THE RELATIONSHIP OF PLASMA AMYLASE TO PANCREATIC DAMAGE INDUCED BY ETHIONINE

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It has been shown repeatedly[1-10] that ethionine will produce pancreatic acinar and hepatocellular damage in rats, presumably by competition with its analogue methionine. In a previous study,[7] it was found that active regeneration of acinar cells of the pancreas occurred after 28 days, while the animals were still being given ethionine. After 63 days of continuous feeding of ethionine, the regenerated areas in the pancreas were more differentiated, and complete acini were formed, giving the appearance of normal pancreas. During the early period of these experiments, the animals had lost considerable body weight, but by the end of the 63-day experimental period most of the weight lost had been regained. These observations raised the possibility of a return of function, concomitant with the acinar regeneration and recovery of body weight. Others have shown that following the administration of ethionine there was a lowering of serum amylase values.[11] However, these were for short term experiments, and no data are available regarding the serum amylase levels in animals given ethionine for prolonged periods. Therefore, the present experiments were undertaken to determine if the regeneration of pancreatic acini which occurs during prolonged ethionine feeding is accompanied by a return of function as indicated by serum amylase values.

The experiment was divided into two parts. Part I was designed to determine the early effects of dl-ethionine upon amylase levels while part II was set up to evaluate the long-term effect of ethionine on amylase levels in the blood.

MATERIAL AND METHODS

Part I

Seventy-two male albino rats of Sprague-Dawley strain, each weighing approximately 330 gm. were used. The animals were housed in groups of 4 per cage, and each animal was weighed daily. Water and food were

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administered *ad libitum*, except for the animals in the weight control group (group III). The rats were divided into 3 groups. Group I contained 24 animals, which were fed a basic diet supplemented with 0.5 per cent dl-ethionine. Group II was made up of 24 animals which were fed the basic diet without ethionine supplement. Group III consisted of 24 animals which were used for weight controls and were fed the basic diet in limited amounts to produce approximately the same weight changes as those in the animals fed ethionine.

The basic synthetic diet had the following composition: glucose, 67 gm.; vitamin free casein, 18 gm.; salt mixture,[12] 4 gm.; corn oil, 11 gm. (containing 0.001 cc. halibut liver oil). Crystalline vitamins were added in the following amounts per hundred gm. of diet: thiamine chloride, 400 μg.; pyridoxine hydrochloride, 400 μg.; riboflavin, 800 μg.; calcium pantothenate, 1,500 μg.; and nicotinic acid, 2,500 μg.

Four animals from each group were sacrificed after experimental periods of the following lengths: 8, 14, 20, 26 hours, and 2 and 3 days. All the rats were fed at 6:00 p.m., as the animals consumed most of their food during the night. The animals were fasted for 2 hours prior to obtaining blood samples, to avoid amylase changes due to very recent consumption of food. It has been reported[11,13,14] that diet affects the serum amylase levels and feeding stimulates enzyme secretion of the pancreas.[15,16] A few drops of blood were collected from a tail vein into a vial containing heparin. The blood was then drawn into capillary tubes, centrifuged, and the plasma separated by breaking the tube. A micromethod was developed for the determination of plasma amylase[17] which was a modification of the method of Myers, Free and Rosinski.[18] Each specimen was run in duplicate together with a plasma blank, a starch blank, and a glucose standard. The amylase determinations were carried out simultaneously on blood samples from the ethionine-fed animals and the normal and weight control animals in order to have comparable results. Amylase was determined daily for 3 days prior to the administration of ethionine, or before the animals were fed the basic diet in limited amounts. These determinations were used to establish a mean amylase value for each animal to serve as a point of reference. Following this, one amylase determination was carried out on each animal sacrificed at 8 hours. Each animal that was allowed to live for 14 hours had 2 serum amylase determinations, one at 8 and the other at 14 hours. Each animal that was allowed to survive the first 2 experimental periods had 3 serum amylase determinations, one immediately preceding sacrifice and one during each of 2 preceding experimental periods, and so on.

The animals were sacrificed under ether anesthesia. At necropsy, salivary glands, pancreas, and liver were removed, fixed in 10 per cent

buffered formalin and weighed. Sections were stained with hematoxylin and eosin. Sections from liver were also stained with Sudan IV for fat. Histologically, the liver fat was graded from 0 to 8+. The histologic alterations in liver and pancreas were graded from 0 to 7+.

Part II

The control groups in part II were set up differently from those for part I. This was done because it was found that there was a reduction in the plasma amylase levels in the animals in part I which were placed on restricted caloric intake in order to serve as weight control animals. For this reason, it was considered advisable to use pair-fed animals as controls rather than animals fed *ad libitum*. Also, it was felt that while the latter were satisfactory for a short-term experiment, pair-fed controls on an isocaloric diet would more truly reflect changes due to the reduced intake of food alone. In this part of the experiment it was not considered necessary to establish a base line for each individual animal prior to the beginning of the experiment, since after such a base line had been established in part I, it was found that the percentage variations in plasma amylase were essentially similar whether they were compared to their own base line or to normal controls.

