The animals were bled by heart puncture under light ether anaesthesia 15, 30 and 60 min after the injection of SC. XD activity was measured in the clear separated serum by measuring the formazan produced by the triphenyltetrazolium chloride reduction in evacuated Thunberg tubes.[2] Figures given represent the mean ± standard deviation found for each particular case, and are expressed as μg formazan/0·5 ml serum/30 min, at 37 °C.

Control rats (16 animals) showed average values of 17·00 ± 0·78. For the injected animals, the following average values were found: 15 min after the injection (7 rats): 4·59 ± 1·03; 30 min after the injection (7 rats): 4·10 ± 0·22.

These results, represented in Fig. 1, clearly indicate that SC inhibits *in vivo* the XD activity of rat serum during the latent period that precedes seizures. After this period, rats died and no more assays were made.

As has been pointed out by Westerfeld *et al.*,[1] SC inhibits the XO activity *in vitro*, but has no effect on the XD activity of milk and of the rat liver enzyme. The *in vivo* XD inhibition found in the present investigation suggests that SC also exerts its effect on the dehydrogenase portion of the enzyme.

Previous findings on the activation of the enzyme by CCl_4[2] and on the electrophoretic distribution of enzyme activity in serum and soluble proteins of rat liver[3] suggested the identity of liver and blood enzymes. The present report furnishes additional evidence on the identity of these enzymes, since XD activity of rat liver is also inhibited *in vivo* by SC, as shown by Westerfeld *et al.*[1]

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Biochemical Laboratory,
Instituto Oswaldo Cruz,
Rio de Janeiro, Brazil.

E. Mitidieri
O. R. Affonso
L. P. Ribeiro

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Action of hepatocatalase on experimental hypercholesterolaemia

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Previous studies on the mechanism of peroxide and lipoperoxide formation in animals have lead us to prepare the enzymes lipoxidase and catalase, and to study their pharmacological actions. As previously reported,[1] hepatocatalase (HC) was obtained from calf liver, following the technique of Sumner and Dounce,[2] and standardized by the method of Beers and Syzer.[3] We accepted as one Pevya unit the minimum quantity of enzyme able to decompose 10 mg of H_2O_2 in 1 min.

The present investigations were made possible through an original method of stabilizing the HC which consisted essentially of lyophylizing (freeze-drying) it from a solution of disacharide, in which it can be kept indefinitely in siliconized bottles. HC obtained under these conditions had such a low general toxity in experimental animals that it was difficult to establish the LD_{50}, and no lesions were observed in the viscera of rats treated for two months with daily subcutaneous injections of up to 25 000 I.U./kg of the product. Previous experimental and clinical studies show that HC decreases the urinary excretion of 17-hydroxycorticosteroids,[4] and the blood level of uric acid.[5] A decrease of blood cholesterol has also been observed in gouty patients threated with HC.

The present study is on the possible influence of HC on experimental hypercholesterolaemia. The results obtained in hypercholesterolaemia induced by the administration of a surface-active agent, Tween 80, are presented.

MATERIALS AND METHODS

Two groups of 6 rabbits (males, of 1·700–1·900 kg) were employed. All of them were intravenously injected with 2·5 ml/kg of a solution of 20% of Tween 80 (T. Schuchardt) in physiological serum (normal saline). The animals of group A were also treated with intramuscular injections of 1·000 Pevya units/kg of HC, administered 1 hr before each blood extraction.

Blood was drawn from the marginal vein of the ear of the animals. Cholesterol was determined in serum by means of the technique of Sols.[6]

RESULTS

The results obtained are shown in Table 1 and Fig. 1. As may be observed in the control group—animals treated only with Tween 80—the maximum increase was produced at 3 hr and reached 205

TABLE 1

Time after injection	Blood cholesterol mg./100 cc											
	Group A						Group B					
	1	2	3	4	5	6	7	8	9	10	11	12
Before	38	90	50	38	68	64	44	54	36	42	42	32
1½ hr	70	144	86	82	86	144	128	118	76	112	128	86
3 hr	108	164	90	86	164	160	150	132	90	144	112	122
4½ hr	76	134	76	76	152	160	128	126	72	122	112	102
6 hr	82	136	60	80	144	150	120	130	86	120	116	*a*108
8 hr	95	135	88	80	124	136	118	134	88	108	88	88

