

soluble derivatives of the vitamin⁹⁻¹¹ or lipid/ether soluble 'active' metabolites¹²⁻¹⁴ cannot be determined by the present experiments¹⁵.

Résumé. Des études autoradiographiques et chimiques montrent que chez le rat, des explants du lobe ventral de la prostate incorporent de l'acétate de rétinyl pendant 48 h. Les résultats différents obtenus par les 2 méthodes utilisées indiqueraient qu'il y a non seulement incorporation de la vitamine A mais aussi transformation de cette dernière en d'autres substances.

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The Lipids of Human Peripheral Lymph

Investigations concerning the total fat content and the specific acid pattern of individual lipid fractions in peripheral human lymph are important for 2 reasons: (1) It is a well documented fact that ingested fat is transported from the intestinal wall mainly by means of the lymph and that therefore this biological fluid plays an important role in fat transportation. (2) Peripheral lymph is held to be a filtrate of blood plasma. The theory of simple filtration, however, which is based on the presence of pores of definite size in the capillary wall provides no explanation for the transportation of chylomicrons from peripheral blood to the lymph¹.

In the present paper some first data concerning the lipids of human peripheral lymph are presented. The results obtained can be explained partly on the basis of selective filtration and partly on that of a direct release of tissue fat into the lymph.

Materials and methods. In order to mark the lymph vessels methylene blue is injected in the interdigital fold between the first and second toe. An incision is then made in the middle third of the anterior surface of the lower leg and a lymph vessel is prepared. The vessel is cannulated so that the lymph flowing from the periphery can be collected and the cannula is held in position by ligation. Approximately 0.5–1.0 ml lymph are obtained within 1 h if the vessel is stroked or massaged gently from the periphery towards the centre from time to time. One drop of sodium citrate is added to the lymph which is then kept at -16°C . A pool of such peripheral lymph samples obtained from 14 patients was employed in the present investigation.

The methods employed for the isolation and determination of the various lipids have been reported elsewhere². Basically total fat was separated by means of silica gel column chromatography in the cholesterol, cholesterol ester, triglyceride, free fatty acid and phospholipid fractions, each of which being then quantitated gravimetrically. Subsequently, after esterification with methanol, the fatty acid pattern of each fraction was determined by means of gas liquid chromatography.

Results. In order to facilitate comparison the results of previous investigations³ concerning the lipids in plasma and in thoracic duct lymph of fasting subjects are shown together with the data obtained in the present study from peripheral lymph in Tables I and II. As may be seen from

⁹ C. F. GARBERS, J. GILLMAN and M. PEISACH, *Biochem. J.* 75, 124 (1960).

¹⁰ P. E. DUNAGIN JR., E. H. MEADOWS JR. and J. A. OLSON, *Science* 148, 86 (1965).

¹¹ R. D. ZACHMAN, P. E. DUNAGIN JR. and J. A. OLSEN, *J. Lipid Res.* 7, 3 (1966).

¹² K. YAGASHITA, P. R. SUNDARESAN and G. WOLF, *Nature* 203, 410 (1964).

¹³ P. R. SUNDARESAN, *Biochim. biophys. Acta* 113, 95 (1966).

¹⁴ M. ZILE and H. F. DE LUCA, *Biochem. J.* 97, 180 (1965).

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Table I the total fat content of peripheral lymph is substantially lower than that of plasma. Similar differences are to be seen in the case of cholesterol esters and free cholesterol, whereas the differences in phospholipids and free fatty acids are not marked. The opposite situation is found where the triglycerides are concerned; here the highest values are found in the lymph of the thoracic duct and the peripheral lymph, whereas plasma values are low.

The fatty acid patterns of the various fractions in plasma, thoracic duct lymph and peripheral lymph are shown in Table II. It is evident that in the case of the cholesterol esters the highest levels of polyenoic acids are found in plasma, whereas in peripheral lymph the saturated acids predominate. The differences are particularly marked for palmitic and linoleic acid. In the case of the triglycerides the situation is reversed: the highest values for saturated acids are found in the plasma, whereas the peripheral lymph triglycerides contain relatively large amounts of highly unsaturated acids. The value for the $\text{C}_{18:2}$ content of the peripheral lymph triglycerides has been put in brackets because the corresponding peak in the gas liquid chromatogram appeared somewhat atypical with a relatively wide basis and with a 'shoulder'. In the fatty acid patterns of free fatty acids and of phospholipids, no significant differences between plasma and lymph could be demonstrated.

Discussion. The results presented in this paper are derived from 2 different groups of individuals. Nevertheless a comparison of the data may be considered permissible as (a) only the mean values are taken into consideration, (b) in both cases fasting (12 h) blood and lymph levels were estimated, and (c) both groups consisted of individuals with the same nutritional background.

The lower levels of total fat in lymph as compared to plasma could be taken as an argument in support of the theory of 'simple filtration' for the passage of fats from plasma to lymph. However the data obtained in the individual lipid fractions demonstrate that this simple

¹ J. M. YOFFEY and F. C. COURTICE, *Lymphatics, Lymph and Lymphoid Tissue*, 2nd edn (Edward Arnold, Publishers Ltd., London, 1956) p. 96.

² G. GRIMMER, A. GLASER, H. OERTEL, K. D. VOIGT and M. APOSTOLAKIS, *Hoppe-Seyler's Z. physiol. Chem.* 333, 232 (1963).

