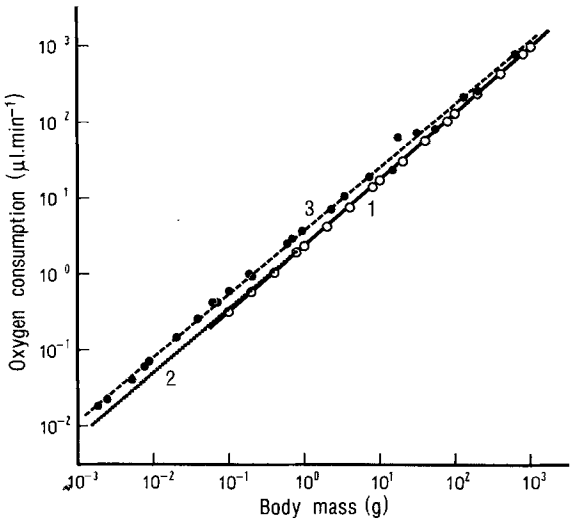

Figure 2. Oxygen consumption of carp in vitro, manometrically determined at 20 °C (line 1: summated for a whole body, present study; line 2: of a chopped whole body prepared by the same method as for tissues, constructed based on our study[10]), and oxygen consumption in vivo of intact carp determined at 20 °C by a constant flow method except for the two smallest size-groups to which a closed method was applied (line 3, present study).

stants, different in different tissues, and W is body mass in g[10]. The relative size of 10 organs, 4 parts and 8 bones were examined in 225 carp of 0.07–1900 g and found to be expressed by another allometric formula, $P = kW^s$, where P is the mass of each organ or part in g, and k and s are constants different in different phases of their change in growth[17]. The values of Q_{O_2} at approximately 200 g in body mass and s-values for various organs and parts of carp of 0.07–1900 g are summarized in figure 1. Organs of high metabolic activity, e.g. brain and kidney, got smaller in mass in proportion to the whole body with growth ($s < 1$, negative allometric growth), while organs and parts of low metabolic activity, e.g. trunk, composed mainly of white muscle (81%), got larger ($s > 1$, positive allometric growth). These results qualitatively support our idea concerning the metabolism-size relationship.

The oxygen consumption in vitro of an organ or a part in $\mu l \cdot min^{-1}$, m, at a given body mass in g, W, was calculated by a combined allometric formula, $m = c \cdot kW^{d+s}$, and the summated oxygen consumption in vitro of a whole body, $M_{in\ vitro}$, in $\mu l \cdot min^{-1}$ at a given body mass was calculated by a formula, $M_{in\ vitro} = W \cdot \Sigma m / \Sigma P$, where Σm is the summation of m of all organs and parts examined and ΣP the summation of mass in g of the organs and parts. The values of c, d, k and s obtained by our studies[10,17] were applied to this calculation. The ratio of ΣP to W was 78–86% in fish of 0.1–1 g and 90% in fish of 2–1000 g. The residual part was head kidney, bulbus arteriosus, atrium, gas bladder, urinary duct, some blood and fat. The relationship between $M_{in\ vitro}$ and W was found to be expressed by an allometric formula, $M_{in\ vitro} = 2.32W^{0.871}$ (line 1 in fig. 2; N = 17, the correlation coefficient, r, between log $M_{in\ vitro}$ and log W was 0.999. This formula is very similar to that for Q_{O_2} of the whole body prepared by the same chopping method as for tissues, $M'_{in\ vitro} = 2.47\ W^{0.847}$ (line 2 in fig. 2; N = 14, r = 0.969), obtained at 20 °C with 600 carp of 0.0016–0.79 g[10]. We found, on the other hand, that the resting rate of oxygen consumption in vivo of an intact carp in $\mu l \cdot min^{-1}$, $M_{in\ vivo}$, determined by a constant flow method at 20 °C with 254 carp of 0.0019–620 g showed a relationship to body mass expressed by another allometric formula, $M_{in\ vivo} = 3.70\ W^{0.832}$ (line 3 in fig. 2; N = 25, r = 0.999). The metabolic rates in vitro (lines 1 and 2) were a little lower than the metabolic rate in vivo (line 3), probably because of a lack of energy expenditure for movement of opercula, heart beat, peristalsis of digestive tract and other physiological and biochemical activities. However, the slopes for the metabolic rate in vitro, 0.871 (summated for a whole body) and 0.847 (of a chopped whole body), were close to the slopes for the metabolic rate in vivo of intact carp at the same temperature, 0.832 (present study), 0.836[18], 0.909[19], and the average slope in many fish species, 0.86[20]. These results are considered to support quantitatively our idea concerning the metabolism-size relationship.

