ACTION OF HUMAN PANCREATIC LIPASE ON SYNTHETIC MIXED SYMMETRICAL TRIGLYCERIDES OF LONG-CHAIN ACIDS AND BUTYRIC ACID.

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Received May 16, 1962

Following the studies of Clement and coworkers (1954), Borgstrom (1954), Savary and Desnuelle (1955, 1956, 1957) and Mattson and Beck (1955, 1956, 1958) it became apparent that the specificity of pancreatic lipase for preferential hydrolysis of the acyl groups on the α,α' carbons of triglycerides could be utilized to determine the structure of natural triglycerides. Thus in 1956 Savary and Desnuelle and Mattson and Beck demonstrated with this technique that the splitting of 12- to 18-carbon fatty acids from randomized glycerides proceeded at equal rates.

Using pure lyophylized human pancreatic juice, we have applied this method to a study of mixed symmetrical triglycerides containing both long- and short-chain fatty acids. It seemed of interest to ascertain for the first time whether this lipase has the same specificity characteristics as pancreatin or the pancreatic juice from other species of animals such as the rat and dog. In particular, we wished to see if the lipase retains its specificity for positions 1 and 3 of triglycerides containing very short-chain acids such as

butyric in position 2 since the rate of enzymatic release of this acid appears to be greater than that of longer chain acids from glycerides of analogous structure.

Finally, we have carried out experiments on lipolysis of mixtures of such mixed triglycerides containing either long-chain fatty acids or both long- and short-chain fatty acids to see if any preferential hydrolysis might occur in such mixtures.

The mixed synthetic triglycerides tested were the following: 2-palmitodibutyrin, B-P-B; 2-butyrodipalmitin, P-B-P; 2-palmitodiolein, O-P-O; 2-oleodipalmitin, P-O-P; 2-oleodistearin, S-O-S; and 2-stearodiolein, O-S-O. The substrate was suspended in 10 or 20 ml. of 20 M NH_4Cl/NH_4OH buffer (containing some gum arabic as emulsifying agent in the case of triglycerides melting above 37° C.) in the presence of calcium chloride, sodium taurocholate and lyophylized human pancreatic juice.[1] The lipolysis was stopped by the addition of a few drops of 3N HCl. The lipids were extracted with methylal: methanol (4:1, V/V). The constituents were separated by silicic acid chromatography using a modification of the technique of Fillerup and Mead (1953), or by the thin-layer technique of Stahl (1956). In either case, the monoglycerides were always purified by thin-layer chromatography. The various fractions were converted to butyl esters and analyzed as such by gas chromatography on a

1. We are grateful to Dr. J. Tremolieres, Director of l'unite of Dietetic Research, National Institute of Hygiene, Bickat Hospital, Paris, for a gift of lyophylized human pancreatic juice.

Barber-Colman Model 10 apparatus at two different temperatures according to the method of Clement and Bezard (1961). In some cases, the β-monoglycerides were isolated directly by thin-layer chromatography after periodate oxidation of the α-monoglycerides (Mattson and Beck, 1955). Butyric acid liberated by hydrolysis of B-P-B or of P-B-P was measured by steam distillation.

The results obtained by hydrolysis of pure substrates (O-P-O, P-O-P, O-S-O, S-O-S, B-P-B) are shown in Table I. Monoglycerides were found to have retained almost exclusively the fatty acid situated on the 2-position of the original triglyceride. There is excellent agreement between the composition of the fatty acids liberated and that of the concomitantly formed monoglycerides. In the case of P-B-P, positional specificity is less straight-forward in that the monoglycerides (total or β) contain an important proportion of acids derived from α positions. Hydrolysis of mixtures of triglycerides containing long-chain acids provides no evidence for substrate discrimination by the lipase. However, when short-chain acids are involved at either the α or β positions, the fatty acid composition of the monoglycerides formed during hydrolyses differs from that involving the β position of the original triglycerides. The method of structure determination of natural triglycerides based on the position-specificity of lipase by examination of the composition of β-monoglycerides or of the liberated fatty acids therefore appears justified in the case of

TABLE I — Composition of Fatty Acids (Molar Percentages) of the Lipid Constituents Present in the Mixture After Pancreatic Lipolysis

Substrates	Triglycerides		Diglycerides		Monoglycerides		Free Fatty Acids	
O P O	64 O	36 P	48 O	52 P	7 O	93 P	92 O	8 P
P O P	36 O	64 P	48 O	52 P	87 O	13 P	90 P	10 O
O S O	63 O	37 P	49 O	51 S	5 O	95 S	95 O	5 S
S O S	32 O	68 S	56 O	44 S	83 O	17 S	17 O	83 S
B P B	67 B	33 P	49 B	51 P	100 P		100 B	
P B P - 20 min.	34 B	66 P	65 B	35 P	81 B	19 P	2 B	98 P
- 2 hrs.	35 B	65 P	32 B	68 P	58 B	42 P	25 B	75 P
O P O + P O P	52 O	48 P	52 O	48 P	50 O	50 P	48 O	52 P
O P O + S O S	47 O	18 P	48 O	25 P	42 O	53 P	48 O	7 P
	35 S		27 S		5 S		45 S	
P O P + B P B	28 O	62 P	20 O	50 P	28 O	72 P	5 O	45 P
	10 B		30 B				50 B	
S O S + B P B	30 O	61 S	16 O	30 S	15 O	3 S	2 O	18 S
	3 P	6 B	27 P	27 B	82 P		80 B	
S O S + P B P - 20 min.	17 O	33 S	15 O	19 S	58 O	10 S	5 O	47 S
	34 P	16 B	19 P	47 B	13 P	19 B	30 P	18 B
2 hrs.	23 O	42 S	27 O	32 S	49 O	11 S	3 O	57 S
	25 P	10 B	34 P	7 B	33 P	7 B	15 P	25 B

Duration of the lipolysis was 2 hours, except where the duration is indicated.

the triglycerides containing only long-chain fatty acids, but may be questioned in the case of triglycerides containing short-chain acids in either position.

We wish to thank Mr. R. Feron, Scientific Director of the Astra Calve Society and his colleagues of the Unilever Research Laboratories for their generosity in preparing the synthetic glycerides.

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