THE EFFECT OF DL-ETHIONINE ON THE CONTENT OF SOME ENZYMES IN PANCREAS AND LIVER

by

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INTRODUCTION

Recently the effect of DL-ethionine on the pancreas and the liver has been reported by FARBER AND POPPER[1], GOLDBERG, CHAIKOFF AND DODGE[2] and BOLLAG[3]. The experiments carried out by these authors revealed that DL-ethionine has a destructive effect on the acinar tissue of the pancreas of rats, and also that the extent of damage to the acinar tissue is dependent upon the dose and duration of treatment. If, for instance, a rat is treated with a sufficiently large dose over a longer period, the acinar tissue becomes completely obliterated and replaced first by infiltrative inflammatory tissue and later by connective tissue. Histological changes are induced also in the liver as well as in the pancreas following treatment with ethionine. Early changes in the former organ are seen two or three days after treatment, in the form of fatty degeneration of the liver cells. This is invariably followed by a focal necrosis and histiocytic interstitial infiltration which develops after the 10th day. It is also of interest to note that there is a sex and species difference in response to treatment with this compound. Female rats show more damage than the male rats, and mice do not show any marked injury to liver and pancreas even when treated with massive doses. It has been shown that the destructive effects of ethionine on liver and pancreas can be completely prevented by the simultaneous administration of equal amounts of methionine[1]. Furthermore, SIMPSON, FARBER AND TARVER[4] have demonstrated that ethionine affects protein synthesis by inhibiting the incorporation of methionine and glycine into proteins in the liver. In order to obtain further data on the action of ethionine, investigations were made on the catalase activity of liver and pancreatic tissue and diastase activity of pancreatic tissue of rats treated with different doses and for different periods of time.

EXPERIMENTAL PART

Albino stock rats, 8 weeks old, weighing 140–160 g at the commencement of the experiment were used exclusively. They were fed on a diet containing 15% protein. DL-ethionine was injected intraperitoneally once daily in a solution of 15 mg per ml. The animals were killed by decapitation after having been previously starved for 3 hours. Histological preparations of liver and pancreas were made by fixing in BOUIN'S aqueous solution and staining by haematoxyline-eosine.

Catalase estimations were carried out as follows: after decapitation the blood was allowed to drain from the carcass. Pieces of liver and pancreas were rapidly dissected, weighed and homogenized in cold-glass distilled water. Catalase has been estimated by the method of VON EULER AND JOSEPH-

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SON[5], measuring the hydrogen peroxide remaining in a given volume of standard solution after allowing the enzyme to act for a given time. The properly diluted homogenate was allowed to react with approximately 0.01 N H_2O_2 at pH 6.8 and at 0° C. Aliquots of the reacting mixture were removed at 3, 5, 7 and 9 minutes time, inactivated with 2 N H_2SO_4 and the residual H_2O_2 titrated with thiosulphate.

Diastase of the pancreatic tissue was estimated after the method devised by WOHLGEMUTH[6]. One diastase unit (WOHLGEMUTH) is the amount of diastase that splits 1 ml of a 0.2% starch solution to such an extent that the blue colour given with a few drops of added $N/50$ iodine does not appear any more. The quantity of diastase is indicated in diastase units per gram of fresh tissue.

RESULTS

The results of the estimation of enzymatic activity in liver and pancreas are to be seen from Tables I and II.

In Table I, the values of enzyme activity of liver and pancreas of 12 control animals consisting of 6 males and 6 females are seen, and, in Table II the values of the treated

TABLE I

CONTROLS

Males			
Weight (grams)	*Pancreas catalase (% of mean value)*	*Liver catalase (% of mean value of males)*	*Pancreas diastase (Wohlgemuth units)*
158	98	108	80 millions
160	92	82	10,000 millions
145	129	124	80 millions
150	82	94	160 millions
142	118	109	40 millions
155	81	83	1200 millions
Females			
Weight (grams)	*Pancreas catalase (% of mean value)*	*Liver catalase (% of mean value of females)*	*Pancreas diastase (Wohlgemuth units)*
156	121	92	40 millions
150	82	67	160 millions
152	155	103	80 millions
146	74	130	10,000 millions
144	76	85	4000 millions
158	92	123	1000 millions

animals are also shown. It must be specially emphasized that the values of liver catalase show a pronounced sex difference, and confirms the previous work of ADAMS[7]. The mean value of males' liver catalase lies 33% higher, that of females' 33% lower than the average of both sexes' values. For this reason all values of liver catalase are expressed in % of the mean value of the concerned sex.