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Marked elevation of HDL-cholesterol in cold-adapted golden hamsters

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Summary. Golden hamsters with spontaneous hypercholesterolemia at 22 °C developed a further increase in plasma cholesterol when they were maintained at 6 °C. This hypercholesterolemia was associated with a redistribution of plasma cholesterol between VLDL and HDL. Plasma cholesterol transported in the VLDL decreased while cholesterol in the HDL increased by 45%. The LDL profile was not significantly modified.
Key words. Cold-acclimatation; lipoprotein profile; HDL-cholesterol; hypercholesterolemia; hamster.

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The hamster is used in our laboratory as a model for studies on cholesterol metabolism because its cholesterol metabolism shows several similarities with that of humans[1,2]. In addition, we have selected a colony of hamsters which develop high levels of cholesterol in plasma and in the liver with aging[3]. However, the percentage of total plasma cholesterol transported in the high density lipoproteins (HDL) remains quite comparable to that found in normocholesterolemic hamsters, as high as 50%[4].

A further increase in cholesterolemia can be induced in these hamsters by exposure to cold[3]. The influence of low temperature on tissue lipid levels has been studied in conventional hamsters[5,6] and in other animal species[7], but investigations on the corresponding modifications in the profile of the various lipoproteins are quite limited. The few results so far reported reveal interesting effects of cold on cholesterol transport in plasma. Cold-acclimated rats display significant rise in the amount of cholesterol transported in the HDL[8]. Also, the increase in plasma HDL observed in highlanders on physical training is partly attributed to the low ambient temperature[9]. Thus, cold could be a HDL-increasing factor. Since a high plasma HDL concentration is recognized as beneficial in the prevention of cardiovascular diseases[10], a confirmation of this phenomenon in various animal species would be desirable. However, to our knowledge no study has yet demonstrated a possible relationship between exposure to cold and the frequency of ischemic heart diseases. Therefore, we decided to determine to what extent our hamsters can increase their HDL-cholesterol in becoming more hypercholesterolemic when they are acclimated to cold. The results reported here were obtained in non-hibernating animals. Hamsters exposed to low temperature acclimate to the new environment in the same manner as the other laboratory homeotherms; some animals can enter hibernation but only after a long period of exposure to cold[11].

Material and methods. The animals used in this experiment were adult male golden hamsters which were spontaneously hypercholesterolemic, bred in our laboratory. They were divided into 2 groups. The first group was placed at 6 °C in individual cages for 1 month. The second group was maintained at 22 °C and served as controls. Food (UAR, 91360 Villemoisson/Orge) and water were available ad libitum. Food intake was regularly measured. At the end of the experimental period, all animals were fasted overnight and sacrificed by a blow on the head. Blood was collected from the neck carotid in tubes containing EDTA (1 mg/ml). Three plasma samples were pooled for determination of cholesterol concentration[12] and analysis of lipoproteins. The lipoprotein fractions: VLDL $d < 1.006$ g/ml, LDL $1.020 < d < 1.063$ and HDL $1.063 < d < 1.180$, were separated by ultracentrifugation (Beckman L 65, SW 41 rotor) on a discontinuous gradient of KBr[13]. To avoid contamination of the HDL by albumin the limit of collection of this fraction was placed at $d = 1.180$ g/ml. Control assays revealed that less than 5% of the total plasma cholesterol was contained in the fraction at the bottom of the tube. The fractions were then dialyzed for 48 h at 4 °C against 0.15 M NaCl solution containing 0.01% EDTA, pH 7.4. The composition of the isolated lipoproteins was then established after the determination of the concentrations of total and free cholesterol[14], of triglycerides[15] of lipid phosphorus[16] and of proteins[17]. The level of oxygen consumption was measured at the neutral thermic temperature in animals fasted for 6 h (09.00–15.00 h).