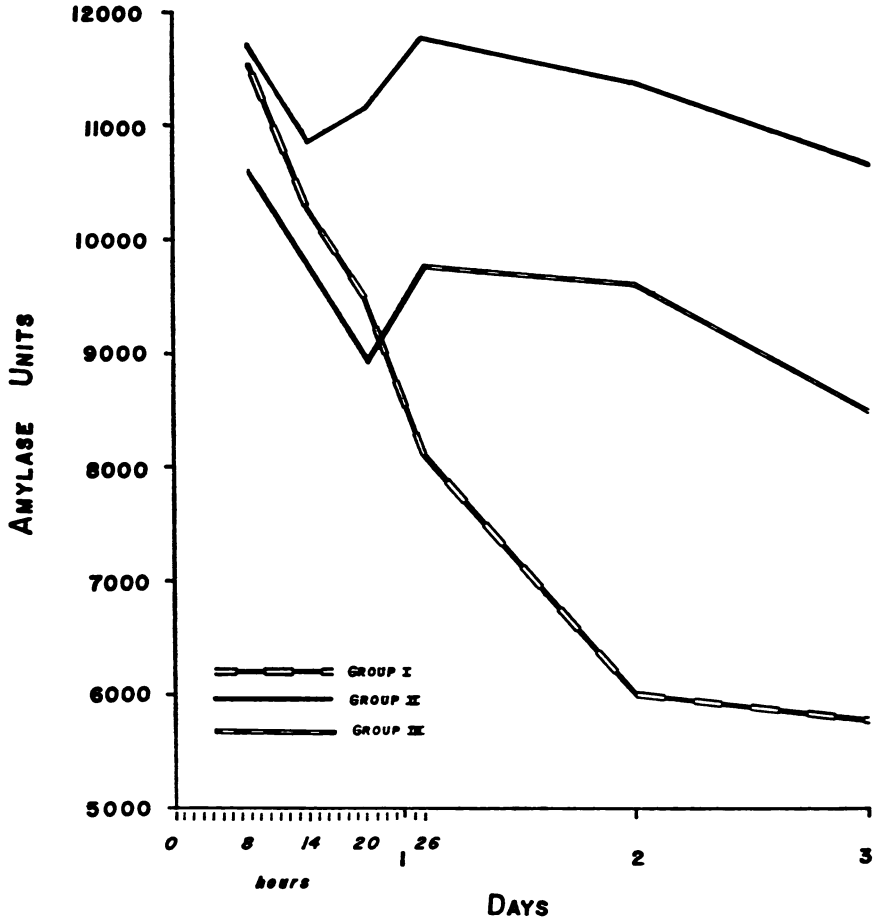
One hundred and fifty-six male albino rats, each weighing approximately 275 gm., were used. They were divided into 3 experimental groups. Group I animals were fed the ethionine-containing diet *ad libitum*. Group II animals were pair-fed controls and received the basic diet. Group III animals were used as control animals for weight loss and received the basic diet in limited amounts to match approximately the weight changes occurring in group I animals. The diets had the same composition as outlined above. Group I and II animals were housed individually, and those of group III were housed 4 per cage. Plasma amylase determinations were done 4, 6, 8, 10, 14, 18, 22, 28, 36, 44, 52, and 60 days following the first administration of ethionine. The experiment was arranged so that at each interval 12 determinations were performed from each group simultaneously. On the fourth day, 8 determinations were performed from each group. At each interval, 4 animals from each group were sacrificed. At 60 days, 12 animals were sacrificed from each group. In all other respects, the experiments were performed essentially as described in part I.

Results

Plasma Amylase Values—Part I

The changes in plasma amylase are indicated in Text-figures 1 and 2 and Tables I to III. The first significant changes in the amylase levels

were observed at 20 hours. These were present in the animals in both the ethionine-fed group (group I) and the weight control group (group III). The amylase level was 9,548 ± 1,657 in the former and 8,963 ± 2,100 in the latter, whereas in the normal controls (group II) it was



Text-figure 1. Changes in plasma amylase values associated with ethionine feeding (group I, basic diet plus 0.5 per cent ethionine; group II, basic diet fed *ad libitum;* group III, basic diet, weight controls).

11,226 ± 1,699. The differences between the ethionine-fed group and normal controls (groups I and II) and weight controls and normal controls (groups III and II) were statistically significant (Table II). There was no significant difference in amylase values between the animals fed ethionine and the weight control rats at 20 hours.

After 26 hours, 2 days, and 3 days, the differences among the 3 groups were significant (Table II). The plasma amylase values were significantly lower in animals fed ethionine (group I) than in normal or weight

control animals. The plasma amylase in the weight control rats (group III) was significantly lower than in normal control animals (group II) but significantly higher than in animals fed ethionine (group I).

TABLE I

PLASMA AMYLASE VALUES
(EXPERIMENT I)

	Time intervals					
	8 hr.	14 hr.	20 hr.	26 hr.	2 days	3 days
	No. of determinations per group					
Group	12	10	12	16	12	8
Group I						
Basic diet + 0.5% ethionine						
Base line	11,597 ± 1,784	11,023 ± 1,490	11,680 ± 1,754	12,411 ± 1,647	12,286 ± 1,278	12,149 ± 1,112
Experimental	11,553 ± 1,966	10,361 ± 1,825	9,548 ± 1,657	8,184 ± 1,473	6,062 ± 1,214	5,803 ± 1,092
Group II						
Basic diet *ad libitum*	11,726 ± 2,166	10,919 ± 1,259	11,226 ± 1,699	11,873 ± 1,433	11,453 ± 1,585	10,715 ± 1,447
Group III						
Basic diet, limited intake						
Base line	11,216 ± 1,797	10,788 ± 1,634	11,358 ± 1,654	11,735 ± 1,314	11,688 ± 936	11,716 ± 910
Experimental	10,610 ± 2,045	9,836 ± 1,763	8,963 ± 2,100	9,887 ± 2,126	9,685 ± 1,008	8,857 ± 436