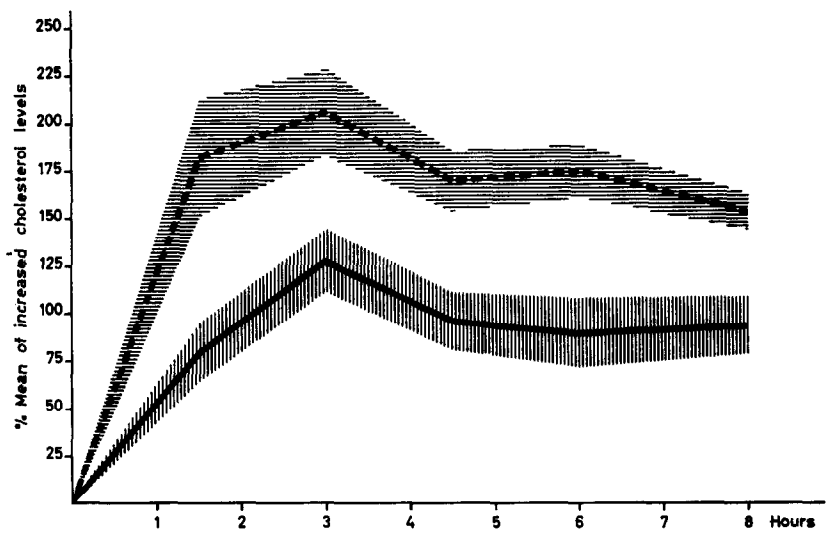

FIG. 1.

per cent. At this time the average increase of the group of animals treated with Tween 80 and HC was only 127 per cent. The differences observed between the means of the two groups were always significant ($0{\cdot}02 > P > 0{\cdot}01$, or $0{\cdot}01 > P > 0{\cdot}001$).

DISCUSSION

The hypercholesterolaemic effect of the administration of the surface-active agent Tween 80 in the rabbit[9] is clearly counteracted by simultaneous intramuscular administration of the enzyme hepatocatalase. The mechanism of action of HC is not yet clearly understood. However, this enzyme might inhibit steroidogenesis by intervening in the peroxidation processes. This inhibitory action may perhaps explain the effects of HC upon experimental hypercholesterolemia.

Catedra de Farmacologia, J. LAPORTE
Facultad de Medicina de Barcelona. P. PUIG MUSET
Departmento de Investigacion del Laboratorio Pevya, F. G. VALDECASAS

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A possible source of error in the measurement of 5-hydroxytryptophan decarboxylase activity in rat tissues*

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THE BIOSYNTHESIS of 5-hydroxytryptamine (5HT) occurs by the decarboxylation of its amino acid precursor, 5-hydroxytryptophan (5HTP). Many investigations on 5HTP decarboxylase have employed homogenate of the rat or guinea pig kidney as a source of the enzyme. The homogenate usually is incubated with the 5HTP, pyridoxal phosphate and iproniazid, or some other monoamine oxidase (MAO) inhibitor in a medium buffered at pH 8·0–8·1. In some instances the incubation is carried out under a nitrogen atmosphere in order to prevent the MAO from inactivating the 5HT that is formed. After incubation, aliquots of the mixture are measured for the quantity of 5HT formed.

In some of our recent work on the influence of pH on the metabolism of 5HT by homogenates of rat tissue, it was observed that, at pH 8·1, 5HT was metabolized even in the presence of MAO inhibitors.[1] This process occurred only at the higher pH levels, 8·1–9·5, and was found mainly with homogenates of heart and kidney. Subsequent work with this system indicated that the enzyme, cytochrome oxidase, might be responsible for attacking the hydroxy group of the indole nucleus. Tnis was supported by the fact that the addition of cytochrome *c* markedly enhanced the metabolism of 5HT and other hydroxylated indole compounds. KCN also was found to be effective in preventing the oxidation of 5HT from taking place.

The present communication describes experiments demonstrating that, when rat kidney homogenate is used as the source of 5HTP decarboxylase, even in the presence of a MAO inhibitor, the 5HT measured after incubation is not a true representation of the actual amount of the amine synthesized. Rather, under these conditions, 5HT is simultaneously being degraded by the cytochrome oxidase present in the kidney homogenate. If, however, the incubation is done under anaerobic conditions, then the amount of 5HT is an accurate measurement of decarboxylase activity. This is shown in Fig. 1, A. The amount of 5HT synthesized by rat kidney homogenate amounted to some 0·8 μmole/90 min/flask in the presence of SKF-385 (tranylcypromine), a potent MAO inhibitor. Under anaerobic conditions (N_2) the 5HT value rose to 1·3 μmoles/90 min/flask. The MAO inhibitor did not block the decarboxylation of 5HTP since, under anaerobic conditions, it has no influence on the biosynthesis of serotonin. KCN, in a concentration of 10^{-4}M, also increased the final amounts of 5HT formed from decarboxylation of 5HTP.

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