Results. After one month of exposure to cold (6 °C), a slight diminution of body weight was observed, about 5%. In spite of a highly increased food intake the animals could not manage to balance their energetic needs, which were markedly increased when the environmental temperature was lowered. Food intake was 10 g/100 g of body weight/day at 6 °C compared to 7 g at 22 °C. The oxygen consumption of cold-adapted animals recorded at their neutral thermic temperature was 151 ± 0.7 ml/h/100 g b.wt in place of 107 ± 2.3 in controls. The plasma cholesterol level increased by about 20%; this rise was relatively modest but significant, $p < 0.01$ (see table 2). There was virtually no variation in the composition of the various lipoprotein fractions except for the VLDL. As indicated in table 1, the percent concentration of cholesterol in the VLDL fraction was augmented, but not significantly, at the expense of the triglycerides. More noticeable were the changes occurring in the contribution of each lipoprotein to cholesterol transport (table 2). There was a redistribution of the cholesterol between the VLDL and the HDL. The amount of plasma cholesterol carried by the VLDL fraction decreased ($p < 0.05$) while HDL-cholesterol clearly increased by about 45% under the influence of cold. On the other hand, exposure to cold did not significantly modify the participation of the LDL fraction in cholesterol transport. Thus, the excess of plasma cholesterol stimulated by the cold was essentially carried by the HDL.

Table 1. Composition of plasma lipoproteins in hamsters exposed to cold (6 °C) and in controls maintained at 22 °C. Values are means ± SEM of the percent of each component: proteins (PR), triglycerides (TG), phospholipids (PL), free cholesterol (FC) and cholesterol esters (CE). p is given by the Student's t-test *$p < 0.05$. Each value was obtained from 6 determinations

	PR	TG	PL	FC	CE
Very low density lipoproteins (VLDL)					
22 °C	9.6 ± 1.2	50.2 ± 3.3	15.8 ± 1.9	3.2 ± 0.6	21.1 ± 2.8
6 °C	9.7 ± 1.4	39.8 ± 6.5*	18.9 ± 1.6	5.5 ± 2.3	26.7 ± 5.0
Low density lipoproteins (LDL)					
22 °C	13.5 ± 2.7	22.8 ± 8.7	18.6 ± 3.1	5.9 ± 1.1	39.2 ± 1.0
6 °C	13.8 ± 3.2	30.5 ± 7.5	18.4 ± 2.2	6.9 ± 1.5	30.2 ± 6.1
High density lipoprotein (HDL)					
22 °C	31.7 ± 2.6	5.5 ± 1.0	27.7 ± 2.3	4.4 ± 0.8	25.1 ± 2.5
6 °C	37.2 ± 2.8	6.9 ± 0.8	28.9 ± 3.6	4.6 ± 0.8	21.8 ± 1.8

Table 2. Distribution of total cholesterol in plasma lipoproteins in hamsters exposed to cold (6 °C) and in controls maintained at 22 °C. Values are means ± SEM of 6 determinations. p is given by Student's t-test. The results are expressed as mg of cholesterol/100 ml of plasma

	Plasma	VLDL	LDL	HDL
22 °C	153 ± 7	25.9 ± 2.4	47.5 ± 4.0	78.0 ± 5.5
6 °C	181 ± 3	17.1 ± 3.0	51.1 ± 8.0	112.6 ± 10.1
	$p < 0.01$	$p < 0.05$	NS	$p < 0.02$

Discussion. This study was undertaken to determine the influence of cold on the plasma lipoprotein profile in hamsters. The animals used were hamsters already exhibiting a spontaneous, but modest, hypercholesterolemia at 22 °C. The exposure to low environmental temperature (6 °C) caused a further increase in plasma cholesterol. This hypercholesterolemia was accompanied by alterations in the VLDL composition and in the distribution of plasma cholesterol between this fraction and the HDL. The percentage of cholesterol esters in the VLDL tended to be increased. Despite this, the amount of plasma cholesterol transported in these lipoproteins was significantly decreased revealing a reduction in the plasma VLDL level. Such a reduction could be the consequence of an enhanced intravascular degradation in relation to a stimulated activity of lipoprotein lipase[18]. Thus, certain elements originating from the VLDL catabolism would be utilized to satisfy the increase in energetic needs while the others, the ones located in the peripheral zone of the lipoprotein, could serve for the transformation of nascent HDL (HDL_3) into mature HDL (HDL_2)[19]. The HDL composition was changed in animals exposed to cold. This suggested a rise in the level of this lipoprotein fraction. The mechanisms responsible for this rise remain to be discovered. In particular, further investigations are needed to define the processes leading to a possible increase of apo AI, the main apoprotein of HDL. Such an increase has been reported in cold-acclimated rats[8]. In these animals, the elevated HDL level is the consequence of a higher synthesis of the apo-

protein prevailing on its degradation. A similar situation might occur in hamsters maintained under the present experimental conditions. It is known that both the liver and the intestine contribute to the formation of apo AI, but attributing the rise in plasma HDL observed in cold-adapted hamsters to one or the other of these two tissues is premature.