TABLE II

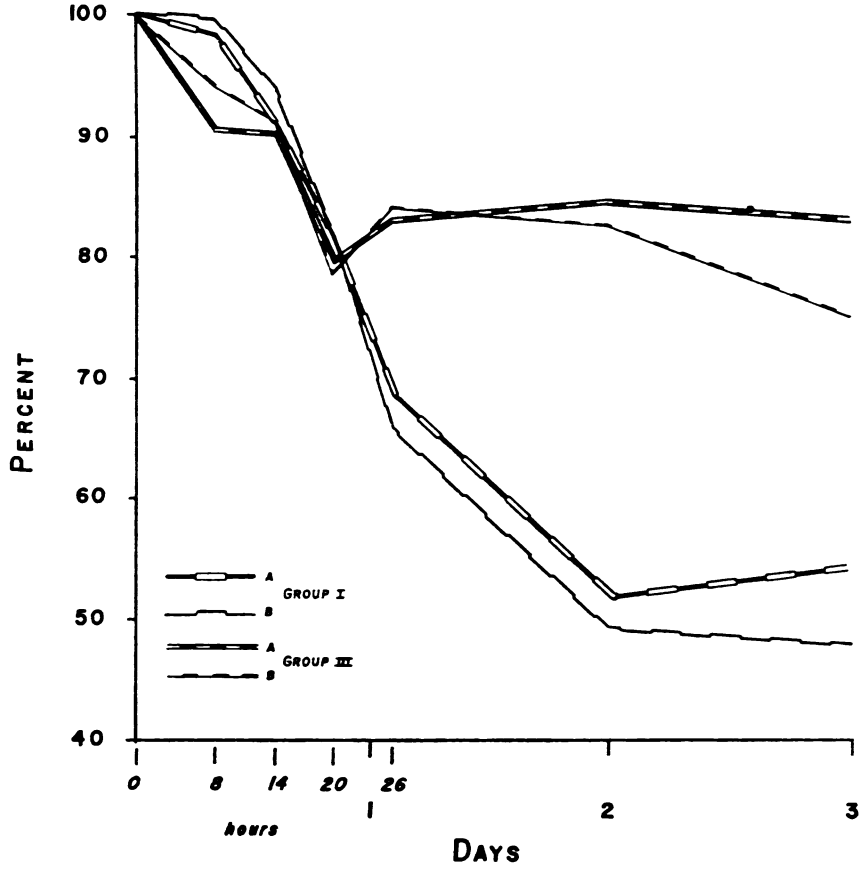
STATISTICAL ANALYSIS (p VALUES) OF PLASMA AMYLASE
(EXPERIMENT I)

	Time					
Compared groups	8 hr.	14 hr.	20 hr.	26 hr.	2 days	3 days
I and II	> 0.1	> 0.1	< 0.05 > 0.01	< 0.01	< 0.01	< 0.01
I and III	> 0.1	> 0.1	> 0.1	< 0.05 > 0.01	< 0.01	< 0.01
II and III	> 0.1	> 0.1	< 0.01	< 0.01	< 0.01	< 0.01

Group I: Basic diet + 0.5% ethionine
Group II: Basic diet *ad libitum*
Group III: Basic diet, weight controls

The amylase values are expressed as absolute values in Table I and Text-figure 1. For purposes of comparison, the amylase values for the group I and III animals at the various intervals were computed in terms

of the per cent of the normal control values and in terms of the per cent of the individual animal's base line which was established before the animal was placed on the experimental regime. This was done to control any variations in the methods due to environmental factors present at the time the determinations were made, as well as variations in individual animals. The curves designated A in Text-figure 2 represent the per cent of the amylase values in the animals fed ethionine and in those used as weight controls when each of these values was compared with the normal controls. At any given time interval, the point of reference



TEXT-FIGURE 2. Changes in plasma amylase. A. Expressed as percentage of normal controls for corresponding time interval. B. Expressed as percentage of values in same animal prior to ethionine administration (group I, basic diet plus 0.5 per cent ethionine; group III, basic diet, weight controls).

for the normal control was considered to be 100 per cent. The curves designated B in Text-figure 2 represent the per cent of the amylase at different times when compared with the values established for each animal at the beginning of the experiment and before the animal had been

placed on an experimental diet. From this it can be seen that the percentages obtained with both methods produce curves that are similar. The per cent decrease from the amylase values obtained for the normal control animals rather than the per cent variations from their own base line was chosen for statistical analysis, since these determinations were done simultaneously (Table III). As noted above, the first significant decrease in amylase values was observed at 20 hours. This decrease was essentially similar in both the weight control and the experimental animals. At 26 hours there was a further drop in the amylase level of the

TABLE III

AMYLASE VALUES
(EXPERIMENT I)

Groups *	Point of reference †	Time Interval					
		8 hr.	14 hr.	20 hr.	26 hr.	2 days	3 days
I	A. Normal controls	99	91	81	69	51	54
	B. Base line	100	94	82	66	49	48
III	A. Normal controls	90	90	80	83	85	83
	B. Base line	95	91	79	84	83	76

* Group I: Basic diet + 0.5% ethionine.
Group III: Basic diet, weight control.
† A: Expressed as percentage of normal controls for corresponding time interval.
B: Expressed as percentage of values in same animal prior to ethionine administration.

ethionine-fed animals, but no further decrease in amylase values in the weight control animals. The amylase levels in the experimental animals continued to fall for 2 days and then leveled off, whereas the amylase levels in the weight control animals remained approximately the same level as at 20 hours (Text-fig. 2).

Plasma Amylase Values—Part II

The results are indicated in Table IV and Text-figure 3.

The values for the animals fed ethionine (group I) were markedly lower than those for either of the 2 control groups. These depressed levels were noted at 4 days and remained low for the duration of the experiment (Text-fig. 3 and Table IV). Further, once these low levels were reached, there were no statistically significant variations at any time during the experiment.

