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THE POTENCY OF CERTAIN DIGESTIVE ENZYMES PREPARED FROM THE WHITE RAT TREATED WITH THE CHEMICAL INSECTICIDE "TAMARON".

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INTRODUCTION

The present work is a part of a series of investigations which deal with the effect of the chemical insecticides on the physiological characterestics of mammals. One of these characterestics is the physiology of digestion. Gabr and Said (1972 A, B and C) found that some chemical insectioides (DDT, lindane and malathione) have a hazardous effect on the structure and function of the peptic and oxyntic cells. In 1972 Garb et al. studied the effect of the previously mentioned insecticides on the potency* and activity of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic lipase in the white rat Rattus rattus. They found that the potencies and activities of these enzymes showed a considerable reduction. Said (1979), carried out experiments on the white rat

* The term potency designates the change in the activity of the enzyme prepared from insecticide treated animals.

Rattus rattus to study the effect of DDT, lindane and malathione on the potency of certain digestive enzymes (maltase and dipeptidase), which are secreted from the crypts of Lieberkühn. He found a reduction in the potencies of these enzymes. In 1981, Said experimented the previously mentioned insecticides on the potency of certain digestive enzymes (liver and ileum esterase and pancreatic lipase) prepared from the domestic pigeon Columba livia domestica. The enzymes of the 1/10 high dose (the approximately lethal dose) treated animals showed a considerable reduction in their potencies.

The present work aimed to study the effect of the chemical insecticide "tamaron" on the potency of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase) prepared from the white rat (Rattus rattus). This study might illuminate the way which leads to the clarification of one of the causes of the digestive troubles widely observed nowadays.

MATERIAL AND METHODS

A. Preparation of enzyme solution:

The animals used through this study were adult male white rats (Rattus rattus) weighing about 130 g each. All

the animals were kept on the same normal diet (bread, milk (NIDO) and water), during the experiment, to avoid the probable interference of the effect of food kind. The animals were kept in the laboratory in cages about one weak before use. In each experiment not less than 30 animals were used after killing them by a blow on their heads.

Certain hydrolytic enzymes (the salivary amylase, pepsin, trypsin, pancreatic amylase and pancreatic esterase) were prepared from these animals. Water extracts of fresh salivary (parotid) glands (1:10) stomach mucosa extract (1:10) and pancreatic extract (1:10) were used. Enterokinase was prepared from duodenal mucosa extract according to the prescription of Waldschmidt-Leitz (1924), treated with acetone and ether.

B. Buffers:

A number of different buffer solutions were used in the present work. The nature of each buffer used in the different experiments is indicated in the tables giving the experimental results. The measurement of the pH values was done by the B.D.H. capillator. The error in this method is about 0.05 pH units.

C. Methods of measurement of the enzymes activity:

1. Peptidases:

The method of Willstätter and Waldschmidt-Leitz(1921)

and Willstätter et al. (1926) was used (with some modifications), for measuring peptic and tryptic activities.

For pepsin, the digestive mixture (total volume 5.0 c.c.) had the following composition.

2.5 c.c. 3 % eggalbumen
1.0 c.c. citrate-HCl buffer
1.0 c.c. distilled water, and
0.5 c.c. enzyme solution

5.0 c.c. Total volume.

The incubation time of 20 h was chosen since it was found to be the most suitable one.

For trypsin, the digestive mixture was made in the following way:

0.3 c.c. enzyme solution
0.1 c.c. enterokinase solution
0.35 c.c. buffer solution (0.2 N ammonia-ammonium chloride).
0.15 c.c. distilled water.

The above mentioned solution were mixed together (total volume 0.90 c.c.) and the pH value of the mixture was 8.0 The mixture was put in the thermostate at 37°C for activation. After 30 minutes, the pH was adjusted to the desired value by the addition of 0.6 c.c. 0.1 N veronal

acetate buffer prewarmed to 37°C, after which 1.5 c.c. of 6 % casein solution were added. (Waldschmidt-Leitz, 1924). The total volume of the digestive mixture was therefore 3.0 c.c. The buffer solutions were prepared according to Michaelis (1922 and 1931). The time of incubation is half an hour.

