ENZYMATIC STUDIES ON THE EXPERIMENTAL ILEUS OF A RABBIT

II. ON THE ENZYMATIC ACTIVITY OF THE FLUID CONTENTS OF THE DUODENAL LOOP TIED-OFF IN EXPERIMENTAL ILEUS *

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It is demonstrated in the previous report (1) (1951) that the activity of the proteinase of the fluid in the tied-off intestinal loops is negligibly weak and that of the peptonase is also remarkably reduced in 48 hours after the operation, while only dipeptidase, halogenacylase and tributyrinase showed a notable activity in the fluid. Therefore it is concluded that there was neither increased activity of protease nor appearance of any strong protease, such as catheptase in the fluid contents of the tied-off intestinal loops of a rabbit. It seems that the proteinase and peptonase were found in a reduced states in the fluid of the ileus intestine of a rabbit.

In these further experiments the activity of proteolytic enzymes in the fluid of the obstructed duodenum, which was tied off by the double upper and lower ligation, was investigated.

EXPERIMENTALS

Experimental ileus made in the rabbit duodenum:

An upper portion (about 15 cm) of the rabbit duodenum, where the bile and the pancreatic duct are opened, was set aside by tying it off with the double ligation to make an obstructed duodenal loops, the anastomosis of the main tract with canalization being made in the similar manner as in the case of the small intestine. In some cases the bile duct, and in the other cases the pancreatic duct was bound up in order to exclude the effect of these juices.

Enzyme sources: The fluid content of the obstructed duodenum loop was taken up 24 or 48 hours after the operation and each 1 ml was used in the experiments.

Test solutions: The solution contains 5 ml 10% protein or peptone or tributyrin solution or 5 ml 0.05 M solution of a synthetic substrate, 5 ml phosphate buffer of a certain pH value and 1 ml fluid of the loop contents (as enzyme source): it was incubated under toluene at 37° C for 24, 48 and 72 hours.

Blanks with the enzyme-buffer solution without the substrate were digested under the same conditions.

The acidity in 2 ml digestate was determined by the formol titration with

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0.1 N NaOH. The acidity increase in 2 ml test solutions is given in ml 0.1 N NaOH in the tables, after correcting for blanks.

Rabbits numbers examined are given in the tables.

1) *Proteinase activity of the fluid of the obstructed duodenum loop with the open or bound pancreatic duct (18 hours after operation)*

TABLE I. Proteinase of the fluid of the ileus duodenum with the open or bound pancreatic duct (18 hours)																			
Acidity increase in 2 ml digestate (ml 0.1 N NaOH)																			
Tests	Substrates		Casein				Gelatin				Ovalbumin				Serumalbumin				
	Animal No.	pH	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96	
A	No. 60* 58 54	4.0	-0.08	—	-0.09	0	-0.03	-0.03	—	-0.02	—	-0.04	-0.04	—	—	—	0.03	—	-0.02
		6.0	-0.02	—	0.21	0.22	—	—	0.32	—	—	—	—	—	—	—	—	—	—
		7.0	0.08	—	0.21	0.33	0.27	0.32	0.38	—	0.47	—	—	—	—	—	—	—	—
		7.2	0.06	—	0.15	0.30	0.28	0.44	—	0.47	—	0.02	0.07	—	0.08	0.01	0.07	—	0.02
		8.0	0.12	—	0.25	0.30	0.28	—	0.38	—	0.38	—	—	—	—	—	—	—	—
	No. 57 59	4.0	—	—	—	—	0.01	-0.01	-0.02	—	—	—	—	—	—	—	—	—	—
		6.0	0.02	—	0.07	—	0.03	0.01	0.07	—	—	—	—	—	—	—	—	—	—
		7.0	0.01	-0.06	0.01	—	0.01	0.08	0.16	—	—	—	—	—	—	—	—	—	—
		7.2	0.08	0.12	0.17	—	0.03	0.12	0.15	—	—	—	—	—	—	—	—	—	—
		8.0	—	—	—	—	0.09	0.16	0.18	—	—	—	—	—	—	—	—	—	—
B	No. 72 77	4.0	0	0.05	0.03	—	0.02	0.08	0.03	—	—	-0.03	-0.03	0.03	—	-0.02	0	—	
		6.0	0.04	0.10	0.12	—	0.03	0.05	0.06	—	—	0	0.05	0.10	0.01	0.06	-0.03	—	
		7.0	0.09	0.10	0.21	—	0.03	0.01	0.07	—	—	0.02	0.06	-0.02	—	0.01	0.07	0	
		7.2	0.15	0.12	0.15	—	0.11	0.16	0.18	—	—	0.02	-0.02	0.02	—	0.02	-0.02	0.04	
		8.0	0.05	0.09	0.14	—	0.06	0.13	0.16	—	—	0	0.01	—	—	0	-0.01	—	