Weight Changes—Part I

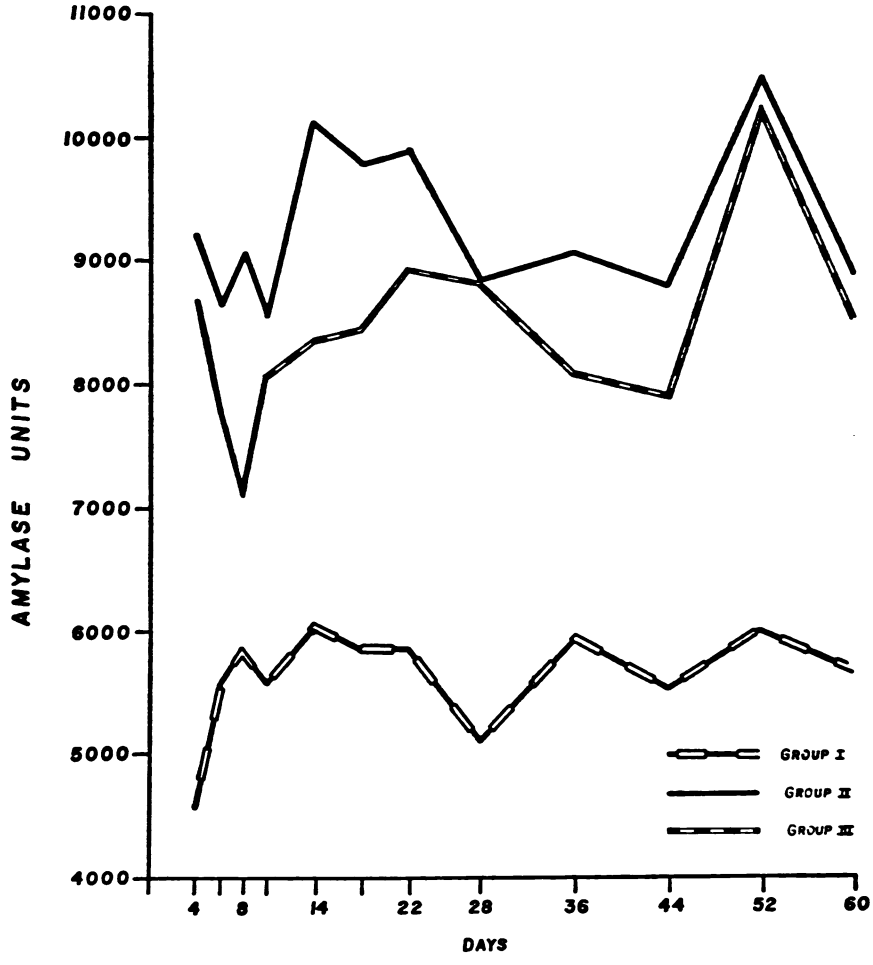
The animals in group I steadily lost weight during the experiment. The normal control animals did not show any significant change in weight. The average weights of livers and pancreases at different ex-

TABLE IV

PLASMA AMYLASE VALUES
(EXPERIMENT II)

Group	Time in days											
	4	6	8	10	14	18	22	28	36	44	52	60
Group I												
Basic diet + 0.5% ethionine	4,504 ± 816	5,631 ± 1,581	5,861 ± 901	5,529 ± 1,177	6,011 ± 1,127	5,867 ± 619	5,366 ± 765	5,115 ± 1,048	5,947 ± 1,859	5,525 ± 974	6,005 ± 1,216	5,709 ± 1,090
Group II												
Pair-fed controls	9,263 ± 1,075	8,731 ± 1,132	9,069 ± 1,472	8,592 ± 736	10,205 ± 697	9,893 ± 1,224	9,913 ± 1,086	8,897 ± 972	9,104 ± 756	8,896 ± 689	10,575 ± 933	8,946 ± 1,638
Group III												
Weight controls	8,769 ± 1,363	7,865 ± 1,543	7,188 ± 1,704	8,054 ± 1,858	8,359 ± 1,282	8,492 ± 1,095	8,949 ± 629	8,853 ± 821	8,153 ± 1,371	7,970 ± 1,216	10,295 ± 885	8,528 ± 816

perimental periods are indicated in Tables V and VI. There were no significant differences in the weights of the pancreases. In the ethionine-fed animals, the liver weights fell below the level of the normal control rats after 3 days. In the weight control animals, the livers weighed less than in the normal control rats and in the ethionine-fed animals.

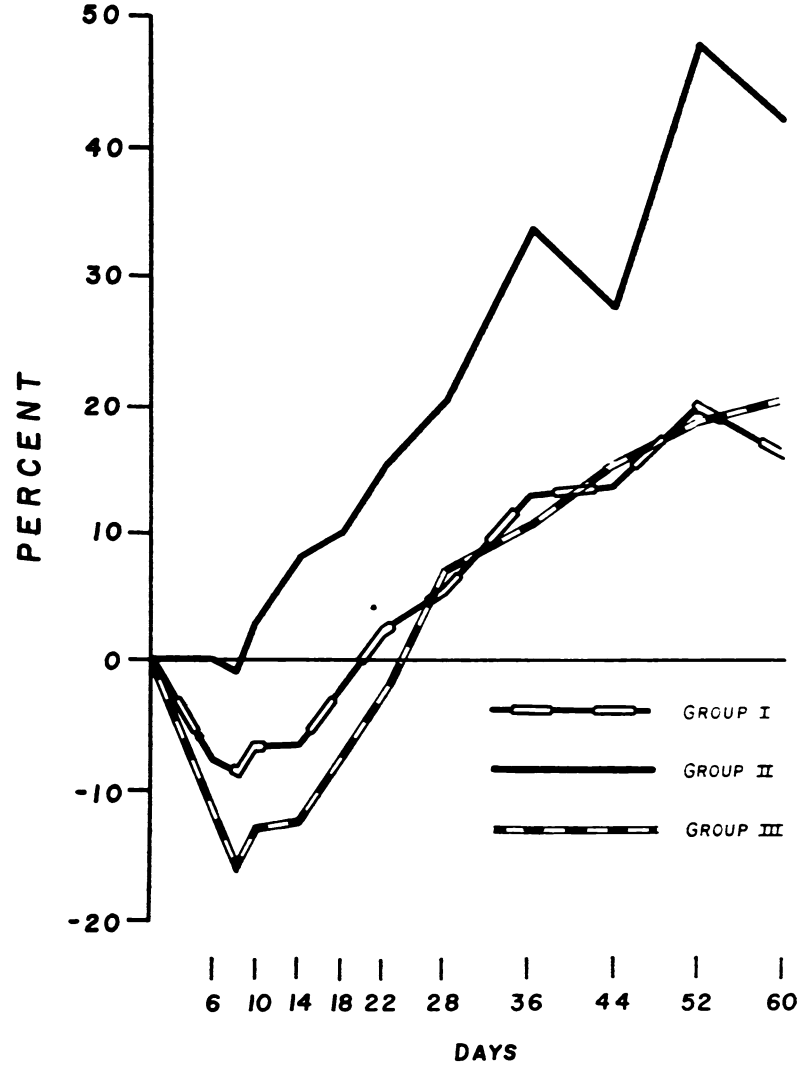


TEXT-FIGURE 3. Changes in plasma amylase values associated with ethionine feeding (group I, basic diet plus 0.5 per cent ethionine; group II, basic diet, pair-fed controls; group III, basic diet, weight controls).