2- Carbohydrases :

Amylase :

The method of Hagedorn and Jensen (1922) was used in the present work.

For salivary amylase, the digestive mixture had the following composition :

2.0 c.c. 2 % starch solution.
0.2 c.c. buffer solution (0.1N veronal-acetate-HCl)
1.3 c.c. distilled water (1.4 c.c. for pancreatic amylase).
0.5 c.c. enzyme solution (0.4 c.c. for pancreatic amylase).

4.0 c.c. Total volume.

The digestive mixture was incubated at 37°C for exactly 10 minutes. The titration sample is of 0.05 c.c. It has been shown by Pucker and Finch (1938) and confirmed by Keddis (1952 and 1956) that each mg maltose has the

same reducing action on potassium ferricyanide as 0.75 mg glucose. Using this factor, the extent of digestion was expressed as "increase in mg maltose" calculated by multiplying each figure of "increase in mg glucose" by the factor 100/75.

3- Esterases :

Esterase:

The method of Willstätter et al. (1923) was followed in the present work. The digestive mixture had the following composition :

1.00 c.c. enzyme solution.
0.05 c.c. ethyl acetate
1.00 c.c. 0.1N veronal-acetate buffer.
1.00 c.c. 2 % $CaCl_2$ and
1.95 c.c. distilled water.

5.00 c.c. Total volume.

The incubation time was one h at 37°C.

D- Estimation of the optimal pH value of the enzymes :

All the experiments of the present study were carried out at the optimal pH of the enzymes. The optimal activity of pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase were 2.1, 8.0, 6.8, 7.1 and 7.5 respectively (Hassan, 1983).

E- Effect of the chemical insecticide on the digestive enzymes :

For studying the effect of tamaron on the potency of the hydrolytic enzymes, 3 doses (1/10, 1/100 and 1/1000 high dose) were tested. The high dose in mg/kg. body weight/day was 30. This high dose is very near to the LD_{50} for tamaron (Hassan, 1983). The animals were given daily the 1/10 high dose, the 1/100 high dose or the 1/1000 high dose orally for 60 days.

RESULTS

a- Peptidases :

1- Pepsin :

Table I displays the data which concern the potency of pepsin prepared from tamaron treated animals as compared with those prepared from normal animals. The 1/10 high dose reduced the potency from 0.80 to 0.47 (=41.3 % reduction), the 1/100 high dose reduced the potency from 0.77 to 0.62 (=19.5 % reduction) and the 1/1000 high dose reduced the potency from 0.81 to 0.70 (=13.6 % reduction).

2- Trypsin:

Table 2 indicates the data which concern the potency of trypsin prepared from chemical insecticide treated animals as compared with those prepared from normal animals.

The 1/10 high dose reduced the potency from 0.97 to 0.78 (=19.6 % reduction) and the 1/100 high dose reduced the potency from 0.98 to 0.91 (=7.1 % reduction), while the 1/1000 high dose reduced the potency from 0.99 to 0.96 (=3.0 % reduction).

b- Carbohydrases:

1. Salivary amylase :

Table 3 shows the potency of the salivary amylase prepared from tamaron treated animals as compared with those prepared from normal animals. The 1/10 high dose reduced the potency from 0.120 to 0.061 (= 49.2 % reduction), the 1/100 high dose reduced the potency from 0.117 to 0.082 (=29.9 % reduction) and the 1/1000 high dose reduced the potency from 0.117 to 0.105 (=7.7 % reduction).

