THE INFLUENCE OF SODIUM TAUROCHOLATE AND COPPER SULFATE ON LIPASE

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The important results obtained by Rona and his collaborators (1–6) in the study of the lipases have prompted us to undertake some investigations along this line. We tried lipases of different origin; namely, water and glycerol extracts of fresh liver and pancreas that were obtained from the slaughter-house, a commercial preparation of liver lipase of the hog, serum lipase of rabbits, and we also studied an extract of adrenal glands. Furthermore, in our experiments we studied the influence of copper sulfate and sodium taurocholate on the lipases of these organs.

The experiments were carried out *in vitro* according to the method of Rona and Michaelis with the stalagmometer of Traube. We thus determined the rate of hydrolysis of tributyrin with the lipase alone and in the presence of copper sulfate or sodium taurocholate. In each experiment we used 50 cc. of a saturated solution of tributyrin, and, by means of 2 cc. of 0.3 M solution of a buffer consisting of primary and secondary sodium phosphates, we maintained the reaction during the experiment constant at pH 7.2. To different concentrations of the substratum and of the buffer solution we added weighed quantities of the preparation to be tested for lipase. The experiments were carried on at a temperature of 36.5°. Every 10 minutes a small volume of liquid was sucked into the stalagmometer and the number of drops counted.

Experiments with Extracts of Adrenal Glands

From the adrenal glands of cattle we prepared water and glycerol extracts and tested the hydrolysis of tributyrin, adding to the 50 cc. of tributyrin solution 0.25 to 0.5 cc. of the extracts that

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corresponded to 0.1 to 0.15 gm. of fresh glands. After 2 hours at a temperature of 36.5° these extracts did not show any action on the tributyrin. These experiments were repeated several times and in no case could we discover the presence of lipase in the adrenal extracts. Using the same method of preparation, we obtained a liver extract that was rich in lipase and an adrenal extract entirely without lipolytic action. Furthermore, we determined whether or not adrenal extract exerts an inhibitory influence on the lipase. For this purpose we tested the hydrolysis of tributyrin with liver lipase alone and with a mixture of liver and adrenal extracts.

In both cases the lipolytic action of the liver extract remained the same. Therefore we conclude that the adrenal extract does not exert an inhibitory influence on the lipolytic power of liver

TABLE I

Action of Extracts of Liver and of Adrenal Glands

Temperature 36.5°; pH 7.2.

Time after beginning experiment	50 cc. tributyrin, 2 " buffer, 1 " liver extract 1:4	50 cc. tributyrin, 2 " buffer, 1 " adrenal extract 1:4	50 cc. tributyrin, 2 " buffer 1 " liver extract 1:4 1 " adrenal extract 1:4
min.	*per cent*	*per cent*	*per cent*
0	100	100	100
10	55	96.5	53.4
20	44.8	96.5	44.8
30	37.9	96.5	39.6

lipase. Our experiments demonstrate also the fact that the adrenal gland does not possess any lipolytic power. The results of these experiments are shown in Table I.

Influence of Sodium Taurocholate on Lipase of Liver and Pancreas

The influence of sodium taurocholate on hydrolysis of tributyrin is shown in Table II. For this experiment we prepared a suspension containing 10 mg. of hog liver lipase (commercial preparation) in 15 cc. of 10 per cent glycerol.

These experiments were repeated many times. In every case we found that sodium taurocholate in concentrations of 1:50,000, 1:100,000, and 1:500,000 considerably accelerated the hydrolysis of tributyrin by the lipases of liver and of pancreas.

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Influence of Copper Sulfate on the Action of Lipase of Liver and Pancreas

Table III shows the influence of copper sulfate on the hydrolysis of tributyrin by liver lipase. In these experiments lipase sus-

TABLE II

Influence of Sodium Taurocholate on Liver Lipase

Temperature 36.5°; pH 7.2.

Time after beginning experiment	50 cc. tributyrin, 2 " phosphate buffer, 0.5 " lipase in suspension (*a*)	Na taurocholate (1:100,000)
		(*a*) plus 0.5 cc. sodium taurocholate (1:1,000)
min.	*per cent*	*per cent*
0	100	100
10	81	60
20	63	52
30	56	41

TABLE III

Influence of Copper Sulfate on Liver Lipase

Temperature 36.5°; pH 7.2.