Weight Changes—Part II

The weight changes are indicated in Text-figure 4. As noted in previous experiments,[7] there was initial weight loss followed by weight gain. The weights of livers and pancreases are indicated in Tables VII and VIII and Text-figures 5 and 6. During the first 8 days the absolute and

relative liver weights decreased (Text-fig. 5 and Tables VII and VIII). After this time there was a steady rise in both the absolute and relative liver weights so that by 44 days the relative liver weights exceeded those of the controls (Text-fig. 5 and Table VIII). On the other hand,



TEXT-FIGURE 4. Weight changes associated with ethionine feeding (group I, basic diet plus 0.5 per cent ethionine; group II, basic diet, pair-fed controls; group III, basic diet, weight controls).

the weights of the pancreases of the animals fed ethionine decreased both absolutely (Table VII) and in relation to body weights (Table VIII and Text-fig. 6). The weights of the pancreases of the pair-fed and weight control animals did not change significantly.

TABLE V

WEIGHTS OF PANCREAS AND LIVER IN GM.
(EXPERIMENT I)

Experimental groups	Organs	Time					
		8 hr.	14 hr.	20 hr.	26 hr.	2 days	3 days
Group I							
Basic diet + 0.5% ethionine	Pancreas	1.30	1.16	1.07	0.97	1.45	1.19
	Liver	12.46	12.26	11.64	10.55	10.45	9.21
Group II							
Basic diet *ad libitum*	Pancreas	1.11	1.09	1.02	1.11	1.48	1.26
	Liver	12.13	11.29	11.22	9.87	11.28	11.27
Group III							
Basic diet, weight controls	Pancreas	1.48	1.07	1.00	1.04	1.37	1.09
	Liver	8.97	9.72	9.96	9.23	9.14	8.34

TABLE VI

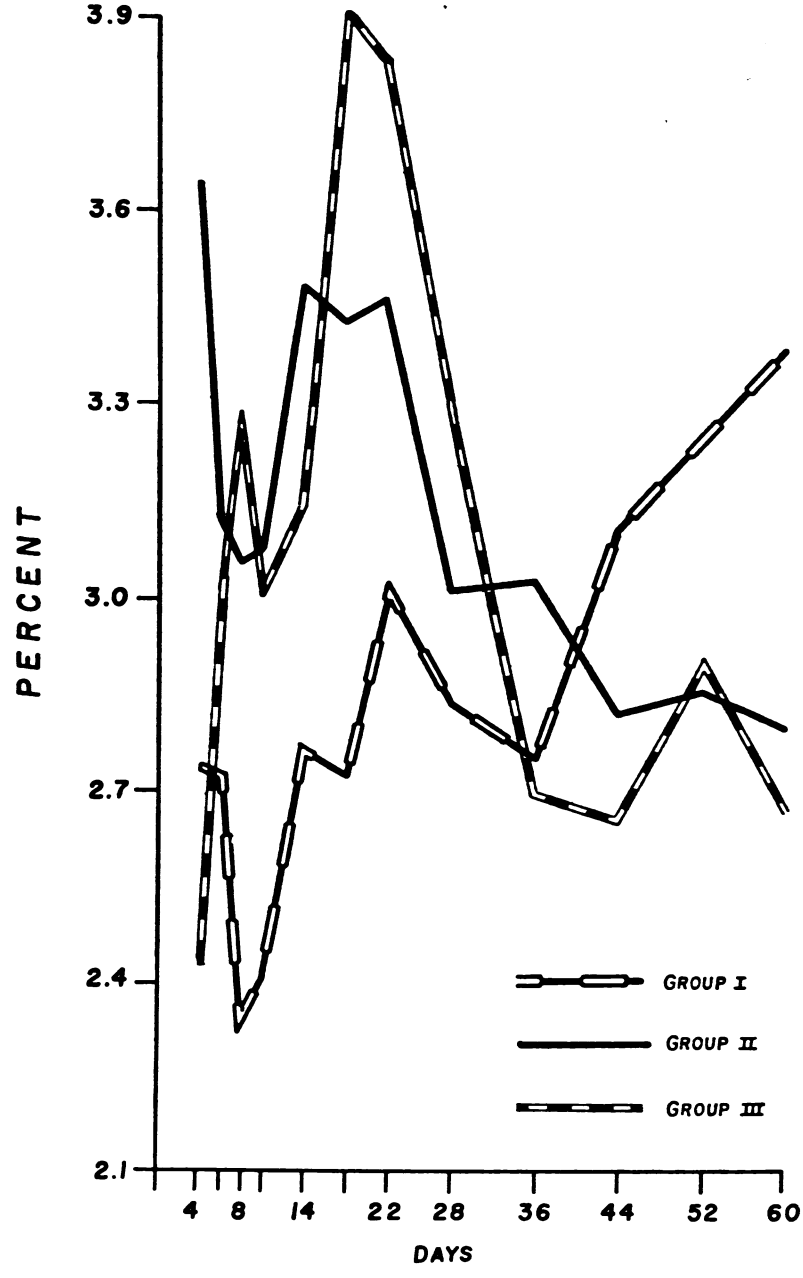
WEIGHTS OF PANCREAS AND LIVER AS PER CENT OF TOTAL BODY WEIGHT
(EXPERIMENT I)

Experimental groups	Organs	Time					
		8 hr.	14 hr.	20 hr.	26 hr.	2 days	3 days
Group I							
Basic diet + 0.5% ethionine	Pancreas	0.41	0.36	0.32	0.29	0.44	0.37
	Liver	3.91	3.77	3.49	3.18	3.15	2.83
Group II							
Basic diet *ad libitum*	Pancreas	0.34	0.34	0.30	0.32	0.43	0.36
	Liver	3.77	3.50	3.35	2.89	3.28	3.24
Group III							
Basic diet, weight controls	Pancreas	0.48	0.34	0.30	0.31	0.42	0.33
	Liver	2.89	3.11	3.02	2.73	2.77	2.55

