INHIBITION OF SQUALENE AND CHOLESTEROL BIOSYNTHESIS BY HEPATOCATALASE (CAPERASE)

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It has been reported recently that hepatocatalase (Caperase) causes a considerable reduction of circulating cholesterol in humans and experimental animals receiving the enzyme by intramuscular injection (Puig-Muset, Martin and Fernandez, 1960). Hepatocatalase was obtained from beef liver by Puig-Muset as a sterile, lyophilized powder which is commercially available in Spain and France under the trade name Caperase. This preparation contains approximately 120 units of hepatocatalase per mg. of protein. An enzyme unit is defined as that amount of protein which will decompose 10 mg. of H_2O_2 per minute under the conditions of Beers and Sizer (1953).

Puig-Muset et al. have postulated that hepatocatalase, which can also act as a peroxidase, would inhibit the cyclization of squalene into lanosterol through the removal of cationic oxygen from the system (Tchen and Bloch, 1957).

This paper presents results from our studies with Caperase (kindly supplied to us by Puig-Muset) both in vivo and in vitro. Evidence is shown that 1) Caperase lowers blood cholesterol levels in rabbits without any major effect on the circulating triglycerides and phospholipids. 2) It inhibits cholesterol biosynthesis between mevalonic acid and squalene. 3) When Caperase is dialyzed against water, a fraction is recovered from the water medium which is highly inhibitory of cholesterol biosynthesis in vitro.

EXPERIMENTAL

In vivo studies. Male white rabbits weighing about 2 kilograms were kept on a Purina rabbit chow and allowed to eat ad libitum. Serum cholesterol, phospholipids and triglycerides were determined before and throughout treatment with Caperase. All animals received 1000 units of hepatocatalase (8.3 mg. of Caperase) daily by intramuscular injection. Serum cholesterol was measured by the procedure of Carr and Drekter (1956). Phospholipids and triglycerides were determined by the methods of Gomori (1941) and Bragdon (1951) respectively. Results are shown in Figure 1, each curve representing average values obtained from six animals.

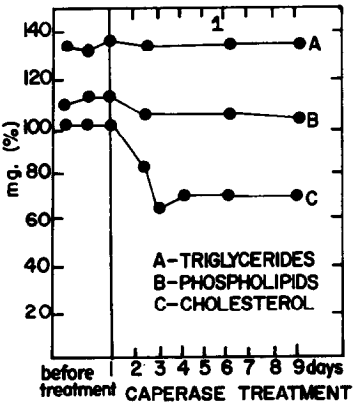

Fig. 1. The effect of Caperase on serum cholesterol, phospholipids and triglyceride levels of normal rabbits. All animals received 1000 units of hepatocatalase daily (8.3 mg. of Caperase).

In vitro studies. Young adult male rats of the Wistar strain were sacrificed by decapitation, the livers excised rapidly, chilled in cracked ice and homogenized in a Potter Elvehjem homogenizer at 300 r.p.m. for 1 minute with 2.5 volumes of medium. The homogenization medium contained phosphate buffer pH 7.4 (0.1M) magnesium (0.006M) and nicotinamide (0.03M). The homogenate was centrifuged at 500 x g for 10 minutes. This entire preparative procedure was carried out at 0°. Diphosphopyridine nucleotide

(DPN) and substrate (labeled acetate or mevalonic acid) were added to an aliquot of the homogenate supernatant fluid prior to incubation. Samples containing Caperase received 3 mg. (360 units of hepatocatalase). The experimental conditions are described in Table 1.

TABLE I

Effect of Caperase on Cholesterol Biosynthesis from Acetate - C^{14} and Mevalonic Acid - C^{14} and on Squalene Biosynthesis from Acetate - C^{14}.

Expt.	Substrate		Caperase added	Recovered radioactivity (cpm./mg.)		% Inhibition
	Acetate	Mevalonic Acid		Squalene	Cholesterol	
1.	+	-	-	19,359		
	+	-	+	9,960		48.5
2.	+	-	-		35,700	
	+	-	+		22,850	35.4
3.	-	+	-		2,810	
	-	+	+		1,930	31.3

The complete system contained the following in a final volume of 2.5 ml: homogenate supernatant fluid, 2 ml; DPN, 2 umoles; and substrate. In experiments 1 and 2, 30 umoles of sodium acetate 1-2-C^{14} containing approximately 1.250.000 c.p.m. were added. In experiment 3, 20 umoles of d-l mevalonic acid - 2-C^{14} containing approximately 190.000 c.p.m. were added. Where indicated, 3 mg. of Caperase containing 360 units of hepatocatalase were added. Incubations were carried out in a Dubnoff shaker at 37° for 2 hours under 100 per cent oxygen when assaying for cholesterol biosynthesis and 100 per cent nitrogen for the determination of squalene biosynthesis.

