The results of the present experiments thus show that porphyrin synthesis takes place in all the organs studied in the albino rats, despite differences in their functions. In relation to ALA dehydratase activity the viscera of albino rats can be arranged in the following descending order: liver > kidneys > lungs > pancreas > small intestine > heart > spleen.

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CHOLESTEROL BIOSYNTHESIS IN THE BLOOD OF RABBITS WITH EXPERIMENTAL ATHEROSCLEROSIS

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Blood of normal rabbits and of rabbits on an atherogenic high-cholesterol diet was incubated with sodium acetate-2-[^{14}C]. After incubation, cholesterol and its precursors (squalene and lanosterol) were found and identified in the unsaponified fractions of leukocytes and platelets. Both in normal rabbits and in rabbits with atherosclerosis the highest specific activity in the leukocytes was found in cholesterol, followed by lanosterol and squalene; in the platelets the label accumulated mainly in lanosterol.

KEY WORDS: experimental atherosclerosis; blood; leukocytes; platelets; cholesterol biosynthesis.

The biological role of cholesterol is largely determined by the fact that it is a key compound in the biosynthesis of the most important steroids. Since cholesterol is a component of the lipid part of the cell membrane, ideas exist on its functional role as transmembrane carrier of various biological substances [8]. Finally, the role of cholesterol in pathology is generally familiar, especially in atherosclerosis and ischemic heart disease [1, 2]. Elevation of the cholesterol level under these circumstances is one of the main risk factors.

It is generally agreed that the principal site of cholesterol biosynthesis in the body is the liver and small intestine, although nearly all organs and tissues are capable of forming this steroid compound [4]. It is difficult at present to decide whether the blood is a site for the biosynthesis of cholesterol or its specific precursors. Yet the solution to this problem is of considerable importance not only from the theoretical, but also from the practical point of view, in connection with the development of new biochemical tests for the diagnosis of atherosclerosis and the production and testing of hypocholesteremic drugs.

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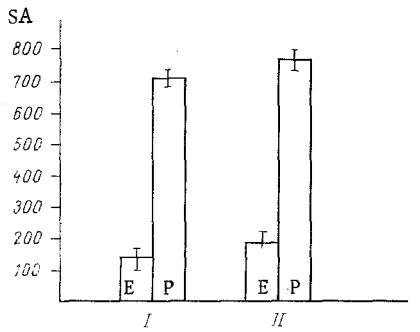

Fig. 1. Specific activity of cholesterol in erythrocytes and plasma of normal rabbits and rabbits with experimental atherosclerosis. SA) Specific activity (in cpm/μg cholesterol); I) normal; II) experimental atherosclerosis; E) erythrocytes; P) blood plasma.

EXPERIMENTAL METHOD

Experiments were carried out on young rabbits aged 8-10 months kept on an ordinary diet and on animals with experimental atherosclerosis induced by daily administration of a 10% solution of cholesterol in sunflower oil (0.1 g/kg) per os for 30 days. The total number of animals in the experiment was 36. The level of hyperlipoproteinemia in the experimental rabbits was monitored in the usual way by determining lipoproteins, cholesterol, triglycerides, and phospholipids. To study cholesterol biosynthesis in the blood, a sample of blood taken from the auricular vein was incubated [5] with sodium acetate-2-[^{14}C] (10 μCi/ml blood). After incubation, the erythrocytes, leukocytes, and platelets were isolated from the blood. The blood cell fractions were subjected to alkaline hydrolysis with a 20% alcoholic solution of KOH in an atmosphere of nitrogen, after which the unsaponified substances were extracted with diethyl ether. These substances were then separated in chloroform by thin-layer chromatography on glass plates (12 × 18 cm) coated with silica gel of the KSK brand and impregnated with $AgNO_3$. The chromatograms were developed with concentrated H_2SO_4. Radioactivity was measured on an SL-20 Intertechnique (France) scintillation counter. Cholesterol [9], lanosterol [11], and squalene [12] were determined quantitatively also. The numerical results were subjected to statistical analysis [3].