2. Pancreatic amylase :

Table 4 shows the potency of pancreatic amylase prepared from tamaron treated animals as compared with those prepared from normal animals. The 1/10 high dose reduced the potency from 0.133 to 0.061 (=54.2 % reduction), the 1/100 high dose reduced the potency from 0.131 to 0.083 (=36.6 % reduction) and the 1/1000 high dose reduced the potency from 0.133 to 0.112 (=15.8 % reduction).

c- Esterases :

Pancreatic esterase :

Table 5 exhibits the potency of pancreatic esterase prepared from tamaron treated animals as compared with those prepared from normal. The 1/10 high dose reduced the potency from 0.40 to 0.18 (=55 % reduction), the 1/100 high dose reduced the potency from 0.38 to 0.25 (=34.2 % reduction) and the 1/1000 high dose reduced the potency from 0.39 to 0.32 (=18.0 % reduction). Table 6 summarized all the previously mentioned results.

DISCUSSION

The results of the present investigation clearly show the reduction in the potencies of the digestive enzymes (pepsin, trypsin, salivary amylase, pancreatic amylase and pancreatic esterase) extracted from tamaron treated animals. These results could be explained as follows:

a) The enzymes of the 1/10 high dose treated animals, showed a considerable reduction in the potencies. It is clear that the pancreatic esterase was the most reduced by this chemical insecticide, while trypsin is the lowest reduced.

b) The enzymes of the 1/100 high dose treated animals, showed a lower reduction in their potencies as compared

with their correspondings for 1/10 high dose treated animals.

c) The enzymes of the 1/1000 high dose treated animals, showed a slight reduction in their potencies as compared with their correspondings for 1/10 and 1/100 high dose treated animals.

The previously mentioned results were in agreement with those obtained by other workers dealing with the effect of certain chemical insecticides on the potency of the digestive enzymes (Platonova, 1970; Gabr and Said A, Gabr et al. 1972; Said, 1979 and 1981).

SUMMARY

1. The enzymes of 1/10 high dosed treated animals with tamaron, showed a considerable reduction in their potencies. The potencies of pancreatic esterase and pancreatic amylase were the most reduced by tamaron, while that of trypsin is of the lowest reduction.
2. The enzymes of 1/100 high dosed treated animals, showed a lower reduction in their potencies than that of 1/10 high dosed treated animals. The potencies of pancreatic amylase and pancreatic esterase are the most reduced by the chemical insecticide, still that of trypsin is of the lowest reduction.
3. The enzymes of the 1/1000 high dosed treated animals, showed a lowest reduction in their potencies as compared

with their correspondings for 1/10 and 1.100 high dosed treated animals.

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Table 1

The potency of pepsin of tamaron treated white rat.

Digestive mixture 5.0 c.c. containing: 0.5 c.c. extract of stomach mucosa (1:10), 2.5 c.c. 3% eggalbumen, 1.0 c.c. citrate-HCl buffer and 1.0 c.c. distilled water.

Titration sample 0.5 c.c. Mean pH value 2.1 (initial pH 2.2 and final pH 2.0). Temperature 37°C . Time of digestion 20 hours.

Condition	Titration (c.c.0.1N KOH)		Potency c.c. 0.1 N KOH
	After 0 hour	After 20 hours	
Normal (control of 1/10 high dose)	5.88	6.68	0.80
Treated 1/10 high dose	6.87	7.34	0.47
Normal (control of 1/100 high dose)	5.91	6.68	0.77
Treated 1/100 high dose	6.69	7.31	0.62
Normal (control of 1/1000 high dose)	5.88	6.69	0.81
Treated 1/1000 high dose	6.66	7.36	0.70

Table 2

The potency of trypsin of tamaron treated white rat.

Digestive mixture 3.0 c.c. containing : 0.3 c.c. water extract of pancreas (1:10), 0.1* c.c. enterokinase solution, 0.35 c.c. 0.2 N ammonium buffer, 0.15 c.c. dist. H_2O, to which after 30 minutes were added 0.6 c.c. 0.1 N veronal-acetate buffer and 1.5 c.c. 6% casein.

Titration sample 0.5 c.c. pH value 8.0. Time of digestion one hour. Temperature 37°C.

