THE PHYTOLYTIC AND ZOOLYTIC ACTIVITY OF BLOOD AMYLASE IN EXPERIMENTAL ETHIONIN PANCREATITIS

A. M. Ugolev and T. M. Chulkova

Laboratory of General Physiology, (Director: Academician V. N. Chernigovskii), Institute of Normal and Pathological Physiology (Director: Active Member of the Academy of Medical Sciences, USSR V. V. Parin), Academy of Medical Sciences, USSR, Moscow
(Presented by Academician V. N. Chernigovskii)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 52, No. 9, pp. 45-50, September, 1961
Original article submitted October 17, 1960

It can at present be regarded as proven that blood amylase, lipase and trypsinogen are enzymes of pancreatic origin and that their level and relative proportion reflect to a certain degree the state of the pancreas [1, 2, 3, 13]. Clinical observations, which showed that changes in the levels of the pancreatic enzymes, particularly amylase, in the blood, frequently are the only reliable criterium enabling us to judge pathological conditions of the pancreas, are of greatest importance [1, 3, 5, 7]. Estimation of the level of amylase and other digestive enzymes in the blood, however, are by no means the only method of assessing the functional state of the pancreas. E. g., we had been able to show that, in the course of the normal function, not only the level of amylase in the blood but also its properties undergo certain changes [8, 9]. In particular, it could be established that the relative activity of amylase, with regard to starch and glycogen, changes, depending on the quality of the food. The amylase of herbivores and of animals kept on a vegetable diet splits starch more intensively than glycogen, whereas the amylase of carnivores, kept on a meat and milk diet, is more active with regard to glycogen than with regard to starch [8, 9]. In this context the question arises whether lesions affecting the pancreas cause changes in the properties of amylase, or whether the pathological processes lead only to changes in the amounts of the enzyme synthetized and secreted by the gland.

In the present paper we discuss changes in the level and in the properties of blood amylase in experimental pancreatitis produced by the administration of ethionin. This method of producing experimental pancreatitis is attractive not only because of its simplicity, but also because it enables us to select the desired degree of disorders in the pancreas function. We used small doses of ethionin which led to short-lasting and transient disorders in the pancreas function, as it is these type of disorders which cause the greatest diagnostic difficulties.

Ethionin, as is well known, inhibits the protein synthesis preventing the incorporation of methionin and glycin into the protein [10, 12]. Ethionin inhibits the exocrine function of the pancreas, and later pancreatitis develops. The production of amylase and lipase are most of all impaired; the production of trypsinogen is affected to a lesser degree [10, 12]. Ethionin is a homolog of methionin; hence it is understandable that equal doses of ethionin combined with a diet, poor in methionin, or with starvation, will cause a more severe pancreatitis [10, 12]. This fact was used in the experiments described below.

METHODS

The experiments were carried out on white rats weighing between 120 and 130 g. In each experimental series the animals were divided into two groups: a control group and an experimental group. Previously the phytolytic and zoolytic* activity of the blood amylase had been estimated; then the experimental rats were given ethionin by intraperitoneal injection (250 mg/kg). After 24.48 and 72 hours respectively the blood amylase activity was estimated in both the experimental and the control rats.

The amylolytic activity was estimated colorimetrically on the basis of the decrease in the concentration of starch and glycogen; (decrease in the coloring of an iodine-starch solution as measured in a photoelectric colorimeter through a red filter and decrease in the coloring of an iodine-glycogen solution measured through a blue filter).

* The term "phytolytic activity" denotes the enzyme activity on substrates of vegetable origin; the term "zoolytic activity" refers to the enzyme activity on substrates of animal origin.

0.15% solutions of starch and glycogen in phosphate buffer (pH 7.2) were used as substrates. 1 ml plasma, previously diluted 50 times with sodium chloride solution was added to 4 ml substrate. The mixture was incubated in a waterbath at 38°C for 30 min under continuous stirring.

The Influence of a Single Injection of Ethionin (250 mg/kg) upon the Amylolytic Activity of Rat Blood during Starvation

No. of animals	Amylolytic activity (hydrolysis of the substrate in %)	Control rats: day of experiment 1st	2nd	3rd	4th	Experimental rats: day of experiment 1st	2nd	3rd	4th
1	P	79	78	56	45	79	24 hrs. after the injection of 30 mf ethionin: 29	48 hrs. after the injection of 30 mg ethionin: 43	72 hrs. after the injection of 30 mg ethionin: 51
	Z	68	64	48	40	68	17	54	44,5
	P/Z	1,16	1,20	1,16	1,12	1,16	The same 0,70	The same 0,80	The same 1,14
2	P	79	74	49	39	79	» » 26,5	» » 35	» » 40
	Z	68	67	41	34	68	» » 34,5	» » 40	» » 34
	P/Z	1,16	1,14	1,20	1,14	1,16	» » 0,76	» » 0,87	» » 1,18
3	P	73	70	47	34	79	» » 29	» » 42	» » 45
	Z	68	52	40	30	68	» » 25	» » 52	» » 38
	P/Z	1,16	1,30	1,17	1,13	1,16	» » 1,16	» » 0,8	» » 1,20
4	P	79	72	40	39	79	» » 20,5	» » 30	» » 37,5
	Z	68	58	32	35	68	» » 23	» » 39	» » 40
	P/Z	1,16	1,20	1,20	1,11	1,16	» » 0,8	» » 0,8	» » 0,95

