Distribution of Dolichol in Human and Rabbit Blood *

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Dolichol is a polyisoprenoid substance present in tissues, mostly in membrane bound form. The biosynthetic pathway for dolichol is in the initial phase identical with that of cholesterol.[1] The condensation of isopentenyl pyrophosphate with farnesyl pyrophosphate is followed by a series of condensation reactions in the microsomes up to the polyprenyl pyrophosphate which is saturated and dephosphorylated to give the alcohol. Dolichol is probably synthesized at several locations but the main organ for its biosynthesis is the liver. Other lipids such as phospholipids, triglycerides and cholesterol are also produced in the liver and released to the blood. The aim of this investigation was to find out whether dolichol, like other lipids, occurs naturally in the blood and whether its concentration is influenced by various physiological conditions.

The amount of dolichol in the blood is low and the large amount of other lipids disturb the isolation of this small component. For these reasons, special modifications in the determination procedure are required. In the routine analyses, 5–10 ml plasma was supplied with dolichol 23 as an internal standard and extracted with diethyl ether after alkaline hydrolysis. After washing with water, the sample was purified and analysed by high performance liquid chromatography (HPLC). Two HPLC runs were employed, the first run being a purification on a short semipreparative C-18 column. The second run was made on a Hewlett-Packard Hypersil ODS 3 μm reversed phase column. This double procedure is necessary to obtain sufficient purity and high sensitivity.

Human blood contains a constant amount of dolichol; 0.13 μg/g whole blood is found in the plasma (Table 1). Practically no dolichol is present in the erythrocyte ghosts. By using gradient centrifugation, the lipoproteins were separated and the dolichol distribution was found to be highly specific. Practically all of this lipid is found to be associated with high density lipoprotein (HDL) and none or very small amounts – perhaps contamination – was recovered in the very low and low density lipoproteins (VLDL and LDL). In rabbit serum, the dolichol content is about the same.

Since the liver is one of the major sites for dolichol production and since blood dolichol is found to be associated with lipoproteins, it is reasonable to assume that the liver discharges the newly synthesized polyprene into the blood. In order to test this idea, rabbits were injected with [^{3}H]mevalonate into the portal vein and the appearance of labeled dolichol in the venous blood was followed (Fig. 1). During the first 25 min only small amounts of labeled dolichol could be detected in the blood but in the following period the specific activity of the lipid increased rapidly and attained a plateau value after 50 min. This type of lipid labeling in the blood is typical. Blood lipids are mainly synthesized in the endoplasmic reticulum of the liver; the finished lipoprotein particles are formed in the Golgi vesicles and are then transported to the blood.[2] This process involves a gradual completion and a transport route within the liver which causes a delayed appeareance of the product in the blood. Similar to other lipids, dolichol obviously also follows this pathway and therefore the polyprenol accumulation in the blood is a time dependent process.

An important point in the discussion of the presence of lipid components in the blood is the extent of physiological variation and the variation connected with pathological conditions. We have analyzed human blood from a number of persons to test various conditions. Blood lipids are sensitive to dietary factors in general and especially large fluctuations are observed during the feeding-starvation cycle. In fact, previous investigations suggested that only α-saturated short polyprenols are taken up from the diet by the liver, where α-saturation and phosphorylation, but not condensation to higher polyprenes, was

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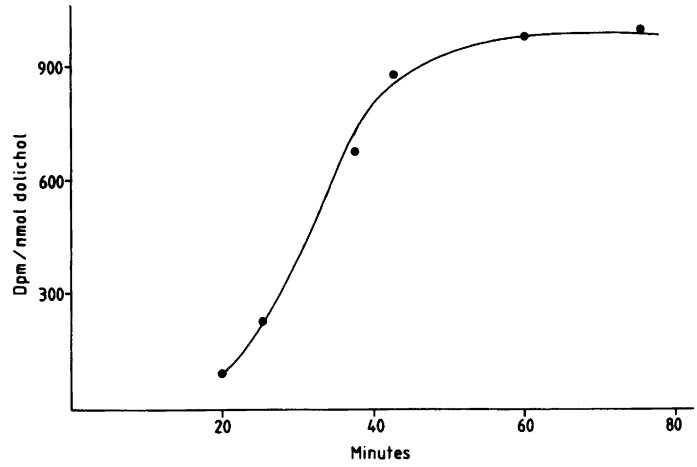