³ M. APOSTOLAKIS, K. D. VOIGT and G. GRIMMER, *Klin. Wschr.* 43, 1094 (1965).

Table I. Total fat and lipid fractions in plasma, thoracic duct lymph and peripheral lymph

	Total fat mg/ml	Cholesterol esters mg/ml	Triglycerides mg/ml	Phospholipids mg/ml	Free fatty acids mg/ml	Cholesterol mg/ml
Blood plasma	7.2	2.3	0.9	1.1	0.4	0.6
Thoracic duct lymph	4.6	1.3	1.7	0.7	0.2	0.3
Peripheral lymph	3.95	0.46	1.77	0.84	0.24	0.1

Table II. Fatty acid pattern of the individual lipid fractions in plasma, thoracic duct lymph and peripheral lymph

		16:0	16:1	18:0	18:1	18:2	18:3	20:4
Cholesterol esters	Plasma	17.4	6.2	1.0	23.1	43.4	1.8	3.0
	Thoracic duct lymph	30.3	4.6	5.6	23.4	29.7	1.1	1.2
	Peripheral lymph	42.1	3.8	9.2	28.0	8.7	2.1	0.9
Triglycerides	Plasma	34.3	6.2	2.9	40.1	9.2	0.7	0.5
	Thoracic duct lymph	30.5	4.2	4.5	30.4	14.2	0.9	1.7
	Peripheral lymph	4.8	0.9	1.0	16.0	'53.4'	7.4	—
Free fatty acids	Plasma	33.8	5.6	9.7	35.6	6.6	0.7	1.2
	Thoracic duct lymph	27.7	6.0	7.0	37.5	9.4	0.6	0.4
	Peripheral lymph	45.1	4.5	7.5	30.8	12.8	—	—
Phospholipids	Plasma	30.3	4.5	22.4	18.9	5.7	0.1	7.6
	Thoracic duct lymph	31.3	3.4	14.2	26.0	10.9	0.5	1.9
	Peripheral lymph	38.3	4.6	13.9	18.7	7.6	2.0	2.2

hypothesis is insufficient. Thus, whereas for the cholesterol esters, free cholesterol, phospholipids and free fatty acids lymph levels are lower than those in plasma, in the case of triglycerides exactly the opposite picture is obtained. As the first 4 fractions named above are to a large extent protein bound in plasma, the degree of the reduction in the concentration levels from plasma to lymph can be considered to be an inverse function of the filtration rate of the corresponding proteins. The situation is completely different in the case of triglycerides which in plasma are to be found mainly in the form of chylomicrons. As triglycerides are found in a higher concentration in lymph than in plasma, and as their presence in the form of chylomicrons with a mean diameter of 0.5μ prohibits any passive passage through the vessel pores, it is evident that other mechanisms beyond simple filtration are involved.

Two possibilities may be envisaged: (1) An active transport of chylomicrons from plasma to lymph against a concentration gradient; (2) a direct passage of triglycerides from the peripheral fat depots to the lymph.

Whereas the first possibility appears rather improbable, the second is further supported by the facts that (a) chylomicrons can pass directly into the intestinal lymph from the mucosa of the intestine and also (b) that lipids derived from the brain and the spinal medulla pass into the liquor cerebrospinalis⁴. A further argument in favour of the second mechanism can be obtained from the fatty acid patterns of the individual fractions. Thus, over and above the pattern differences between plasma and lymph triglycerides, the presence of large amounts of polyenoic acids in the latter agrees very well with the assumption that they originate to a great extent from the fat depots. We are not yet in a position to decide however whether the light massage of the lower leg which was carried out in order to obtain the peripheral lymph has exercised any mechanical effect in this connection, i.e. has caused an increased release of fat from the depots into the peripheral lymph.

The fatty acid patterns of free fatty acids and phospholipids are similar in all 3 biological fluids examined; this finding is in complete agreement with the 'simple filtration' theory as far as these protein bound lipids are concerned. The differences of the fatty acid pattern of the cholesterol esters on the other hand can only be explained on the basis of 'selective filtration'. Whether this 'selection' is due simply to the differences in the filtration rate between α_1 - and β -lipoproteins – with both of which cholesterol esters are associated – is at present an open question. There is no doubt, however, that the fatty acid pattern of the thoracic duct lymph cholesterol esters is influenced significantly by endogenous cholesterol ester synthesis in the intestine⁵. The same holds true for the triglyceride fraction of the thoracic duct lymph⁶.

Zusammenfassung. Fettgehalt und -zusammensetzung der peripheren Lymphe von 14 Patienten wurden untersucht und mit früheren Ergebnissen an peripherem Blut und Thoracicuslymphe verglichen. Der Triglyceridgehalt der Lymphe war grösser, der aller anderen Fettfraktionen niedriger als im peripheren Blut. Zusätzlich wiesen die Triglyceride der Lymphe einen relativ hohen, die Cholesterolester einen relativ tiefen Gehalt an Polyensäuren auf.

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⁴ O. SCHRAPPE and H. G. STÖCKERT, Tagung der Ges. für Physiol. Chem., Oktober 1966, Marburg/Lahn.

⁵ M. APOSTOLAKIS, G. GRIMMER, A. GLASER and K. D. VOIGT, Biochem. Z. 336, 1 (1962).

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