Remark: P = phytolytic activity; Z = zoolytic activity.

The reaction was stopped by addition of HCl. The coloring of a solution, containing the same ingredients but treated with HCl before the incubation, was defined as 100%. As we said above, identical doses of ethionin caused more severe lesions in the pancreas of starving animals than in the pancreas of well fed animals. For that reason we used in some experiments starving animals, (in both the experimental and the control group) and in others, animals kept on a normal diet.

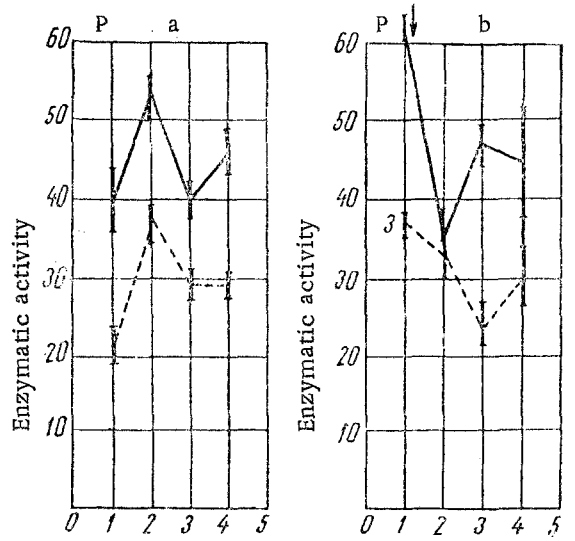
RESULTS

The data set forth in the table and in the figure show that, before the administration of ethionin in all, control and experimental animals, the phytolytic activity was stronger than the zoolytic activity.

In the control animals, which were fed normally throughout the experiment, the amylase activity with regard to starch predominated as usual. The variations in the amylase level did not exceed the range of the usual daily variations. In the experimental rats a marked fall in the amylase activity with regard to starch, and - in some cases - a slight fall in the amylase activity with regard to glycogen could be observed within 24 hours after the injection of ethionin. In consequence both types of activity were approximately at the same level*. Later, the amylase activity with regard to starch increased, and after 48-72 hours the relative proportion of the phytolytic and zoolytic activity had returned to the original values (see figure).

The disorders in the pancreatic function, developing after a single small dose of ethionin, are, as is well known, of transient character [9, 12]. The restoration of the normal proportion between the phytolytic and the zoolytic activity, observed 48 hours after the injection of ethionin (and later) apparently reflects the normalization of the pancreatic function.

In the control rats, which were starved throughout the period of the experiment, the activity with regard to starch was consistently higher and the daily variations in the amylolytic activity were of more regular character; this became manifest in a slight, gradual and parallel decrease of both activities (see table). In the starting rats of the experimental group, the fall in the amylase activity observed 24 hours after the injection of ethionin, both with regard to starch and to glycogen, was more marked than in the well fed rats; the phytolytic activity showed a more marked fall than the zoolytic activity. On the second day the activity was gradually restored; the zoolytic activity was more rapidly restored. In consequence, 48 hours after the injection of ethionin the activity with regard to glycogen was in all rats higher than the activity with regard to starch (see figure).



The influence of a single injection of ethionin (250 mg/kg) upon the amylolytic activity of the blood of rats kept on a normal diet. (Average values, M±m). a) Control group; b) experimental group; the arrow indicates the moment of ethionin injection; P) phytolytic activity; Z) zoolytic activity (hydrolysis of the substrate in %).

Above we said that ethionin pancreatitis takes a more severe course in starving rats than in well fed animals. The relative proportion between the phytolytic and zoolytic amylase activity reverted to its normal value 3 days after the injection of ethionin. (See table).

The data quoted above warrant the conclusion that in experimental pancreatitis, not only the level, but also the properties of blood amylase undergo changes. The changes in the amylase level on the one hand, and the changes in the properties of the enzyme on the other hand, are probably caused by different factors. E.g. the increased blood amylase level was in most cases due to the obstruction of the outflow of pancreatic juice i.e. to disorders in the excretory ducts rather than to disorders in the secretory apparatus. Direct evidence for this view was supplied by the experiments of F.G. Milyushkevich, who showed that a temporary obstruction of the outflow of pancreatic juice in dogs with a chronic pancreatic duct fistule causes a considerable amylasemia [6]. Changes in the permeability of the secretory cells may also lead to an increase in the blood amylase level as shown by the findings of Egdahl [11].