Condition	Titration (c.c.0.02N KOH)		Potency c.c. 0.02N KOH
	After 0 hour	After one hour	
Normal (control of 1/10 high dose)	1.19	2.16	0.97
Treated 1/10 high dose	1.24	2.02	0.78
Normal (control of 1/100 high dose)	1.19	2.17	0.98
Treated 1/100 high dose	1.28	2.19	0.91
Normal (control of 1/1000 high dose)	1.18	2.17	0.99
Treated 1/1000 high dose	1.29	2.25	0.96

* Prepared from normal animals.

Table 3

The potency of salivary amylase of tamaron treated white rat.

Digestive mixture 4.0 c.c. containing: 0.5 c.c. patrotid glands extract (1:10), 2.0 c.c. 2% starch solution, 0.2 c.c. 0.1 N veronal-acetate buffer and 1.3 c.c. dist. H_2O.

Titration sample 0.05 c.c. Time of digestion 10 minutes. Temperature 37°C. pH value 6.8.

Condition	Digestion (mg glucose)		Potency	
	After 0 minute	After 10 minutes	mg glucose	mg maltose
Normal (control of 1/10 high dose)	0.032	0.122	0.090	0.120
Treated 1/10 high dose	0.029	0.075	0.046	0.061
Normal (control of 1/100 high dose)	0.032	0.120	0.088	0.117
Treated 1/100 high dose	0.031	0.093	0.062	0.082
Normal (control of 1/1000 high dose)	0.034	0.122	0.088	0.117
Treated 1/1000 high dose	0.034	0.113	0.079	0.105

Table 4

The potency of pancreatic amylase of tamaron treated white rat .

Digestive mixture 4.0 c.c. containing: 0.4 c.c. pancreatic extract (1:10), 2.0 c.c. 2% starch solution, 0.2 c.c. 0.1 N veronal-acetate buffer and 1.4 c.c. dist. H_2O.

Titration sample 0.05 c.c. Time of digestion 10 minutes. Temperature 37°C. pH value 7.1.

Condition	Digestion (mg glucose)		Potency	
	After 0 minute	After 10 minutes	mg glucose	mg maltose
Normal (control of 1/10 high dose)	0.124	0.224	0.100	0.133
Treated 1/10 high dose	0.129	0.175	0.046	0.061
Normal (control of 1/100 high dose)	0.124	0.222	0.098	0.131
Treated 1/100 high dose	0.129	0.191	0.062	0.083
Normal (control of 1/1000 high dose)	0.124	0.224	0.100	0.133
Treated 1/1000 high dose	0.127	0.211	0.084	0.112

Table 5

The potency of pancreatic esterase of tamaron treated white rat.

Digestive mixture 5.0 c.c. containing: 1.0 c.c. pancreatic extract, 0.05 c.c. ethyl acetate , 1.0 c.c. 0.1 N veronal acetate buffer, 1.0 c.c. 2% $CaCl_2$ and 1.95 c.c. dist. H_2O.

Titration sample 0.5 c.c.Time of digestion one hour. Temperature 37°C.pH value 7.5.

Condition	Titration (c.c.0.01N KOH)		Potency c.c. 0.01N KOH
	After 0 hour	After one hour	
Normal (control of 1/10 high dose)	1.14	1.54	0.40
Treated 1/10 high dose	1.18	1.36	0.18
Normal (control of 1/100 high dose)	1.20	1.58	0.38
Treated 1/100 high dose	1.19	1.44	0.25
Normal (control of 1/1000 high dose)	1.17	1.56	0.39
Treated 1/1000 high dose	1.19	1.51	0.32

Table 6

Effect of the chemical insecticide "tamaron" on the potency of the digestive enzymes.

Enzymes	Percentage of reduction of the potency of the extract of treated animals		
	1/10 high dose	1/100 high dose	1/1000 high dose
Pepsin	41.3	19.5	13.6
Trypsin	19.6	7.1	3.0
Salivary amylase	49.2	29.9	7.7
Pancreatic amylase	54.2	36.6	15.8
Pancreatic esterase	55.0	34.2	18.0