and to different forms of apoprotein C-III (bands V, VI, and VII).

In the 1.006–1.063 g/ml class, the human profile was as usual reduced to traces of apoproteins C-I and A-I, and to small amounts of apoproteins C-II and C-III. On the other hand, the badger pattern was characterized by a large amount of apoprotein A-I-equivalent (band III), while the peptides corresponding to apoproteins C-II (band IV) and C-III (bands V, VI, VII) were present in larger quantities and in proportions apparently similar to those observed in the HDL class.

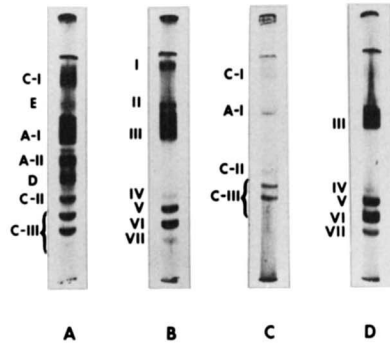

Fig. 9. Electrophoresis of TMU-soluble apoproteins according to the method of Kane (16). Samples (ca. 250 μg total protein for HDL and 300 μg for LDL) are: A, human HDL; B, badger HDL; C, human LDL; D, badger LDL. Gels were fixed in 10% trichloroacetic acid, stained with 0.5% Coomassie brilliant blue and destained for 48 hours in 10% trichloroacetic acid. Results were entirely consistent when examining lower amounts (ca. 70 and 150 μg) of protein.

No difference could be noted between badger apoprotein samples when studied in the presence or absence of reducing agents, or when electrophoretic experiments were conducted on different amounts of protein (about 70–300 μg protein per gel). Although circumstances and availability of material did not afford us the opportunity of repeating these experiments at frequent intervals, it appears that the aforementioned characteristics of the badger apoprotein pattern remained qualitatively identical along the annual cycle.

DISCUSSION

The present observations indicate a rather complex transport system for the plasma lipids in the badger. Thus, it is already evident that phenomena of great interest occur during the course of what appears to be an annual cycle. As early as 1957, Johansson (5) established evidence for seasonal variations of plasma cholesterol in the badger. Unfortunately, his experiments seem to have been conducted at only three dates in a year, and on very small groups of animals. However, this author reported an evolution rather consistent with our own findings, noting a mean cholesterolemia of 385 mg/dl in October 1955, while in May 1956 this value had fallen to 153 mg/dl. To our knowledge, no other data regarding seasonal variations in badger plasma lipids or lipoproteins, or both, exist in the literature.

The characteristic shared in common by our animals is a seasonal hyperlipidemia occurring during the cold months; this is primarily a hypercholesterolemia but also a hyperphospholipidemia. The quantitative importance of plasma triglycerides is much less, as are its seasonal modifications, but the separate contributions of free glycerol and of triglyceride glycerol remain to be established.

As customary when assaying non-laboratory-selected animals, important differences between individual animals may occur, together with modifications in the amplitude of the seasonal variations and dates of occurrence of the maxima and minima. These latter factors may be particularly correlated with the precocity and length of the cold season. However, it is of note that increases and decreases of cholesterol, phospholipids, and glycerol occurred at the same time. This is unlike the feature noticed in the woodchuck *Marmota monax* (26), where total lipid levels were observed to decrease in the cold while plasma cholesterol continued increasing for two further months. Hyperlipidemia in several other hibernators has already been described. In *Citellus columbianus,* triglycerides seem to represent the principal class among the plasma lipids, even in subjects maintained on a low-fat diet (27). The thirteen-lined ground squirrel, studied by Galster and Morrison (28), exhibited total lipid levels quite comparable to those of badgers, both with regard to the seasonal variations and dates of extrema (late fall maximum: 2700 mg/dl, spring minimum: 700 mg/dl). In the hedgehog, *Erinaceus europaeus,* some similarities with the badger in the plasma levels and seasonal variations regarding cholesterol and triglycerides can be noted from the paper of Johansson and Johansson (29). Finally, and though an annual cycle of the body weights indeed occurred in our badgers, it is noteworthy that a readily-observable relationship between modifications in plasma lipids and body weight was not evident in some cases.

