3. G. Geyer, Electron Histochemistry [Russian translation], Moscow (1974), p. 264.
4. I. B. Zbarskii, in: The Cell Nucleus. Morphology, Physiology, and Biochemistry [in Russian], Moscow (1972), p. 229.
5. S. N. Kuz'mina, T. V. Bul'dyaeva, and I. B. Zbarskii, Biokhimiya, 45, 1417 (1980).
6. R. Berezney and D. S. Goffey, J. Cell Biol., 73, 616 (1977).
7. P. Engelhardt, U. Plagens, I. B. Zbarskii (I. B. Zbarsky), et al., Proc. Natl. Acad. Sci. USA, 79, 6937 (1982).
8. K. Furukawa and H. Terayama, Biochim. Biophys. Acta, 585, 575 (1979).
9. T. Kawasaki and I. Yamashina, J. Biochem. (Tokyo), 72, 1517 (1972).
10. W. Rizzo and M. Bustin, J. Biol. Chem., 252, 7062 (1977).
11. G. S. Stein, R. M. Roberts, J. L. Davis, et al., Nature, 258, 639 (1975).
12. G. S. Stein, R. M. Roberts, J. L. Stein, et al., in: The Cell Nucleus, H. Busch, ed., Vol. 9, New York (1981), p. 341.
13. R. W. Stoddart, Biol. Rev. Cambridge, Philos. Soc., 54, 199 (1979).
14. I. Virtanen, Biochem. Biophys. Res. Commun., 78, 1411 (1977).
15. I. Virtanen and J. Wartiovaara, J. Cell Sci., 22, 335 (1976).
16. J. G. Wood and F. O. Sarinana, Anal. Biochem., 69, 320 (1975).

ACTION OF HIGH-DENSITY LIPOPROTEINS ON CHOLESTEROL BIOSYNTHESIS IN THE RAT LIVER

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High-density lipoproteins (HDL) have a protective action and prevent the development of atherosclerosis. The attention of research workers has been drawn to the ability of HDL to accept excess cholesterol in the intima and media of the vascular wall [14]. HDL loaded with cholesterol are transported to the liver where some of them give up their excess of cholesterol esters and make good the deficiency of phospholipids, whereas some are assimilated by the liver cells and undergo catabolism [14].

The liver metabolizes not more than 5-8% of the total HDL pool [15], but nevertheless it is the principal site of HDL biosynthesis. The liver also plays a special role in cholesterol catabolism. Interaction of HDL with liver cells both *in vivo* and *in vitro* is accordingly interesting. Data in the literature on this question are contradictory. It has been shown that HDL activate cholesterol biosynthesis in the hepatocytes [5] but do not change cholesterol synthesis *in vitro* [11] or even inhibit it [4]. In an attempt to discover the cause of these contradictions, a method of conducting experiments *in vivo* to study the effect of HDL on cholesterol biosynthesis in the liver was devised.

The object of this investigation was to study cholesterol biosynthesis in the rat liver *in vivo* during a temporary but considerable rise in the blood HDL level.

EXPERIMENTAL METHOD

Male Wistar rats weighing 230-250 g were used. The experimental and control groups each contained eight animals. Sixteen rats were used as donors. The control and experimental animals were lightly anesthetized with pentobarbital two weeks before the experiment and a polyethylene catheter 0.3 mm in diameter was introduced into the femoral vein and immediately filled with a 6% aqueous solution of polyvinylpyrrolidone. The catheter was fixed by ligatures. The free end of the catheter was sealed with hot forceps and brought out through the skin at the base of the tail, where it was secured with a tantalum clip. The point of emer-

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TABLE 1. Lipid Content in Liver Tissue (in mg/g) and Blood Serum (in mg/ml) of Rats (M ± m)