Histologic Alterations—Part I

The histologic changes found in the experimental animals fed ethionine were identical to those previously reported.[1–10] Briefly, in the pancreas these consisted of decrease in basophilia and vacuolation of acinar cells. There was decrease or absence of zymogen granules in the acinar

cells which had undergone degenerative alterations, as evidenced by pyknotic nuclei and vacuolation of cytoplasm. There was no histologically demonstrable reduction of zymogen granules in other acinar cells,



TEXT-FIGURE 5. Weight changes of liver expressed as per cent of total body weight (group I, basic diet plus 0.5 per cent ethionine; group II, basic diet, pair-fed controls; group III, basic diet, weight controls).

TABLE VII

WEIGHTS OF PANCREAS AND LIVER
(EXPERIMENT II)

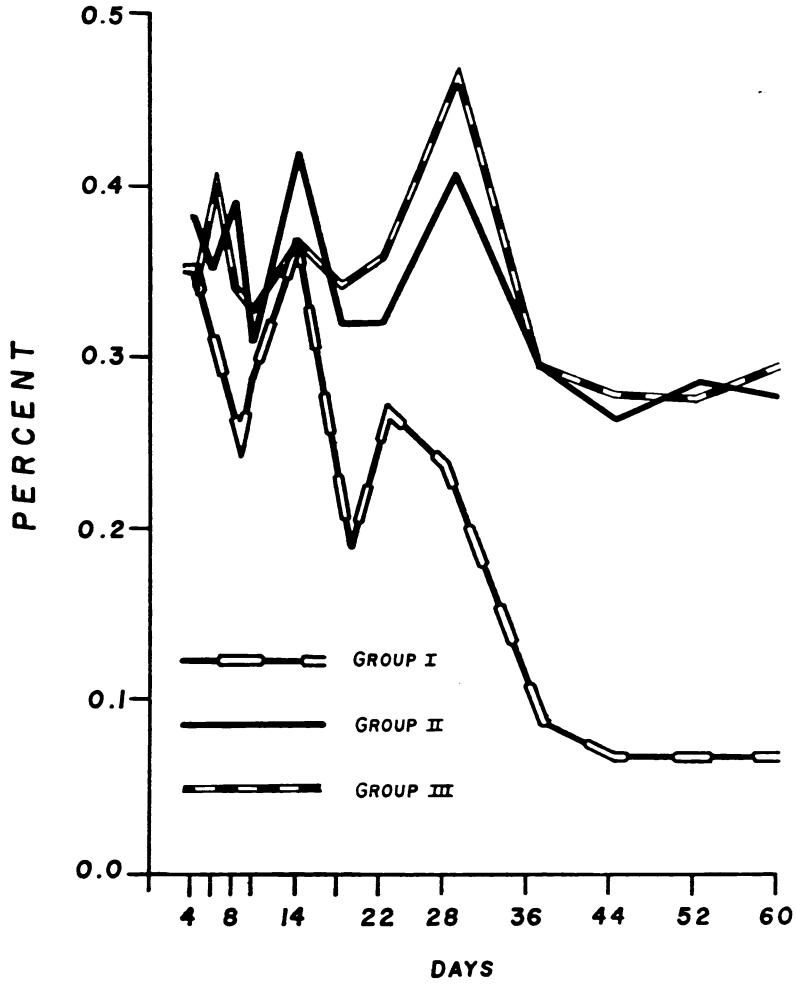
Experimental groups	Organs	Time in days											
		4	6	8	10	14	18	22	28	36	44	52	60
Group I													
Basic diet + 0.5% ethionine	Pancreas	1.08	0.75	0.61	0.75	0.95	0.51	0.74	0.68	0.28	0.21	0.22	0.22
	Liver	8.48	6.91	5.82	6.15	7.04	7.41	8.36	8.16	8.32	9.20	9.93	10.21
Group II													
Basic diet, pair-fed controls	Pancreas	1.29	0.96	1.06	0.88	1.25	0.99	1.02	1.35	1.06	0.92	1.13	1.06
	Liver	12.37	8.64	8.35	8.66	10.39	10.45	10.94	9.89	10.80	9.49	11.11	10.39
Group III													
Basic diet, weight controls	Pancreas	0.98	0.97	0.81	0.77	0.88	0.85	0.97	1.34	0.90	0.82	0.87	0.92
	Liver	7.07	7.38	7.70	7.23	7.46	9.81	10.25	9.48	8.02	7.76	8.87	8.21

TABLE VIII

WEIGHTS OF PANCREAS AND LIVER AS PER CENT OF TOTAL BODY WEIGHT
(EXPERIMENT II)

Experimental groups	Organs	Time in days											
		4	6	8	10	14	18	22	28	36	44	52	60
Group I													
Basic diet + 0.5% ethionine	Pancreas	0.35	0.30	0.25	0.29	0.37	0.19	0.27	0.24	0.09	0.07	0.07	0.07
	Liver	2.74	2.72	2.35	2.40	2.76	2.72	3.01	2.85	2.75	3.12	3.25	3.28
Group II													
Basic diet, pair-fed controls	Pancreas	0.38	0.35	0.39	0.31	0.42	0.32	0.32	0.41	0.30	0.27	0.29	0.28
	Liver	3.65	3.14	3.06	3.09	3.49	3.42	3.47	3.01	3.03	2.82	2.86	2.79
Group III													
Basic diet, weight controls	Pancreas	0.34	0.40	0.34	0.32	0.37	0.34	0.36	0.47	0.30	0.28	0.28	0.30
	Liver	2.43	3.01	3.28	3.01	3.15	3.91	3.85	3.30	2.69	2.65	2.89	2.67

which only had loss of basophilia. In the liver there were varying degrees of fatty metamorphosis, necrosis of parenchymal cells and infiltration by round cells. No lesions were noted in the salivary glands.