From a static point of view, the plasma lipoprotein spectrum in the badger exhibits some features, such as low amounts of VLDL or elevated levels of high density components, which have already been noted in many animal species (1–3). On another hand, this spectrum becomes very unusual when its spontaneous modifications are considered during the period of a year. The major characteristic is certainly the cyclic redistribution between the LDL and HDL density classes according to the season, manifested as increased amounts of both lower-density HDL and of LDL during the pre-winter period of the year. Detailed measurements of cholesterol concentrations through the entire density spectrum also provide evidence for the alternate prominence of the fraction of this lipid belonging to the HDL or LDL density classes. These seasonal modifications may be accounted for by at least two different mechanisms, namely the intermittent occurrence of one or several supplementary cholesterol-rich lipoprotein species, or periodic changes in the turn-over rates, or both.

Indeed, analytical ultracentrifugal scans, as well as electrophoresis, assay of cholesterol concentrations in the chromatographic fractions, and comparison of the chemical compositions of the different density classes at several periods of the year, consistently suggest that before and during the season of maximum hyperlipidemia, a spectrum of cholesterol-rich lipoproteins may progressively extend largely below the density of 1.063 g/ml. This may be compared with the previously-reported occurrence of lipoproteins of similar density, and termed HDL_c, in several species (dog (30), swine (31), Patas monkey (32), and rat (33)), when maintained on a high-cholesterol diet. Secondly, an increase in the plasma concentration of "true" LDL, (i.e., of lipoproteins whose protein moiety primarily consists of an apoB-like protein), and

of IDL has also been regularly observed in the aforementioned species and probably occurs as well in the badger, as judged by our experiments. This could result from rapid catabolism of increased concentrations of VLDL, or eventually from a direct synthesis of IDL and LDL, as first demonstrated in squirrel monkeys by Illingworth and Portman (34). However, as this enrichment in LDL occurs simultaneously with an increase in the concentration of the aforementioned cholesterol-rich lipoproteins, this may equally suggest that slower clearing of the LDL could be a consequence of competition between these two lipoprotein species for binding to cell receptors. Such a mechanism has already been demonstrated in cholesterol-fed swine by Bersot et al. (35), while Innerarity and Mahley (36) have shown that, in the swine, apoprotein E-containing HDL_c is far more potent than LDL in displacing iodinated LDL from cellular receptors.

Irrespective of the species considered, no such spontaneous behavior seems to have been reported to date in the literature. However, among the numerous papers dealing with animal lipoproteins and experimental atherosclerosis, the process related by Mahley, Weisgraber, and Innerarity (30), when giving a hypercholesterolemic diet to thyroidectomized foxhound dogs, appears to display certain aspects in common with the phenomena occurring naturally in the badger. On an unknown segregation basis, some of these authors' animals, referred to as "hyperresponders," developed severe atherosclerosis together with a dramatic shift in their lipoprotein density spectrum, characterized by an extension of HDL_c from their original density interval (1.025–1.100 g/ml) to the VLDL class, where some 65% of their plasma cholesterol became located. Other dogs ("hyporesponders") exhibited no significant atherosclerosis, while their HDL_c did not massively reach the $d < 1.006$ g/ml region. It is of note that, in a recent paper, Maurel and Boissin (37) provided evidence for considerable seasonal variations of thyroid activity in badgers bred in the same colony as our own animals. Although some chronological differences were noted in individual animals and years of experiments, these authors clearly observed a maximum of thyroid activity at the end of summer and in autumn, together with a winter minimum followed by a significantly greater spring level. On the other hand, study of plasma testosterone concentrations showed that the annual cycle of this hormone presented a long minimum, lasting from July to December, while the greatest values were situated between February and May (38). The precise interrelations between these endocrine factors and the simultaneous modifications of plasma lipoproteins will certainly be of interest.

The supplementary cholesterol-rich lipoproteins noted in the badgers thus share several characteristics in common with dog HDL_c, namely a density-interval overlapping with both the LDL and HDL density classes, an electrophoretic migration in polyacrylamide gel intermediary between LDL and HDL, an occurrence leading to a large enrichment in cholesterol in the 1.006–1.063 g/ml density class, and the presence in plasma of zero (dogs surgically thyroidectomized) or very low levels (badgers during late fall and winter) of thyroid hormones.

However, important differences must be considered, primarily regarding the apoprotein content. Indeed, typical HDL_c are apoprotein E-rich, while in the badger, apart from the apoprotein B counterpart, the quantitatively most important peptide noted in the 1.006–1.063 g/ml class exhibits the electrophoretic behavior of human apoprotein A-I. Furthermore, assay of the lipid composition reveals that the proportion of esterified cholesterol was much lower than that observed by Mahley et al. (30) in typical HDL_c. Thus the possibility that surface material-rich remnants could be responsible, at least for a part of the changes observed in the lipoprotein pattern of the badger during the cold months, must be kept in mind for future experiments.