Test object	Experimental conditions	Cholesterol esters	Free cholesterol	Mono-glycerides	Diglycerides	Triglycerides	Fatty acids	Phospho-lipids
Liver tissue	Control	1,84±0,33	3,44±0,21	0,89±0,02	0,55±0,18	4,80±0,41	0,66±0,05	14,46±0,24
	Experiment	1,69±0,16	1,47±0,28 p<0,02	0,88±0,30	0,50±0,04	4,63±0,19	0,71±0,09	15,12±1,03
Blood serum	Control	0,76±0,18	0,55±0,06	—	—	0,34±0,11	0,36±0,04	0,94±0,09
	Experiment	0,91±0,08	0,64±0,04	—	—	0,32±0,07	0,40±0,06	1,12±0,08

TABLE 2. Specific Radioactivity of Lipids (in cpm/mg lipid) from Rat Liver and Blood Serum (M ± m)

Test object	Experimental conditions	Cholesterol esters	Free cholesterol	Mono-glycerides	Di-glycerides	Tri-glycerides	Fatty acids	Phospho-lipids
Liver tissue	Control	501±68	1388±92	1812±110	1240±12	1020±56	815±18	1151±108
	Experiment	563±34	2894±108 p<0,01	2203±146	1306±81	985±47	797±34	1024±52
Blood serum	Control	415±18	1535±64	—	—	1425±60	660±81	920±201
	Experiment	384±37	1480±44	—	—	1340±101	610±122	1004±77

gence of the catheter was periodically painted with iodoform. Patency of the catheter was preserved for several weeks after the operation. For a long time before the experiment the animals were accustomed to wearing a wire vest which did not restrict their mobility but, at the same time, did not allow the rats to tug on the catheter with their teeth. During the experiment the rats were kept in a specially designed cage which restricted their mobility somewhat. Animals accustomed to these conditions behaved relatively quietly throughout the experiment (5 h). By taking these precautions the effects of stress were avoided.

HDL were isolated from the blood serum of donor rats by preparative ultracentrifugation in salt solution with a density of 1.210 g/cm^3. To preserve the physiological properties of the HDL, reduced glutathione was added to the blood serum in a concentration of 1.5 mg/ml. The HDL thus obtained were dialyzed against 0.15 M NaCl containing EDTA in a concentration of 1 mg/ml. After dialysis the HDL were concentrated by ultrafiltration and the protein content in the HDL was determined by Lowry's method [12]. The HDL solution contained 0.12 mg of apoproteins in 1 ml. During infusion the outer end of the catheter was connected to a peristaltic pump and HDL, in the above-mentioned concentration, were injected at a rate of 1 ml/h. Animals of the control group were injected for 2 h with 0.15 M NaCl to which reduced glutathione was added in the same concentration. Since each experimental animal received HDL obtained from two donor rats, their blood HDL level was more than doubled.

The animals were given [2-^{14}C]acetate (from Upjohn, England) 30 min after infusion of HDL. After exposure for 2 h, rats of the control and experimental groups were decapitated, their blood was collected, and the liver removed and washed with 0.15 M NaCl. Serum lipids and liver lipids after homogenization of the tissues were extracted [7]. Neutral lipids and phospholipids were separated [1] by linear two-stage thin-layer chromatography on silica-gel (Kieselgel, from Merck, West Germany). Lipids were removed from the layer, transferred to porous glass filters, and extracted. The lipid content was determined by the sulfophospho-vanillin method, using an individual standard for lipids of each class [16]. Radioactivity of the samples was counted on a liquid scintillation spectrometer. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Data showing changes in the lipid content in the liver and blood serum of rats after infusion of HDL for 2 h are given in Table 1. The free cholesterol content in liver tissue of the experimental rats was reduced. Changes in the level of other classes of lipids were not found in either liver or blood serum. The fall in the level of free cholesterol in the liver, accompanied by no change in the content of its esters, led to some decrease in the total cholesterol content. In response to infusion of HDL the free form of cholesterol is evidently more active metabolically. Data on the rate of incorporation of [2-^{14}C]acetate

into fractions of neutral lipids and phospholipids in the liver tissue and blood serum are given in Table 2. Under the influence of a high concentration of HDL and of moderate hypocholesterolemia, cholesterol biosynthesis was activated in the liver. Changes in specific radioactivity of other classes of lipids were not significant. Examination of the mean values shows very slight activation of monoglyceride biosynthesis. Specific radioactivity of lipids in the blood of the experimental animals was reduced. This was because the injected HDL contained each of the test lipids. Specific radioactivity of free cholesterol was reduced at the same time.