Time after beginning experiment	50 cc. tributyrin, 2 " phosphate buffer, 0.5 " lipase in suspension (*a*)	$CuSO_4 \cdot 5H_2O$ (1:100,000)	$CuSO_4 \cdot 5H_2O$ (1:500,000)
		(*a*) plus 1 cc. $CuSO_4 \cdot 5H_2O$ (1:2,000)	(*a*) plus 1 cc. $CuSO_4 \cdot 5H_2O$ (1:10,000)
min.	*per cent*	*per cent*	*per cent*
0	100	100	100
10	67	74	74
20	52	63	67
30	45	60	69
40	41	52	

pension prepared from commercial brands, as described above, was used.

In all of our experiments copper sulfate inhibited the action of liver lipase as well as that of pancreas lipase. The inhibitory action was manifest in the following concentrations of copper sulfate: 1:50,000, 1:100,000, and 1:500,000.

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Influence of Copper Sulfate and Sodium Taurocholate on Pancreas Lipase

Table IV shows the influence of copper sulfate and sodium taurocholate on the activity of the pancreas lipase. The copper sulfate

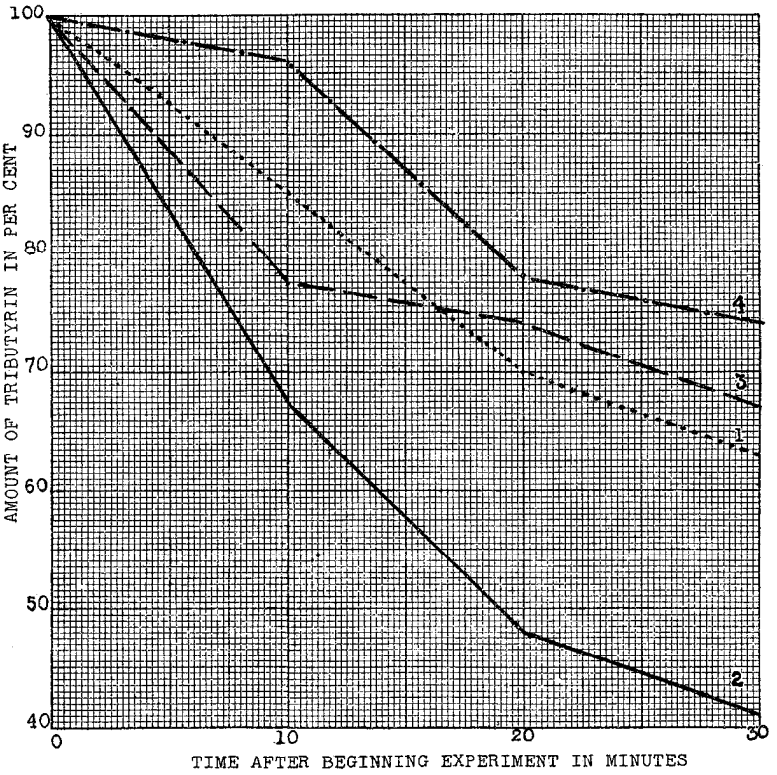

FIG. 1. The antagonistic action of copper sulfate and sodium taurocholate on hydrolysis of tributyrin by pancreatic lipase. Curve 1, control; Curve 2, with sodium taurocholate; Curve 3, with copper sulfate and sodium taurocholate; Curve 4, with copper sulfate. Temperature 36.5°; pH 7.2. Sodium taurocholate, 1:50,000; copper sulfate, 1:50,000.

considerably inhibited the work of pancreas lipase. On the contrary, the stimulating effect of Na taurocholate on the action of pancreas lipase is very pronounced.

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Antagonism between Copper Sulfate and Sodium Taurocholate in Lipolysis

Further experiments were carried out on the antagonistic action of copper sulfate and sodium taurocholate. In these experiments

TABLE IV

Influence of Copper Sulfate and Sodium Taurocholate on Pancreas Lipase

Temperature 36.5°; pH 7.2.

Time after beginning experiment	50 cc. tributyrin, 2 " phosphate buffer, 1 " glycerol pancreas extract = 0.3 gm. fresh pancreas (*a*)	$CuSO_4 \cdot 5H_2O$ (1:50,000) (*a*) plus 1 cc. $CuSO_4 \cdot 5H_2O$ (1:1,000)	50 cc. tributyrin, 2 " phosphate buffer, 0.5 " glycerol pancreas extract = 0.15 gm. fresh pancreas (*b*)	Sodium taurocholate (1:50,000) (*b*) plus 1 cc. sodium taurocholate (1:1,000)
min.	*per cent*	*per cent*	*per cent*	*per cent*
0	100	100	100	100
10	81	85	85	67
20	52	78	70	48
30	48	67	63	41

TABLE V

Influence of Sodium Taurocholate and Copper Sulfate on Serum Lipase

Temperature 36.5°; pH 7.2.