Whatever the case, and despite their repeated seasonal hypercholesterolemias, badgers do not seem to develop spontaneous premature atherosclerosis. This fact suggests that these animals may represent naturally-occurring "hyporesponders", thereby justifying the exploration in depth of their lipid transport system and its seasonal variations. We therefore envisage the study of badger apoproteins, of lipoprotein interconversions and rates of metabolism, of the endocrine regulation of these processes, and of the consequences of the repeated hyperlipidemia on the arterial walls. Apart from the obvious interest of purely comparative biochemistry, some more general conclusions may then be reached. Indeed, in man, lipoproteins of hydrated density 1.063–1.21 g/ml, which have been reported in numerous papers to play a protective role against atherosclerotic processes (39, 40), are now considered as highly heterogeneous. This is especially true for the HDL_2, about which Anderson et al. (41) have reported that they may represent the most important factor in determining the apparent inverse correlation between coronary heart disease and HDL cholesterol levels. Heterogeneity in this subclass of lipoproteins was demonstrated as early as 1963 by Barclay et al. (42) and it is now well established that, in this part of the density

spectrum, several species are coexistent, including lipoprotein (a). More recently, Innerarity et al. (43) established evidence for the existence in man, in this same density fraction, of a minor subclass (HDL I), binding to the LDL receptors. Moreover, Mahley et al. (44), when submitting young, healthy human individuals to a high cholesterol-content diet, observed the appearance of HDL_c-like lipoproteins in man. It is thus suggested that lipoproteins appearing to be of great importance in human atherosclerotic processes may find their counterpart in the badger, where the large spontaneous modifications observed should provide a convenient model for studying their metabolism and physiopathological implications.

We wish to thank Dr. M. J. Chapman for helpful criticism and careful reading of the manuscript. The skilled technical assistance of Mrs. M. Breton, of Mr. J. C. Fage, and of Mr. J. P. Rambaut is gratefully acknowledged.

Manuscript received 8 November 1979 and in revised form 31 March 1980.

REFERENCES

1. Delcourt, R. 1969. Les lipoprotéines sériques des vertébrés. Etude électrophorétique et immunologique. *Acta Zool. Pathol. Antverp.* **48:** 197–220.
2. Mills, G. L., and C. E. Taylaur. 1971. The distribution and composition of serum lipoproteins in eighteen animals. *Comp. Biochem. Physiol.* **40B:** 489–501.
3. Alexander, C., and C. E. Day. 1973. Distribution of serum lipoproteins of selected vertebrates. *Comp. Biochem. Physiol.* **46B:** 295–312.
4. Audy-Relexans, M. C. 1972. Le cycle sexuel du blaireau mâle (*Meles meles* L.). *Ann. Biol. Anim. Biochim. Biophys.* **12:** 355–366.
5. Johansson, B. 1957. Some biochemical and electrocardiographical data on badgers. *Acta Zool. (Stockholm).* **38:** 205–218.
6. Edam, K. 1966. Lipoidphosphor als Einglasmethode und anorganischer Phosphor aus dem gleichen Ansatz. *Arztl. Praxis.* **18:** 3356–3357.
7. 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.
8. Fruchart, J. C. 1976. Exploration biochimique des lipides et des lipoprotéines. *In* "Lipides et Lipoprotéines." G. Sezille, J. C. Fruchart, J. Jaillard and P. Dewailly, editors. Crouan et Roques, Lille, France. 92–95.
9. Rudel, L. L., J. A. Lee, M. D. Morris, and J. M. Felts. 1974. Characterization of plasma lipoproteins separated and purified by agarose column chromatography. *Biochem. J.* **139:** 89–95.
10. 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.
11. Hatch, F. T., and R. S. Lees. 1968. Practical methods for plasma lipoprotein analysis. *Adv. Lipid Res.* **6:** 1–68.
12. Ewing, A. M., N. K. Freeman, and F. T. Lindgren. 1965. The analysis of human serum lipoprotein distributions. *Adv. Lipid Res.* **3:** 25–61.
13. Lindgren, F. T., N. K. Freeman, and A. M. Ewing. 1967. Ultracentrifugal analysis of serum lipoproteins. *Prog. Biochem. Pharmacol.* **2:** 475–499.
14. 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.
15. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4411.
16. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
17. Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* **121:** 404–427.
18. 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.
19. Tayeau, F., J. Marquevielle, R. Nivet, and M. Dumas. 1959. Recherches sur le système lipo-protéidique du sérum de Blaireau (*Meles meles*). *Bull. Soc. Pharm. Bordeaux.* **98:** 97–106.
20. Puppione, D. L., G. M. Forte, A. V. Nichols, and E. H. Strisower. 1970. Partial characterization of serum lipoproteins in the density interval 1.04–1.06 g/ml. *Biochim. Biophys. Acta.* **202:** 392–395.
21. Lindgren, F. T., and A. V. Nichols. 1960. Structure and function of human serum lipoproteins. *In* The Plasma Proteins. A. Putnam, editor. Academic Press, New York. **2:** 1–58.
22. Eisenberg, S., and R. I. Levy. 1975. Lipoprotein metabolism. *Adv. Lipid Res.* **13:** 1–89.
23. 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.
24. Weisgraber, K. H., T. P. Bersot, and R. W. Mahley. 1978. Isolation and characterization of an apoprotein from the $d < 1.006$ lipoproteins of human and canine lymph homologous with the rat A-IV apoprotein. *Biochem. Biophys. Res. Commun.* **85:** 287–292.
25. McConathy, W. J., and P. Alaupovic. 1976. Studies on the isolation and partial characterization of apolipoprotein D and lipoprotein D of human plasma. *Biochemistry.* **15:** 515–520.
26. Wenberg, G. M., and J. C. Holland. 1973. The circannual variations in the total serum lipids and cholesterol with respect to body weight in the woodchuck (*Marmota monax*). *Comp. Biochem. Physiol.* **44A:** 577–583.
27. Bragdon, J. H. 1954. Hyperlipemia and atheromatosis in a hibernator, *Citellus columbianus. Circ. Res.* **11:** 520–524.
28. Galster, W. A., and P. Morrison. 1966. Seasonal changes in serum lipids and proteins in the 13-lined ground squirrel. *Comp. Biochem. Physiol.* **18:** 489–501.
29. Johansson, B. G., and B. W. Johansson. 1972. Fat