Biosynthetically formed cholesterol and squalene were assayed at the end of the incubation period. When cholesterol biosynthesis was to be measured the samples were saponified with alcoholic KOH, extracted with petroleum ether, the ether extract evaporated to dryness, taken up in acetone-ethanol

and cholesterol isolated as the solid digitonide and counted. An aliquot of the digitonide was dissolved in glacial acetic acid and the cholesterol content determined colorimetrically. Squalene was separated by chromatography on alumina according to the procedure of Goodman (1962) and its C^{14} activity determined in a measured aliquot.

Assay of Purity. Caperase moves as a single band when subjected to paper electrophoresis at pH 8.6. However, five distinct bands can be detected on disc electrophoresis using acrylic columns according to the procedure of Ornstein and Davis (1962). Two of these bands have been identified as being lactic dehydrogenase and malic dehydrogenase and a third and main component as hepatocatalase. The nature of the other two contaminants has not yet been determined.

When a concentrated solution of Caperase is dialyzed against water a fraction is recovered after concentration of the water medium which is highly inhibitory of cholesterol biosynthesis in vitro. This fraction reacts positively with the ninhydrin and biuret reagents and its absorption spectrum shows a maximum peak at 280 mμ suggesting the presence of a peptide or small protein. Unlike Caperase it does not show a porphyrin band. (Fig. 2) When

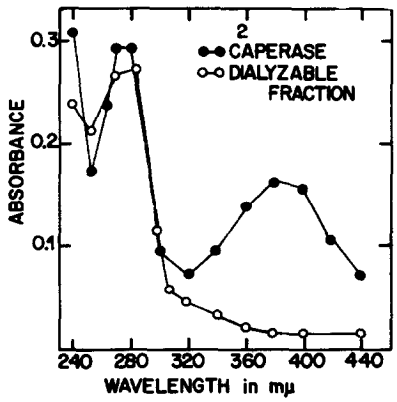

Fig. 2. Absorption spectra of Caperase and of the dialyzable fraction at pH 2.5

treated with sulfosalicylic or phosphotungstic acids it yields a precipitate which is soluble in H_2O and shows the same properties as the original fraction.

The effect of this dialyzable fraction on cholesterol biosynthesis in vitro is shown in Table II. The addition of 0.02 mg. to the system caused approximately the same degree of inhibition as 5 mg. of Caperase.

TABLE II

Effect of Caperase and of Dialyzable Fraction on Cholesterol Biosynthesis from Acetate-1, 2-C^{14} by Rat Liver Homogenate

Exp. No.	Caperase Added mg.	Dialyzable Fraction Added mg.	Cholesterol Found (C. P. M. /mg.)	% Inhibition
1	--	--	8515	--
2	5	--	6250	36
3	--	0.02	6190	37.5

The experimental conditions were as described in Table I. The amounts of Caperase and of the dialyzable fraction added are expressed in mg. of protein or peptide as measured by the biuret test.

RESULTS AND DISCUSSION

As shown in Fig. 1 there is a fast and quite marked reduction of serum cholesterol in rabbits treated with Caperase. A decrease of approximately 40% occurs after two days of treatment with little further change during the rest of the experimental period. There is no appreciable effect on the circulating phospholipids and triglycerides.

Cholesterol biosynthesis from labeled acetate and mevalonic acid is inhibited to approximately the same degree (30 to 35%) by Caperase. This is shown in Table I. Caperase did not inhibit cholesterol biosynthesis from labeled squalene, nor did it have any effect on labeled cholesterol added to the homogenate supernatant fluid.

From these results it is apparent that the site(s) of action of Caperase must be located below mevalonic acid. The fact that squalene biosynthesis is considerably inhibited by Caperase suggests that its inhibitory action occurs between mevalonic acid and squalene.

The foregoing evidence indicates that although the hypocholesterolemic action of Caperase results from interference with cholesterol biosynthesis this effect may not be directly related to its hepatocatalase content but rather to the presence of an active moiety, possibly a peptide.

Further studies directed towards the isolation and identification of this compound are in progress.

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