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A thiol protease of peritoneal macrophages in the guinea pig[1]

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Summary. Proteolytic enzymes of the guinea pig peritoneal exudate macrophages were investigated using synthetic fluorogenic peptide substrates. Among several enzymes, t-butyloxycarbonyl-phenylalanyl-seryl-arginine 4-methylcoumaryl-7-amide cleaving enzymes had the highest activity, and the activity in exudate macrophages was about 3 times stronger than that in resident macrophages. The molecular weight of the enzyme was around 35,000 and optimal pH around 6.5–7.0. It was inhibited by thiol-blocking reagents, suggesting a thiol protease.
Key words. Protease; amidase; macrophage; guinea pig.

The role of macrophages in cellular immune reactions and inflammations is the focus of much current interest. However, the underlying biochemical reactions are still poorly understood. In a series of experiments designed to explore the chemical mediation of the delayed hypersensitivity reaction (DHR)[3], one of the authors (T.K.) reported the presence of at least three chemotactic factors for macrophages at DHR skin sites (MCFS-1, 2, and 3) in the guinea pig and suggested that these factors participate in mononuclear cell accumulation in their lesions[4–7]. The strongest one (MCFS-1) was a protein of molecular weight (MW) 150,000, and was found to be generated from a precursor protein in the plasma by limited proteolysis by an endogenous trypsin-like protease which hydrolyzed t-butyloxycarbonyl-phenylalanyl-seryl-arginine 4-methylcoumaryl-7-amide[7]. A similar protease was found in the DHR skin sites but the MW was around 600,000; it consisted of a complex of MW 31,000 protease with acidic carrier molecules[8]. Since extractable protease activity in DHR skin sites was chronologically paralleled with the macrophage infiltration, it is, therefore, possible to estimate that the trypsin-like protease in DHR skin sites would derive from emigrated macrogphages in vivo.

In the present experiments, we examined the neutral proteases in macrophages (oil-induced peritoneal macrophages) in guinea pigs to examine whether macrophages had a trypsin-like protease(s), and found a thiol protease.

Materials and methods. Animals. Hartley guinea pigs, 300–500 g, of both sexes, were injected intraperitoneally with 20 ml of sterile liquid paraffin. Four days later, the animals were exsanguinated under ether anesthesia, and their peritoneal cavities were washed three times with 20 ml Hanks balanced salt solution (HBSS) containing 10 IU/ml of heparin. After centrifugation, the cells (1×10^7 cells/ml in HBSS) were homogenized by ultrasonication using a Cell Disruptor, Model W-225R, Heat Systems-Ultrasonics, Inc., New York, U.S.A., and the clear supernatants were recovered by centrifugation for 20 min at 15,000 rpm at 4 °C and used as the extracts.

Determination of protease activities. The protease activities were measured by amidolytic activities with the use of recently developed synthetic fluorogenic peptide substrates which had high specificity and sensitivity; t-butyloxycarbonyl-phenylalanyl-seryl-arginine 4-methylcoumaryl-7-amide (Boc-Phe-Ser-Arg-MCA); succinyl-glycyl-prolyl-leucyl-glycyl-proline 4-methylcoumaryl-7-amide (Suc-Gly-Pro-Leu-Gly-Pro-MCA); carbobenzoxy-phenylalanyl-arginine 4-methylcoumaryl-7-amide (Z-Phe-Arg-MCA); succinyl-glycyl-proline 4-methylcoumaryl-7-amide (Suc-Gly-Pro-MCA); succinyl-alanyl-alanyl-prolyl-phenylalanine 4-methylcoumaryl-7-amide (Suc-Ala-Ala-Pro-Phe-MCA), and succinyl-alanyl-prolyl-alanine 4-methylcoumaryl-7-amide (Suc-Ala-Pro-Ala-MCA) (Protein Research Foundation, Osaka, Japan). For the study, 10 µl of substrate stock solution (5mM) in dimethylsulfoxide (DMSO) or water were mixed with 440 µl of assay buffer (final concentration of substrate during reaction, 100 µM) and preincubated for 5 min at 37 °C. Then 50 µl of enzyme solution was added and incubated for 10 min at 37 °C, and the reaction was terminated by the addition of 8 M guanidine. A concentration of 50 mM Tris-HCl