Time after beginning experiment	50 cc. tributyrin, 2 " phosphate buffer, 1 " rabbit serum (1:7) (*a*)	Sodium taurocholate (1:50,000) (*a*) plus 1 cc. sodium taurocholate (1:1,000)	50 cc. tributyrin, 2 " phosphate buffer, 1 " serum lipase (1 serum : $8H_2O$) (*b*)	$CuSO_4 \cdot 5H_2O$ (1:50,000) (*b*) plus 1 cc. $CuSO_4 \cdot 5H_2O$ (1:1,000)
min.	*per cent*	*per cent*	*per cent*	*per cent*
0	100	100	100	100
10	63	63	92	95
20	41	44	90	90
30	33	31	86	86

first sodium taurocholate and copper sulfate, and subsequently the lipase preparation, were added to the tributyrin solution. In Fig. 1 the results of one of these experiments are shown.

From these and other analogous experiments we conclude that the addition of sodium taurocholate to some extent protected the lipase from the inhibitory influence of copper sulfate.

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Influence of Sodium Taurocholate and Copper Sulfate on Serum Lipase

In these experiments we used rabbit serum previously diluted 1:7 with water. Sodium taurocholate and copper sulfate in concentrations of 1:50,000, 1:100,000, and 1:500,000 were tested separately as to their influence on serum lipase. Table V shows the results of two of many similar experiments.

In accordance with this series of experiments it can be stated that copper sulfate and sodium taurocholate in the concentrations mentioned in the text have no effect on the serum lipase. The addition of glycerol in small quantities (0.2 to 50 cc.) to tributyrin, as was done in our experiment with the glycerol suspension of the commercial preparation of hog lipase, did not change the susceptibility of serum lipase toward either of the two substances.

DISCUSSION

A review of the literature (7–14) concerning the accelerating or inhibiting action of various substances on lipase indicates that our knowledge concerning the influence of various chemicals on lipase is in general inadequate and somewhat contradictory, although Rona and his collaborators (1–6) contributed much to our understanding of the action of some substances on lipase. As regards sodium taurocholate, many authors have noted its accelerating effect on lipase action. We found this statement to hold true only for liver and pancreas lipase, for we discovered that the serum lipase is not influenced by sodium taurocholate. We think it improbable, therefore, that, as Morse (15) believes, the accelerating effect of sodium taurocholate depends only upon its action on tributyrin.

There were no definite data that existed so far, concerning the effect of copper on lipolysis. We established the great toxicity of copper salts on liver and pancreas lipase, and their inhibitory effect even in very low concentrations on lipase action. Furthermore, we succeeded in diminishing this toxic effect of copper sulfate by the addition of sodium taurocholate. Our experiments show that, if we wish to explain the toxic action of copper on the organism, the influence of this metal on enzymes must be taken into consideration. In addition we were able to establish the specific

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influence of copper sulfate on lipase of different origin. While it inhibited the action of liver and pancreas lipase, it had no effect on serum lipase.

In the literature dealing with lipase we found no data concerning the presence of this enzyme in the adrenal glands. Our experiments show that the adrenal glands of cattle are devoid of lipolytic action.

SUMMARY

1. Our experiments prove the absence of a lipolytic enzyme in the adrenal glands.

2. Sodium taurocholate acts differently on the lipase originating in various organs and on that lipase found in blood serum. It accelerates the activity of the pancreas and liver lipase and has no influence on the serum lipase; it accelerates the action of liver and pancreas lipase even in such low concentrations as 1:50,000 and 1:500,000.

3. Copper sulfate also acts differently on the lipase of certain organs and on that of the blood stream. It is toxic for liver and pancreas lipase and does not affect the serum lipase. This inhibiting action of copper sulfate is likewise evident in concentrations as low as 1:50,000 and 1:500,000.

4. The inhibitory action of copper sulfate on liver and pancreas lipase can be partly counteracted by the addition of sodium taurocholate.

5. From these findings it follows that the serum lipase is distinguished by its stability toward the compounds tested so far by us.

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