A) The pancreatic duct was open

B) The pancreatic duct was bound up

* The fluid was obtained 22 hours after operation

In the cases of the open pancreatic duct (No. 58 and 60) it is observed that the fluid of the obstructed duodenum showed its activity of caseinase and gelatinase in a moderate degree, whilst in the other cases (Table I, A, No. 57 and 59) their activity was very low, showing different values. Albumins were all resistant to the fluid enzyme. These results agree with the intestinal erepsin. The fluid of the ileus duodenum with the bound pancreatic duct showed a very slight activity of the proteinase (Table I, B, No. 72 and 77). From these results, obtained in both cases with the open or with the bound pancreatic duct, it will be questionable as to whether this moderate proteinase activity of the fluid observed in the open duct was due to the pancreatic juice secreted into the obstructed duodenum loop: but the activity must be much stronger, if it were to come from the normal activated trypsin or chymotrypsin of the pancreas.

Otherwise no appearance of catheptic enzymes was recognized.

However, it must be taken into consideration here that the activity appeared is at a very low rate and that it is nearly almost missed even in the other cases with the open pancreatic duct (Table I, A, No. 57 and 59). These observations suggest that the pancreatic enzyme, if appeared, is also in an abnormal state or perhaps in an injured state. The injury seems to be partial or almost total. A question now arises as to whether it comes from the inhibited activation of the pancreatic trypsinogens or disturbed secretion of the pancreatic juice or from the secondary damaged formation of the pancreatic enzymes owing to the obstructed duodenum. No conclusions have been decided. After all, the proteinase activity of the fluid of the ileus duodenum appears only in a slight or negligible state, although the appearance of some pancreatic proteinase is not excluded.

2) *Action of peptonase, dipeptidase and tributyrinase of the fluid of the obstructed duodenum loop with the open or with the bound pancreatic duct (18, 20 and 22 hours after operation)*

TABLE II. Peptonase, dipeptidase and tributyrinase of the fluid of ileus duodenum

Acidity increase in 2 ml digestate (ml 0.1 N NaOH)										
Substrates		Peptone-Witte				Diglycine				Tributyrin
Tests	pH \ Hours	24	48	72	96	24	48	72	96	24
A*	4.0	0.04	—	0.10	0.19	—	—	—	—	—
	6.0	0.19	—	0.23	0.26	0.02	—	0.14	0.19	—
	7.0	0.30	—	0.33	0.32	0.24	—	0.43	0.43	—
	7.2	0.30	—	0.40	0.37	0.12	—	0.36	0.36	2.17
	8.0	0.26	—	0.33	0.40	0.28	—	0.33	0.44	1.88
B**	4.0	0.01	0.01	0.03	—	—	—	—	—	—
	6.0	0.13	0.22	0.25	—	0.01	0.10	0.11	—	—
	7.0	0.20	0.29	0.34	—	0.16	0.14	0.25	—	—
	7.2	0.25	0.29	0.35	—	0.09	0.07	0.21	—	1.37
	8.0	0.16	0.30	0.31	—	0.15	0.17	0.27	—	2.24

A) The pancreatic duct was open

B) The pancreatic duct was bound up

* The fluid No. 60 for peptone and diglycine was obtained 22 hours and No. 50 and 54 for esterase 20 and 18 hours after the operation respectively.

** The fluid No. 75 for erepsin was taken 18 hours and No. 70 and 75 for esterase 20 and 18 hours after the operation respectively.

An activity of peptonase and dipeptidase was ascertained in the fluid of the duodenum ileus loop with the open pancreatic duct (Table II, A). As the latter, which showed almost 100% hydrolysis of diglycine, is not contained in the pancreatic juice of a rabbit (1936) (2), it must be due mainly to the duodenal enzyme erepsin. The peptonase activity should be, however, explained by the occurrence of both the duodenal and pancreatic enzymes. Since it appeared to the same extent as in the case of the duodenum ileus with the bound pancreatic duct or of the small intestine ileus, the duodenal enzyme would be responsible for the peptonase activity in these cases (Table II, B).

The fluid content of the duodenum ileus exhibited a notable activity of tributyrinase as in the case of the obstructed small intestine (Table II, A). The esterase can also be influenced by the pancreatic juice. The fluid of the duodenum ileus with the bound pancreatic duct has shown almost a similar activity of the fluid esterase of the duodenum with the open pancreatic duct. These results will be explained by the assumption that the esterase activity of the fluid is mainly due to the duodenal enzyme, but not to the pancreatic enzyme.