EXPERIMENTAL RESULTS

The study of cholesterol synthesis from labeled sodium acetate showed that the highest specific activity (SA) of cholesterol both under normal conditions and in atherosclerosis was found more often in the plasma than in the erythrocytes (Fig. 1). SA of cholesterol in the plasma was 4.2 times higher in atherosclerosis than SA for cholesterol in the erythrocytes. Keeping the animals on an atherogenic high-cholesterol diet had a particularly marked effect on the SA level of the plasma cholesterol, and as Fig. 1 shows, this was statistically significantly higher than in the control. With this fact in mind, in subsequent experiments to study cholesterol biosynthesis leukocytes and platelets were isolated separately from the plasma after incubation of the blood with labeled sodium acetate. Both in the normal group with atherosclerosis, besides cholesterol, its precursors squalene and lanosterol were found and identified in unsaponified fractions of leukocytes and platelets. The results of the study of incorporation of the [^{14}C] label into sterols of leukocytes and platelets of normal rabbits and of rabbits receiving cholesterol for 30-40 days are given in Table 1.

TABLE 1. Specific Radioactivity of Cholesterol and Its Precursors in Leukocytes and Platelets of Normal Rabbits and Rabbits with Experimental Atherosclerosis (in cpm/μg sterols)

Group of rabbits from which blood was obtained	Leukocytes			Platelets		
	squalene	lanosterol	cholesterol	squalene	lanosterol	cholesterol
Control	109±10	298±16	408±11	210±8	472±13	282±16
Atherosclerosis	160±16*	349±20*	420±18	290±24*	549±16*	319±20

Legend. Each series of experiments carried out on nine animals. Data for which $P < 0.05$ marked by asterisk.

As the results in Table 1 show, the distribution of SA of the sterols differed in leukocytes and platelets. Cholesterol biosynthesis in these blood cells followed a different course at the stages of steroid formation studied. For instance, in leukocytes incorporation of the radioactive label was highest in cholesterol both in normal rabbits and in rabbits with atherosclerosis; it was rather lower in lanosterol, and lowest in squalene. Keeping rabbits on an atherogenic diet led to a statistically significant increase in SA of squalene by 46% and of lanosterol by 17%, whereas SA of cholesterol showed no significant change. In platelets the highest SA in both normal rabbits and rabbits with atherosclerosis was found in lanosterol. Here, just as in the leukocytes, the production of experimental cholesterol atherosclerosis led to accumulation of radioactive label in squalene (by 38%) and lanosterol (by 17%) only.

Oral administration of cholesterol to rabbits, while causing no significant change in the character of cholesterol biosynthesis in the leukocytes and platelets, nevertheless thus increases SA of its precursors (squalene and lanosterol). This fact may be indirect evidence that the hyperlipidemia has an inhibitory effect on cholesterol formation in the blood. This was shown more demonstratively by the writers previously in the tissues of the liver and aorta [6, 7]. Inhibition of cholesterol biosynthesis in experimental atherosclerosis may perhaps take place, as the results described above indicate, at the stage of conversion of lanosterol into cholesterol. Attention should also be directed to differences in cholesterol biosynthesis in the leukocytes and platelets. Whereas in the former sterol biosynthesis proceeds as far as the end product, namely cholesterol, in the platelets the process ends at the stage of lanosterol, in which most of the radioactive label was incorporated both in normal rabbits and in rabbits with atherosclerosis. Derksen and Cohen [10], in experiments in vitro, showed that human and monkey platelets cannot form $^{14}CO_2$ during conversion of lanosterol into cholesterol through oxidation of C_4 and C_{14} methyl groups from lanosterol-^{14}C. It can accordingly be postulated that cholesterol biosynthesis in rabbit platelets is inhibited at the stage of demethylation of lanosterol.

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