Fig. 1. Appearance of labeled dolichol in rabbit serum after *in vivo* labeling. Rabbits were injected with 37 MBq [^{3}H]mevalonate/100 g body weight into the portal vein and after various intervals blood samples were taken from the ear vein. Total dolichol was isolated and determined as described in the text.

observed.[3] We have compared the blood from a group of starved and fed persons, respectively, and we were not able to find any difference in blood dolichol content. Furthermore, the distribution of individual dolichols also speaks against a dietary origin since, contrary to the short polyprenols present in the diet, blood dolichols have a composition similar to the ones found in the tissues, consisting mainly of 18, 19 and 20 isoprene residues.

Age appears to be a factor of importance influencing blood lipid content. As is apparent from Fig. 2A, the dolichol content continuously increases with age and a linear relationship can be statistically established. The values on the figure demonstrate at the same time that no sex differences exist since the blood samples that were analyzed originated from a mixed population containing both males and females.

In contrast to the age dependency, a negative correlation is present between blood triglycerides and dolichol (Fig. 2B). A sharp decrease of the dolichol content is observed

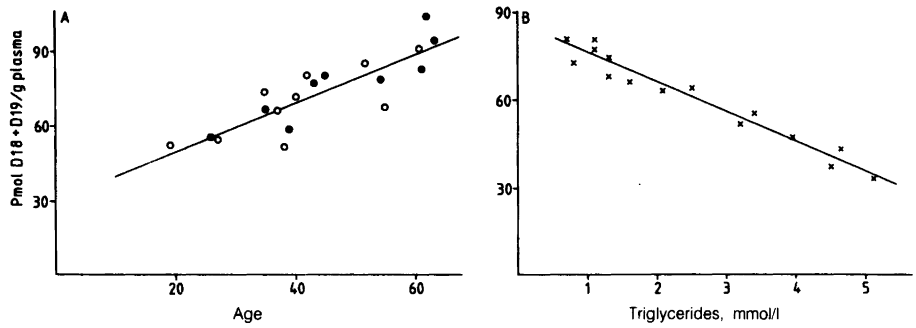

Fig. 2. Dependency of blood dolichol content on age and triglycerides. A. Blood samples of both male and female at different ages were collected and the dolichol content was determined. The values that are given represent individual samples. ○, Male; ●, female. B. Blood samples which exhibited different triglyceride content were collected and analyzed for dolichol concentration.

Table 1. Distribution of dolichol in human and rabbit blood. The appropriate fractions were subjected to alkaline hydrolysis, extracted and then the amount was determined by HPLC as described in the text. Preparations of the 3 lipoprotein fractions and ghosts were made as before.[4] The results are the mean values of 8–15 analyses.

Fractions	Amount μg/g whole blood	% distribution
Human		
Plasma	0.13	100
VLDL		0
LDL		13
HDL		87
Serum	0.15	
Ghosts	0.02	
Rabbit		
Serum	0.11	

with increasing triglyceride concentration; the relationship is closely linear. It is not clear at present whether the polyprenol content has a direct relationship to the triglyceride content as such or whether the relationship is indirect and depends primarily on HDL concentrations.

The cholesterol content was also analyzed but, unlike the triglycerides, no correlation to dolichol amount could be detected. Another important aspect was a possible correlation to the body weight since, at excess weight, lipid metabolic disturbances are common. Blood from persons with a broad body weight variation was analyzed and no correlation was detected, *i.e.* the polyprenol concentration was unchanged.

It appears from the results, that dolichol is a component consistent with human blood and that it is associated with a single lipoprotein fraction. The amount in the blood is probably regulated by the biosynthetic mechanism of the endoplasmic reticulum of the liver, which, however, does not necessarily mean that the blood dolichol content is easily influenced by a number of various conditions. The positive correlation with age and the negative correlation with triglyceride content seems to be linear, while other factors that were investigated do not influence its content or distribution. Dolichol biosynthesis and metabolic regulation is closely interrelated with that of cholesterol and ubiquinone and it is very possible that specific metabolic diseases may in the future be attributed to an abnormal blood dolichol content and distribution.

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