It thus appears that the blood amylase level characterizes the conditions for the excretion of the enzyme rather than the functional state of the cellular elements themselves. And indeed: pancreatitis caused by injection of ethionin is followed by a certain decrease in the amylolytic activity of the blood and not by an increase, notwithstanding the striking structural and metabolic disorders of the pancreas.

The most general principle detected by us in our studies concerning the relative proportion of the phytolytic and zoolytic amylase activity in cases of pancreatitis can be summed up as follows: It could be observed that, inde-

* The marked fall in the level of the amylolytic activity caused by the administration of ethionin, which was observed by other authors as well, can be explained by the interference with the enzyme synthesis in the pancreatic cells.

dependent from the severity of the condition, the differences between the two types of activity were levelled out in contrast to the usual marked predominance of the phytolytic activity. This came about by a marked fall in the phytolytic activity; in the majority of cases the fall in the zoolytic activity was insignificant and in some animals activity of the latter type did not decrease at all. Hence, it follows that ethionin impairs to a greater degree those stages of the synthesis which secure the phytolytic properties of amylase. In other words, the disorders in the enzyme synthesis, developing in case of experimental pancreatitis, are not only of quantitative but also of qualitative character.

In more severe forms of pancreatitis, the synthesis of both the phytolytic and the zoolytic amylase were impaired. Recovery led to the restoration of the normal proportions between the phytolytic and zoolytic activity.

In our opinion it is important to underline that changes in the relative proportion of the two types of activity became clearly manifest even in those cases in which the changes in the amylase level did not exceed the range of normal variation. In consequence it seems advisable to investigate not only the amylase level, but also the properties of the enzyme for diagnostic purposes; the amylase level throws light upon the conditions governing the outflow of the secretion, whereas the relative proportion between the phytolytic and zoolytic activity reflects the course of the synthetic process in the pancreas.

The changes in the properties of amylase observed by us in cases of pancreatitis enable us, in our opinion, to voice yet another important assumption: it is generally believed that in disorders of the digestive glands the properties of the enzymes do not as a rule change, compared to the normal state. This conception was being used for the construction of theories concerning the pathogenesis of various pancreatic and gastric disorders. From the findings reported in the present paper, however, it appears that changes in the properties of digestive enzymes ("Pathology of Enzymes") do take place even in those cases in which other manifestations of the condition are insignificant. Should it appear that under pathological conditions changes occur not only in the properties of amylase, but also in the properties of proteolytic enzymes, this fact will, to a certain degree, change our views at least with regard to those pancreatic and gastric disorders in which the digestion of tissues by digestive enzymes plays an important part.

SUMMARY

As shown, even with mild forms of ethionine pancreatitis there occur, apart from the changes in the blood amylase level, significant changes in the spectrum of its fermentative activity. This has led to a conclusion that it is not only the content of digestive enzymes that changes in pathological conditions but also their properties; this may be useful for understanding the pathogenesis of these disease and their diagnosis.

LITERATURE CITED

1. V. M. Voskresenskii, Acute Pancreatitis [in Russian] (Moscow, 1951).
2. M. D. Kiverin and M. A. Ritt, Biokhimiya, 1953, No. 3, p. 257.
3. N. I. Leparskii, Diseases of the Pancreas [in Russian] (Moscow, 1951).
4. V. I. Lagutin, Dissertation: On the Influence of Food of Different Quality upon the Amylase Content in the Saliva of Dogs [in Russian] (Moscow, 1955).
5. S. V. Lobachev, Klin. med. 1951, No. 3, p. 19.
6. G. F. Milyushkevich, Scientific Conference on Problems concerning the Physiology and Pathology of Digestion [in Russian] (Tartu, 1957), p. 179.
7. N. N. Samarin, The Diagnosis of the "Acute Abdomen" [in Russian] (Leningrad, 1940), p. 117.
8. A. M. Ugolev, AN SSSR, 1957, Vol. 113, No. 2, p. 478.
9. A. M. Ugolev, Izv. AN SSSR, Seriya biol., 1960, No. 5, p. 768.
10. V. Becker, Sekretionsstudien am Pankreas. Stuttgart, 1957.
11. R. H. Egdahl, Ann. Surg., 1958, v. 148, p. 389.
12. G. Gambassi and L. Del Gatto, Z. ges. exp. Med. 1956, Bd. 128, S. 128.
13. W. E. Knox, et al., Physiol. Rev., 1956, v. 36, p. 164.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.