It was shown previously *in vitro* that HDL can increase the outflow of cholesterol from extrahepatic tissues, as a result of which there is a marked increase in the level of labeled cholesterol in the incubation medium [9, 10]. It has also been shown that cholesterol emerging from cells is the lipid component of HDL [3, 8]. It has been suggested [8] that HDL play the role of acceptor of cholesterol for which the tissues have no further need, or which simply cannot be retained in the presence of HDL. The excessive loss of cholesterol by the cell under these circumstances can induce synthesis of the sterol, by the feedback principle, in the cell itself [8]. The possibility cannot be ruled out that in the present experiments, when a high blood level of HDL was created, a fall in the free cholesterol level in the hepatocytes and subsequent activation of cholesterol biosynthesis took place on the basis of these same principles. However, unlike those of previous studies, our data were obtained in experiments *in vivo*. Injection of native HDL obtained from donors into rats, and their increase in the blood HDL concentration of the recipients create real conditions for increased acceptance of cholesterol from hepatocyte plasma membranes. At the same time, we know that when HDL loaded with cholesterol, in which the ratio free cholesterol/phospholipids exceeds 1.5-2, are utilized the HDL can behave as donors of the free sterol for plasma membranes [2].

It can be postulated that the increase in the rate of cholesterol biosynthesis in the liver is a response to the hypocholesterolemic action of HDL. This is in agreement with data obtained by other workers *in vitro* and during perfusion of the liver [5]. Meanwhile Anderson et al. [4], who studied cholesterol synthesis in rat liver *in vivo*, found an extremely weak inhibitory effect of HDL. In the course of their experiments these workers injected low concentrations of human HDL into rats over a long period of time. In an investigation in which no effect of HDL on cholesterol synthesis in the liver was observed [11], synthesis of the sterol after injection of HDL was studied in liver tissue slices *in vitro* during incubation with labeled precursors, i.e., synthesis took place in a medium which no longer contained a high HDL concentration. *In vitro*, absence of effect of HDL on cholesterol synthesis was demonstrated in a fibroblast culture during short-term culture of hepatocytes [17]. Meanwhile an activating effect of HDL on the cholesterol biosynthesis was found in a hepatocyte culture [6]. Disagreement between these results, in our opinion, is due to the experimental conditions (different HDL concentrations, different exposures). Addition of HDL to medium in which human skin fibroblasts and rat smooth muscle cells were cultured caused a decrease in the intracellular cholesterol concentration [17].

High HDL concentrations evidently increase the rate of outflow of free cholesterol from the membrane and inhibit interaction of low-density lipoproteins (LDL), the main supplier of cholesterol for the cells, with plasma membrane receptors [13]. These two factors, acting as synergists, probably cause a decrease in the free cholesterol concentration in the cells, and this in turn acts as trigger mechanism for intracellular cholesterol biosynthesis. However, during the longer action of HDL, synthesis of LDL receptor proteins may be activated [13], the flow of exogenous sterol into the cell increased, and endogenous cholesterol synthesis inhibited.

HDL under physiological conditions thus accept cholesterol not only from membranes of peripheral tissues, but also of liver cells. As a result of the development of intracellular hypocholesterolemia, synthesis of the sterol is activated. These data were obtained for the first time *in vivo* in rats in which a high concentration of native HDL was created. They show that a fall in the intracellular cholesterol content in peripheral tissues can be achieved only when the blood HDL concentration is optimal.