The histological changes induced in both the pancreas and liver following this treatment have previously been described[1,2,3]. Therefore these results will be only briefly summarised. The pancreas showed disintegration of the acinar tissue finally leading to complete destruction and replacement by inflammatory and connective tissue. The liver

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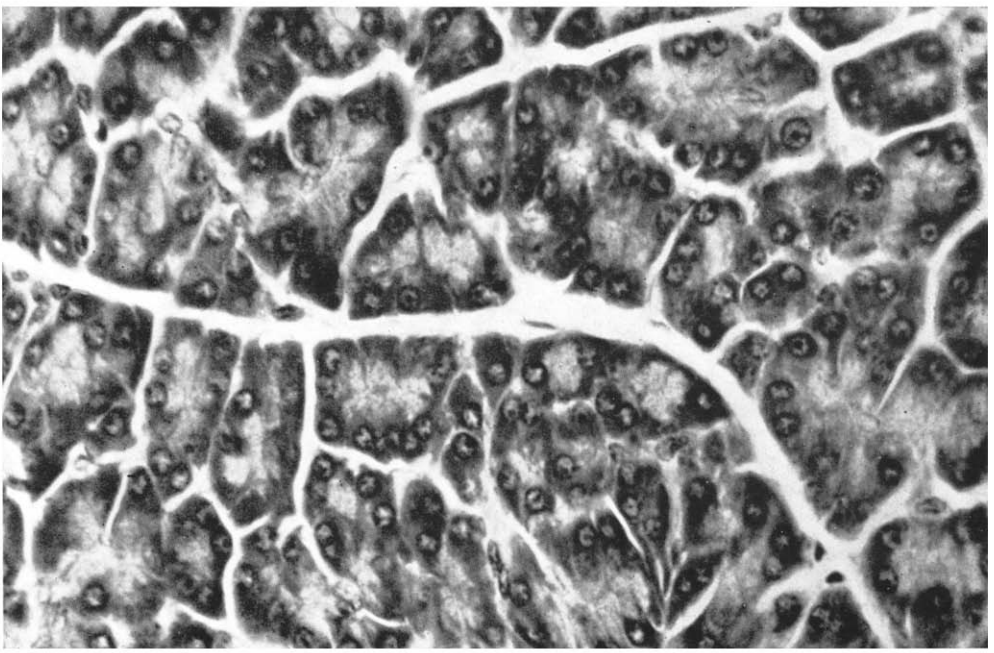

Fig. 1. Normal pancreas of a female rat. Pancreas diastase: 10,000 million units. Pancreas catalase: 74% of mean value. H. -E.: × 720.