metabolism. I. Plasma lipids and lipoproteins. *Acta Physiol. Scand. Suppl.* **380:** 49–53.
30. 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.
31. 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.
32. Mahley, R. W., K. H. Weisgraber, and T. Innerarity. 1976. Atherogenic hyperlipoproteinemia induced by cholesterol feeding in the Patas monkey. *Biochemistry.* **15:** 2979–2985.
33. 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.
34. Illingworth, D. R., and O. W. Portman. 1973. Secretion of newly synthesized apo low density lipoprotein (LDL) into the plasma of squirrel monkeys (SM). *Circulation Suppl.* **4:** 111.
35. Bersot, T. P., R. W. Mahley, M. S. Brown, and J. L. Goldstein. 1976. Interaction of swine lipoproteins with the low density lipoprotein receptor in human fibroblasts. *J. Biol. Chem.* **251:** 2395–2398.
36. Innerarity, T. L., and R. W. Mahley. 1978. Enhanced binding by cultured human fibroblasts of apo-E-containing lipoproteins as compared with low density lipoproteins. *Biochemistry.* **17:** 1440–1447.
37. 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.
38. 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 D.* **284:** 1577–1580.
39. Miller, G. J., and N. E. Miller. 1975. Plasma high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet.* **1:** 16–19.
40. Carew, T. E., T. Koschinsky, S. B. Hayes, and D. Steinberg. 1976. A mechanism by which high-density lipoproteins may slow the atherogenic process. *Lancet.* **1:** 1315–1317.
41. Anderson, D. W., A. V. Nichols, S. S. Pan, and F. T. Lindgren. 1978. High density lipoproteins distribution: resolution and determination of three major components in a normal population sample. *Atherosclerosis.* **29:** 161–179.
42. Barclay, M., R. K. Barclay, O. Terebuskekish, E. B. Shah, and V. P. Skipski. 1963. Disclosure and characterization of new high-density lipoproteins in human serum. *Clin. Chim. Acta.* **8:** 721–726.
43. Innerarity, T. L., R. W. Mahley, K. H. Weisgraber, and T. P. Bersot. 1978. Apoprotein (E-AII) complex of human plasma lipoproteins. II. Receptor binding activity of a high density lipoprotein subfraction modulated by the apo (E-AII) complex. *J. Biol. Chem.* **253:** 6289–6295.
44. Mahley, R. W., T. L. Innerarity, T. P. Bersot, A. Lipson, and S. Margolis. 1978. Alterations in human high-density lipoproteins with or without increased plasma-cholesterol induced by diets high in cholesterol. *Lancet* **2:** 807–809.