LITERATURE CITED

1. V. N. Titov and D. G. Pitsin, Biokhimiya, No. 1, 83 (1978).
2. V. N. Titov, V. I. Rudnev, and M. G. Tvorogova, Kardiologiya, No. 5, 103 (1981).
3. V. N. Abdulla and C. W. Adams, Atherosclerosis, 31, 473 (1978).

4. J. M. Andersen, S. D. Turley, and S. Dietschy, Proc. Natl. Acad. Sci. USA, 76, 165 (1979).
5. A. J. Barak, M. E. Sorrel, and D. J. Tuma, Lipids, 14, 883 (1979).
6. P. A. Edwards, Biochim. Biophys. Acta, 409, 39 (1975).
7. J. Folch, M. Lees, and G. H. Sloane-Stanley, J. Biol. Chem., 226, 497 (1957).
8. D. S. Fredrickson and R. I. Levy, in: Metabolic Basis of Internal Disease, J. B. Stanbury and J. B. Wangarden, eds., New York (1972), p. 545.
9. J. K. Ho, M. S. Brown, and J. L. Goldstein, J. Lipid Res., 21, 39 (1980).
10. R. L. Jackson and C. J. Glueck, Lipids, 15, 130 (1980).
11. M. R. Lakshmanan and R. A. Muesing, J. Biol. Chem., 52, 6581 (1977).
12. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
13. N. E. Miller, Biochim. Biophys. Acta, 529, 131 (1978).
14. J. L. Miller and N. E. Miller, Lancet, 4, 16 (1975).
15. G. Sigurdsson, S. P. Noel, and R. I. Havel, J. Lipid Res., 20, 316 (1979).
16. E. B. Tapscott, J. Chromatogr., 107, 470 (1975).
17. J. J. Volp and S. W. Henessy, Biochim. Biophys. Acta, 486, 408 (1977).

EFFECT OF SUPERHIGH-FREQUENCY ELECTROMAGNETIC RADIATION AND OF SOME HORMONES ON OSMOTIC RESISTANCE OF MOUSE ERYTHROCYTES

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KEY WORDS: microwaves; hormones; osmotic resistance; erythrocytes.

Some hormones increase the resistance of animals to superhigh-frequency electromagnetic (or microwave) irradiation [4, 6, 12, 13] which, inter alia, affects the osmotic resistance of erythrocytes (ORE) [2, 3, 7, 9-11]. Changes in ORE in animals exposed to microwave (MW) irradiation in response to injection of hormones have received little study.

The aim of this investigation was to study the effect of adrenal hormones (hydrocortisone and adrenalin) and a pituitary hormone (ACTH) on ORE in mice during repeated MW irradiation.

EXPERIMENTAL METHOD

Experiments were carried out on 594 noninbred albino mice of both sexes (80% males), weighing 22-26 g, in spring.

In the experiments of series I 168 mice of one group received intraperitoneal injections of adrenalin (1 mg/kg), hydrocortisone (1 mg/kg), or ACTH (15 U/kg) on alternate days for 12 days (each hormone was given to 56 mice), in the form of solutions in isotonic NaCl solution, in a volume of 1 ml/100 g body weight (control), whereas mice of another group (42 animals) received injections of the corresponding volume of solvent (healthy animals) according to the same scheme. In the experiments of series II the mice were irradiated with MW by the method described previously [5] for 8 min daily for 12 days, with an intensity of 62 ± 5 mW/cm^2 (frequency 2374 MHz). After the first session of irradiation and subsequently every other day, the mice of one group (342 animals) were injected with the above-mentioned hormones (114 mice received each hormone — experiment), whereas the mice of another group (42 animals) received an injection of 0.9% NaCl solution (irradiated control) in the doses given above.

ORE of all mice was determined on the 2nd, 4th, 6th, 8th, 10th, 12th, and 17th days after the beginning of the experiments, 30 min after MW irradiation or injection of the sub-

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