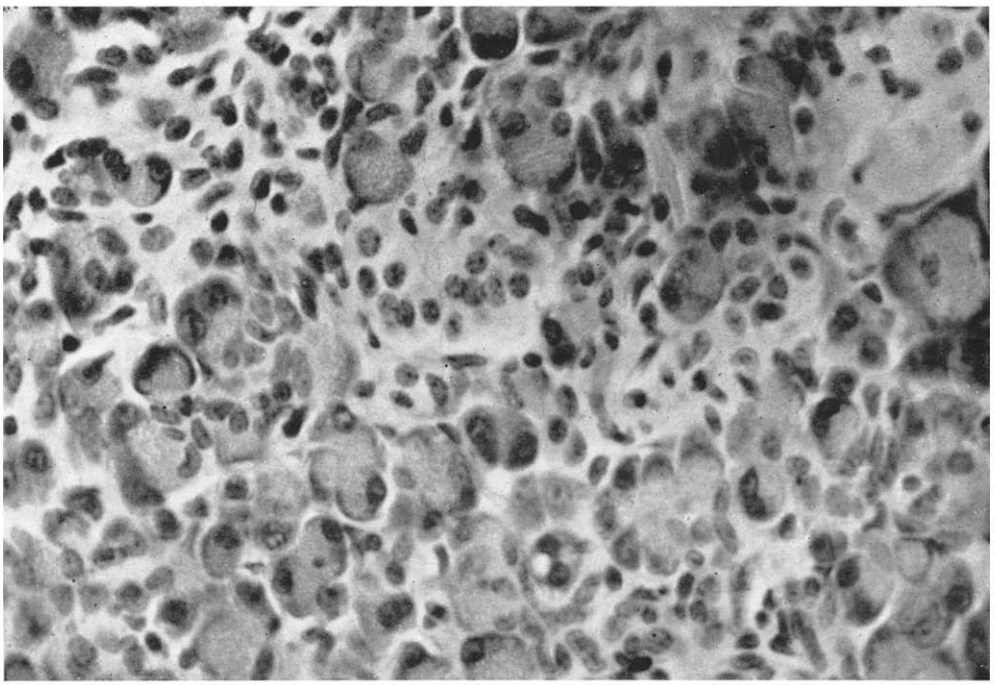

Fig. 2. Pancreas of a female rat treated with 10 mg. DL-ethionine daily for 23 days. Marked destruction of acinar tissue. Pancreas diastase: 1 million units. Pancreas catalase: 389% of mean value of normal pancreas. H. -E.: × 720.

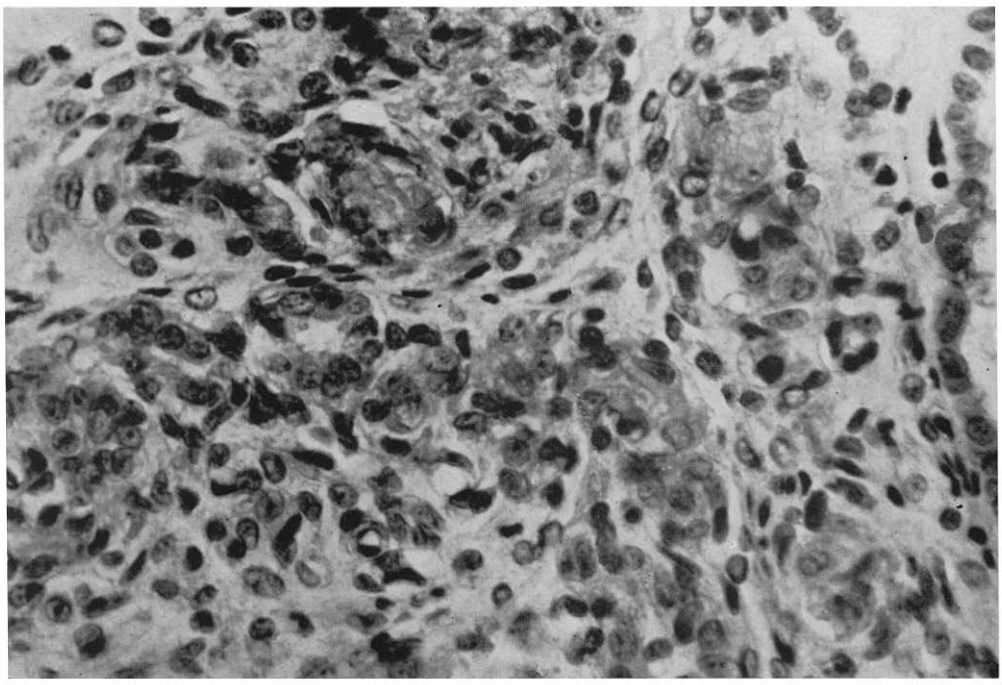

Fig. 3. Pancreas of a female rat treated with 30 mg DL-ethionine daily for 13 days, showing destruction of acinar tissue and replacement by inflammatory and connective tissue. Pancreas diastase: 0 units. Pancreas catalase: 522% of mean value of normal pancreas. H.-E.: × 720.

TABLE II

Treatment	*Weight (grams)*	*Pancreas catalase (% of mean value)*	*Liver catalase (% of mean value of males)*	*Pancreas diastase (Wohlgemuth units)*
Males				
30 mg daily for 13 days	136	496	25	0
20 mg daily for 23 days	180	555	28	20
10 mg daily for 23 days	186	351	26	100,000
60 mg 24 hours before estimation	154	294	48	5 millions
100 mg 24 hours before estimation	154	260	60	2.4 millions
100 mg 48 hours before estimation	156	371	49	40 millions
Females				
30 mg daily for 13 days	126	522	22	0
20 mg daily for 23 days	106	530	22	0
10 mg daily for 23 days	176	389	43	1 million
60 mg 24 hours before estimation	142	166	64	2.5 millions
100 mg 24 hours before estimation	150	592	56	5 millions
100 mg 48 hours before estimation	144	407	47	1,200 millions

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undergoes fatty degeneration which is later followed by focal necrosis of liver cells and histiocytic infiltration. The liver and pancreas of females were found to be more damaged than those of the males.