TEXT-FIGURE 6. Weight changes of pancreas expressed as per cent of total body weight (group I, basic diet plus 0.5 per cent ethionine; group II, basic diet, pair-fed controls; group III, basic diet, weight controls).

The histologic changes in the pancreas and liver were graded and recorded in Table IX.

Histologic Alterations—Part II

The lesions present in the pancreas were similar in kind to those seen in the animals in part I but were more marked. In addition, as the experimental periods became longer there was shrinkage and loss of acinar

TABLE IX

HISTOLOGIC CHANGES AND PLASMA AMYLASE VALUES
(EXPERIMENT I)

	Time intervals					
	8 hr.	14 hr.	20 hr.	26 hr.	2 days	3 days
Pancreas						
Decrease of basophilia						
Group I	0	1+	2+	4+	5+	5+
Group II	0	0	0	0	0	0
Group III	0	1+	3+	4+	4+	3+
Vacuolation						
Group I	0	0	1+	1+	2+	2+
Group II	0	0	0	0	0	0
Group III	0	0	1+	1+	1+	1+
Nuclear pyknosis						
Group I	0	0	0	1+	2+	2+
Group II	0	0	0	0	0	0
Group III	0	0	1+	1+	1+	1+
Liver						
Fat						
Group I	2+	3+	4+	4+	6+	5+
Group II	1+	1+	1+	1+	1+	2+
Group III	2+	2+	2+	2+	2+	1+
Round cell infiltrate						
Group I	0	1+	3+	4+	2+	1+
Group II	0	0	0	0	0	0
Group III	0	0	0	0	0	0
Necrosis						
Group I	0	1+	2+	3+	3+	3+
Group II	0	0	0	0	0	0
Group III	0	0	0	0	0	0
Plasma amylase						
Group I	11,553	10,361	9,548	8,184	6,062	5,803
Group II	11,726	10,919	11,226	11,873	11,453	10,715
Group III	10,610	9,836	8,963	9,887	9,685	8,857

cells as well as entire acini, replacement of pancreatic tissue by fat, and later regeneration of acini. With the shrinkage and loss of the acinar cells as well as with the loss of acini, there was overall decrease of zymogen granules when compared with the control animals. In the younger cells undergoing regeneration there were relatively few zymogen granules, but in the more mature cells which had regenerated, zymogen granules were present in the same number as in the control animals. In the liver, in addition to the changes described in part I, there was bile duct proliferation and regeneration. The changes were graded and are recorded in Table X.

No striking differences were found between the salivary glands of the experimental animals and the 2 control groups during the first 18 days of the experiment. After 22 days of ethionine administration, there was a reduction in size of the acinar cells and acini of the submaxillary gland. No changes were seen in the parotid and sublingual glands at this time.

After 36 days, the alterations in the submaxillary gland were more marked, and the cytoplasm was more eosinophilic. At this time, the first changes in the parotid and sublingual glands were noted. The nuclei became rounded, increased in size, and tended to be more centrally located. These changes were less apparent in the sublingual glands, which did not show any further alterations up to the end of the experiment. Loring and Hartley[9] also described more severe lesions in the submaxillary glands than in the sublingual glands.

After 52 and 60 days, the changes in the submaxillary gland were more marked than at 36 days. The lobules were spaced farther apart. In the parotid glands, the most marked changes were seen during these periods. The nuclei were larger, rounded, and less peripherally placed in the cell. The cytoplasm had lost some of its basophilia and in some situations was occasionally eosinophilic.

Discussion

The histologic changes which developed in the pancreas during ethionine administration were similar in kind to those that have been observed by ourselves and others.[1-10] Also, it has been shown that many of the pancreatic acini of animals during ethionine administration regenerated to such a degree that they resembled functioning acini.[1,7] Similar evidence of regeneration was found in the present experiments. Regeneration of acini first was noted in the animals after 18 days, and this became more marked as the experiments progressed, so that the animals showing the greatest degree of regeneration were those on the experimental regime for the longest periods of time. However, there was loss of pancreatic substance at the end of the 60-day experimental period as indicated by the lower weights of the pancreases when compared with the weights of the pancreases of the pair-fed control rats. The loss of weight of the pancreases in the experimental group becomes even more significant when the increase of fat tissue in the pancreas is considered. This suggests that the loss of acinar tissue is even greater than is reflected by the lower weights of these organs in the experimental group since the increase in fat indicates a further replacement of acinar tissue.

There was a progressive decrease of plasma amylase in the animals fed ethionine, beginning 20 hours after the start of the experiment. This fall in the amylase values continued until the second day when the values

TABLE X

HISTOLOGIC CHANGES AND PLASMA AMYLASE VALUES
(EXPERIMENT II)

	Time interval in days											
	4	6	8	10	14	18	22	28	36	44	52	60
Pancreas												
Decrease of basophilia												
Group I	6+	6+	6+	5+	4+	4+	3+	3+	3+	3+	4+	3+
Group II	0	0	0	0	0	0	0	0	0	0	0	0
Group III	4+	2+	1+	1+	2+	1+	0	1+	2+	0	0	0
Vacuolation												
Group I	2+	3+	3+	3+	2+	2+	1+	4+	3+	2+	2+	2+
Group II	0	0	0	0	0	0	0	0	0	0	0	0
Group III	1+	0	0	0	0	0	0	0	0	0	0	0
Nuclear pyknosis												
Group I	2+	3+	3+	3+	2+	2+	1+	4+	3+	2+	2+	2+
Group II	0	0	0	0	0	0	0	0	0	0	0	0
Group III	1+	0	0	0	0	0	0	0	0	0	0	0
Shrinkage of acini												
Group I	0	0	1+	2+	3+	4+	3+	4+	3+	4+	4+	3+
Group II	0	0	0	0	0	0	0	0	0	0	0	0
Group III	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis												
Group I	1+	1+	2+	3+	2+	2+	0	4+	3+	2+	2+	2+
Group II	0	0	0	0	0	0	0	0	0	0	0	0
Group III	0	0	0	0	0	0	0	0	0	0	0	0
Loss of acini												
Group I	0	0	1+	2+	2+	3+	2+	4+	4+	5+	6+	6+
Group II	0	0	0	0	0	0	0	0	0	0	0	0
Group III	0	0	0	0	0	0	0	0	0	0	0	0

