BIOCHEMISTRY

Fatty Acid Specificity for the Esterification of Vitamin A and Cholesterol by Intestinal and Pancreatic Enzymes in Rats

VITAMIN A and cholesterol esters have been shown to undergo extensive hydrolysis in the lumen of the small intestine during the process of absorption; they are re-esterified to appear in the lymph mostly as esters[1,2]. However, the vitamin A esters of the lymph, blood and liver of the rat are formed by long-chain fatty acids[3] and in the normal rat liver, probably as palmitates[4]. On the other hand, cholesterol esters are usually made up of poly-unsaturated fatty acids in the lymph and blood of rats[5]. For the absorption of the two lipid materials, the enzymes of the pancreas have been largely implicated, while not much attention has been paid to the possible role of the mucosal enzymes. From the behaviour of the mucosal enzymes, as presented here, it appears that probably these enzymes play a more important part in the re-esterification of the two lipid materials during their absorption.

Acetone-dried powder was prepared from the intestinal mucosæ and pancreas of normal stock rats of this Institute by grinding the tissues in 10 vol. of acetone pre-cooled to −15°. The powder was suspended in cold distilled water in the proportion of 1 gm. powder per 10 ml. water for 1 hr. and was then centrifuged for 10 min. at 10,000*g* in the cold. The clear supernatant liquid was suitably diluted and used as the source of the enzymes. The reaction mixtures for cholesterol esterification contained 5 μmoles of free cholesterol and 10 μmoles of the given fatty acid in 0·5 ml. of ethanol, 3 ml. of Michaelis veronal buffer (0·1 *M*), *p*H 6·1, 25 mgm. of sodium taurocholate and the enzyme extracts representing 10 mgm. of pancreatic or 25 mgm. of intestinal powder. The final volume was made up to 5 ml. and incubation was for 30 min. for pancreas and 60 min. for intestine at 37°. The reaction mixtures for vitamin A esterification contained 1 μmole of vitamin A alcohol and 2 μmoles of the given fatty acids in 0·5 ml. ethanol, 3 ml. of Michaelis veronal buffer (0·1 *M*), *p*H 6·6 and the enzyme extract representing 2 mgm. of pancreatic or 5 mgm. of intestinal powder. The final volume was made up to 5 ml. and both were incubated for 30 min. at 37°. The reaction mixtures were extracted twice with light petroleum ether (40–60°) after stopping the reactions by the addition of 5 ml. of ethanol. We have been using chromatographic procedures for the separation of vitamin A ester and alcohol, and we have found that the same method can be used for the quantitative separation of free and esterified cholesterol also. The two forms of vitamin A and cholesterol were, therefore, separated on alumina columns[6] and estimated as described previously[7].

Results summarized in Table 1 clearly show that both pancreatic and intestinal enzymes esterify cholesterol preferentially with unsaturated fatty acids. Our findings with the pancreatic enzymes agree broadly with the earlier reports of Hernandez and Chaikoff[8] and Swell *et al.*[9], who have shown that the presence of unsaturation in the fatty acid is necessary for the effective esterification of cholesterol by the pancreatic enzymes. However, according to Hernandez and Chaikoff[8], while oleic and linoleic acids showed almost the same rates of esterification, linolenic acid was only 13 per cent as active as oleic acid, and our results with the pancreatic enzymes show linoleic and linolenic acids to be 68 and 40 per cent as efficient as oleic acid. In contrast, as demonstrated here, the mucosal enzymes exhibit progressively increased esterification with increase in the unsaturation of the fatty acid. If the pancreatic enzymes were to re-esterify cholesterol for its transport during absorption, the oleate should be the major ester in the lymph and blood, but in these tissues the cholesterol is found esterified mostly with polyunsaturated fatty acids[10–12]. It thus seems clear that the mucosal enzymes, rather than the pancreatic enzymes, are responsible for the re-esterification of cholesterol during absorption.

Table 1. FATTY ACID SPECIFICITY FOR THE ESTERIFICATION OF VITAMIN A AND CHOLESTEROL BY THE ENZYMES OF INTESTINE AND PANCREAS OF RATS

Fatty acid	mμmoles cholesterol esterified/mgm. protein/hr.		mμmoles vitamin A alcohol esterified/ mgm. protein/hr.	
	Pancreas	Intestine	Pancreas	Intestine
Acetic	0·0	0·0	3·0	2·3
Butyric	0·0	0·0	2·3	1·5
*Iso*valeric	0·0	0·0	2·3	2·3
Caproic	0·0	0·0	2·3	1·5
Lauric	60·0	7·5	938·0	285·0
Myristic	40·0	5·0	868·0	142·5
Palmitic	47·5	5·5	896·0	159·0
Stearic	50·0	5·7	798·0	139·5
Oleic	1,313·0	50·0	840·0	130·5
Linoleic	885·0	71·5	854·0	135·8
Linolenic	515·0	82·0	882·0	132·0

Regarding the esterification of vitamin A, neither tissue showed any preference for unsaturation, and it was effectively esterified with fatty acids containing more than 12 carbon atoms by both the tissues. It should be mentioned here that Pollard and Bieri[13] had similar experience with chick pancreas. This again would explain the presence of higher fatty acid esters of vitamin A in the blood and lymph of rats[3].

This work was supported by a research grant from the Indian Council of Medical Research, New Delhi.

S. K. MURTHY
S. MAHADEVAN
P. SESHADRI SASTRY
J. GANGULY

Department of Biochemistry,
Indian Institute of Science,
Bangalore 12.

[1] Siperstein, M. D., Chaikoff, I. L., and Reinhardt, W. O., *J. Biol. Chem.*, **198**, 111 (1952).
[2] Thompson, S. Y., Braude, R., Coates, M. E., Cowie, A. T., Ganguly, J., and Kon, S. K., *Brit. J. Nutrition*, **4**, 398 (1950).
[3] Mahadevan, S., Krishnamurthy, S., and Ganguly, J., *Arch. Biochem. Biophys.*, **83**, 371 (1959).
[4] Gray, E. L., and Cawley, J. D., *J. Nutrition*, **23**, 301 (1942).
[5] Klein, P. D., and Janssen, E. T., *J. Biol. Chem.*, **234**, 1417 (1959).
[6] Thompson, S. Y., Ganguly, J., and Kon, S. K., *Brit. J. Nutrition*, **3**, 50 (1949).
[7] Krishnamurthy, S., Seshadri Sastry, P., and Ganguly, J., *Arch. Biochem. Biophys.*, **75**, 6 (1958).
[8] Hernandez, H. H., and Chaikoff, I. L., *J. Biol. Chem.*, **228**, 447 (1957).
[9] Swell, L., Flick, D. F., Field, jun., H., and Treadwell, C. R., *Amer. J. Physiol.*, **180**, 124 (1955).
[10] Klein, P. D., *Arch. Biochem. Biophys.*, **76**, 56 (1958).
[11] Lough, A. K., and Garton, G. A., *Biochem. J.*, **67**, 345 (1957).
[12] Swell, L., Law, M. D., Field, jun., H., and Treadwell, C. R., *J. Biol. Chem.*, **235**, 1960 (1960).
[13] Pollard, C. J., and Bieri, J. G., *Arch. Biochem. Biophys.*, **87**, 9 (1960).