DISCUSSION

The activity of all the three enzymes we measured was found to have a wide range of variation in normal rats, and the liver catalase in every instance showed a sex difference. For this reason, one has to be very cautious in the interpretation of these results. Therefore, small differences in enzymatic activity cannot be evaluated to be of significance. We chose liver catalase as an example for a liver enzyme, because the liver catalase can be diminished to a great extent even under conditions when no morphological damage to the liver tissue is visible by the routine histological examination. This catalase depression is well known in tumour-bearing animals[7,8,9]. Diastase of the pancreas was selected for this work as being one of the most significant enzymes produced by this particular organ.

Following destruction of the acinar tissue by treatment with ethionine, pancreatic diastase ceased. This was the case in the male and female treated with 30 mg for 13 days and in the female treated with 20 mg for 23 days. In another instance a male treated with 20 mg for 23 days had only the minimal amount of 20 units diastase left in its pancreas although the histological picture revealed still intact acinar tissue in some areas of the organ. Apparently the function of this tissue to produce diastase was reduced. The rats treated with 10 mg for 23 days and the rats killed 24 hours after a single dose of 60 and 100 mg showed a pancreas diastase which was slightly diminished. It is therefore assumed that the diastase values correspond in general to the extent of morphological damage to the acinar tissue of the pancreas, depending on dose and duration of treatment (Figs. 1, 2 and 3).

The catalase content of the pancreas is in normal rats a very low one, about 1/120th of the catalase content of liver. In all the DL-ethionine-treated animals the catalase content of the pancreas was increased even following small doses and after a short duration of treatment. It may be that the source for this increase in catalase can be attributed to the cells of the inflammatory interstitial infiltration or to an increased number of red blood cells. In this respect it is of interest to note that peroxydase which is known to occur in leucocytes has the same hydrogen-peroxide-splitting activity as catalase and also that this enzyme may be responsible for the increased values.

The liver catalase is reduced to about 1/4–1/5 in the animals treated with 20 mg for 23 days or 30 mg for 13 days, respectively; but even 24 to 48 hours after a single high dose the catalase content is markedly reduced. Therefore it is probable that this early effect on the liver enzyme is the consequence of the direct action of ethionine on the liver rather than an indirect one.

This investigation has been supported by grants to the Royal Cancer Hospital from the British Empire Cancer Campaign, the Jane Coffin Childs Fund for Medical Research the Anna Fuller Fund and the Division of Research Grants of the U.S. Public Health Service.

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SUMMARY

The effect of DL-ethionine on liver and pancreas catalase and on pancreas diastase has been investigated. The decrease in pancreas diastase activity was found to correspond roughly to the extent of destruction of acinar tissue. The catalase content of the liver is markedly reduced by the administration of DL-ethionine. Pancreas catalase activity is increased possibly as a consequence of the appearance of inflammatory cells or red blood cells in the pancreas.

RÉSUMÉ

Nous avons examiné l'effet de DL-ethionine sur la catalase du foie et du pancréas et sur la diastase du pancréas. La réduction de l'activité de la diastase du pancréas, correspond à l'étendue de la destruction du tissu acinaire. L'administration de DL-ethionine réduit l'activité de la catalase du foie. Le pancréas contient plus de catalase après traitement avec DL-ethionine, probablement à cause de la présence de cellules inflammatoires et d'erythrocytes dans le pancréas.

ZUSAMMENFASSUNG

Die Wirkung von DL-Aethionin auf Leber- und Pankreas-Katalase und auf Pankreas-Diastase wurde untersucht. Die Pankreas-Diastase Werte fallen ab, je nach Ausdehnung der Zerstörung des azinären Gewebes des Pankreas. Die Katalase Aktivität der Leber wird durch DL-Aethionin beträchtlich reduziert. Die Katalase Aktivität des Pankreas wird nach Behandlung der Ratten mit DL-Aethionin erhöht gefunden. Dies ist wahrscheinlich auf die Anwesenheit von Entzündungszellen oder roten Blutkörperchen im Pankreas zurückzuführen.

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Received November 29th, 1951