3) *Activity of proteinase, peptonase and tributyrinase of the fluid of the obstructed duodenum loop with the open or the bound bile duct (15 hours after operation)*

TABLE III

Acidity increase in 2 ml digestate (ml 0.1 N NaOH)										
Substrates		Casein			Gelatin			Ovalbumin		
Tests	<div>Hours pH</div>	24	48	72	24	48	72	24	48	72
A No. 82 83	4.0	-0.03	-0.07	-0.05	0.02	0.03	0	0.02	0.06	0.04
	6.0	0.01	0.05	0.07	-0.01	0.03	0.04	-0.01	0.03	-0.03
	7.0	0.03	0.08	0.02	0.01	0.07	0.07	0.07	0.05	0.07
	7.2	-0.05	0.05	0.07	-0.04	0.03	0.01	-0.05	0	-0.02
	8.0	0	0.03	0.03	0.04	0.05	0.06	0.03	0.03 _g	0.03
B No. 90 91	4.0	—	—	0.07	—	—	—	—	—	—
	6.0	0.05	-0.01	0.03	0.07	0.02	0.08	0.01	0.05	0.08
	7.0	-0.06	-0.04	0	0.03	0.03	0.04	-0.01	-0.02	-0.07
	7.2	-0.07	0	0	0.05	0.04	0.04	-0.03	0.01	0.02
	8.0	0.03	0.03	0.04	0.03	0.04	0.05	-0.01	-0.01	-0.01

(Continued from p. 32)									
Serumalbumin			Peptone-Witte			Diglycine			Tributyrin
24	48	72	24	48	72	24	48	72	24
0	0.03	-0.02	-0.06	0.04	-0.07	—	—	—	—
-0.02	0.01	-0.05	0.11	0.21	0.17	0.13	0.13	0.15	—
-0.03	0.02	0.03	0.16	—	0.33	0.21	0.23	0.20	—
-0.06	-0.05	-0.04	0.07	0.17	—	-0.01	0.07	0.10	1.25 *
0.02	0.02	0.01	0.11	0.15	0.18	0.09	0.09	0.09	1.13 *
—	—	—	—	—	—	—	—	—	—
0.11	0.09	0.12	0.23	0.23	0.24	—	—	—	—
-0.02	-0.02	0.05	0.12	—	0.22	—	—	—	—
0	0.02	0.02	0.18	0.22	0.18	—	—	—	1.80 **
0.02	0.02	0.02	-0.01	-0.01	0.12	—	—	—	1.36 **

A) The bile duct was open

B) The bile duct was bound up

* It is the data of No. 83

** Data of No. 91 is given

In these cases of the obstructed duodenum, the author has examined the protease activities, when the bile duct was bound. Since the bile has hardly any influence on the protease action as reported by Itzioka (3) (1937), there is no difference between the fluids, obtained from the obstructed duodenum loops with the open or the bound bile duct. The fluids showed only a very slight or nearly a negative activity of the proteinase and a weak activity of the peptonase as well as of the dipeptidase, as shown in the previous experiments.

It must be pointed out here that the tributyrinase of the fluid has a notable activity, even when the bile duct was bound.

SUMMARY

These investigation showed the enzymatic activity of the fluid contents of the duodenum of a rabbit laid in the experimental ileus by the ligation. In some cases the pancreatic duct or the bile duct was bound, in order to exclude the effect of these juice.

1. The activity of caseinase and gelatinase are found only in a slight degree, sometimes even in a negligible values in the fluid of the duodenum ileus loops with open pancreatic duct. Albumins are resistant to the duodenal enzyme, even when the pancreatic duct is open. No appearance of the tissue protease, such as catheptase, was found in the fluid of the tied-off duodenum.

2. The fluid showed a weak activity of peptonase, which did not, however, seem to be strengthened by the experimental ileus.

3. The dipeptidase has restored its activity in an appropriate strength, but sometimes in a very weak degree in the fluid of the experimental duodenum ileus.

4. Tributyrinase was found always in a notable activity, as shown in the case of the tied-off small intestine loops.

5. It appeared to be no difference between the fluid enzymes obtained from the tied-off duodenum loops with the open or the bound duct of the pancreas or of the bile bladder.

REFERENCES

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- 2) ITZIOKA, F.: *J. Biochem.* 24, 237 (1936).
- 3) ITZIOKA, F.: *Ibid.* 26, 37 (1937).