Pancreatic duct proliferation and regeneration												
Group I	o	o	o	o	o	1+	2+	2+	3+	4+	5+	5+
Group II	o	o	o	o	o	o	o	o	o	o	o	o
Group III	o	o	o	o	o	o	o	o	o	o	o	o
Liver												
Fat												
Group I	5+	8+	8+	8+	5+	4+	3+	3+	3+	3+	3+	3+
Group II	2+	2+	2+	4+	3+	3+	2+	4+	4+	2+	3+	3+
Group III	1+	2+	2+	1+	2+	2+	2+	1+	2+	2+	3+	2+
Round cell infiltrate												
Group I	1+	1+	o	o	o	o	o	o	o	o	o	o
Group II	o	o	o	o	o	o	o	o	o	o	o	o
Group III	o	o	o	o	o	o	o	o	o	o	o	o
Necrosis												
Group I	3+	4+	5+	6+	7+	6+	5+	4+	3+	2+	2+	2+
Group II	o	o	o	o	o	o	o	o	o	o	o	o
Group III	o	o	o	o	o	o	o	o	o	o	o	o
Regeneration												
Group I	1+	1+	1+	2+	3+	4+	5+	6+	6+	7+	7+	7+
Group II	o	o	o	o	o	o	o	o	o	o	o	o
Group III	o	o	o	o	o	o	o	o	o	o	o	o
Plasma amylase												
Group I	4,504	5,631	5,861	5,529	6,011	5,867	5,366	5,115	5,947	5,525	6,005	5,709
Group II	9,263	8,731	9,069	8,592	10,205	9,893	9,913	8,897	9,104	8,896	10,575	8,946
Group III	8,769	7,865	7,188	8,054	8,359	8,492	8,949	8,853	8,153	7,970	10,295	8,528

became stabilized at a reduced level and did not vary significantly throughout the duration of the experiment. When the levels of blood amylase are matched with the histologic lesions in the pancreas, the first significant drop in amylase corresponded to a marked reduction in basophilia and slight vacuolation, slight nuclear pyknosis and slight loss of zymogen granules of the pancreatic acinar cells. There was greater loss of basophilia, and the extent of vacuolation, nuclear pyknosis, and loss of zymogen granules became progressively more marked as the experiment continued, but the amylase values remained essentially the same.

From a study of the results, it appeared that vacuolation and nuclear pyknosis tended to parallel the amylase values more closely than basophilia. The latter was decreased in equal degree in the experimental and weight control animals in part I for the first 26 hours of the experimental period. While basophilia and amylase values decreased in the weight control animals during the first 20 hours, there was no further decrease in the plasma amylase levels in these control animals even though basophilia was decreased. However, loss of basophilia did appear to parallel approximately the loss of weight in both the experimental and weight control animals.

Significant histologic changes in the livers of the experimental animals were first noted after 20 hours. There was moderate increase in fat, focal round cell infiltration, and slight central necrosis. As the experimental period continued, the fatty changes increased up to 10 days, and necrosis became more pronounced until the third week of the experiment. After those periods, these lesions became less marked. Regeneration of the liver was noted early and became more marked as the experiment progressed. The fall in plasma amylase values coincided with the first appearance of significant liver damage at 20 hours and continued to parallel the severity of damage up to 48 hours. After this, although the liver damage became more pronounced and the liver weights in relation to body weights decreased, there was no further reduction of plasma amylase levels, nor was there a corresponding rise in amylase values during the period of liver regeneration and liver weight increase.

The histologic changes in the parotid glands of the experimental animals were never marked. The first alterations noted in the submaxillary glands appeared after 22 days and in the parotid and sublingual, after 36 days. No correlation could be found between the histologic changes in the salivary glands and the serum amylase levels. These findings are in agreement with those of Wiberg and Tuba,[11] who found there were no significant changes in the amylase levels of parotid gland tissue during ethionine administration.

It has been demonstrated that starvation brings about a reduction in serum amylase levels.[11,19] While none of the animals in the present experiment were starved, there was a significant lowering of plasma amylase after 20 hours in the animals whose food intake was restricted. These lower levels were maintained throughout the experiment, but they remained considerably higher than the plasma amylase levels of the animals fed ethionine. The only histologic change noted in these animals not found in the animals fed *ad libitum* in part I or in the pair-fed control animals in part II was a slight loss of basophilia of the acinar cells of the pancreas.

There is no general agreement regarding the origin of amylase found in the blood serum.[11,20–30] It has been assumed by some[11,20] that the pancreas contributes small quantities of amylase to the blood, and it is known that when extensive acute inflammation of the pancreas or a sudden obstruction of its ducts takes place, the amount of amylase entering the blood is greatly increased.[31] However, no satisfactory information is available regarding the fraction of the serum amylase that is contributed by the pancreas.

As the experiment progressed and the pancreases became smaller and smaller, it was puzzling to find that the amylase values did not continue to drop. Further, histologic evidence of regeneration of the pancreas was noted in all animals after 18 days on the experiment, but there was no change in plasma amylase values. It is possible that from this time on, the number of regenerating acini kept pace functionally with the number of degenerating acini so that the amylase values were maintained. However, it is difficult to accept this explanation in its entirety, since the actual weight of pancreatic tissue continued to decline. Another possible explanation for these unchanging low amylase levels is that they represent minimal amounts of the enzyme synthesized elsewhere in the body and present in the serum and that little or no amylase was being produced by the pancreas.

It is not possible to compare the present observations directly with results obtained by others, since the purpose and design of the experiments are not comparable. Nevertheless, there are certain observations which are of interest. Other investigators[3,5,6,8] have found that after an initial decrease there was a transient elevation of serum amylase levels following ethionine administration. Sidransky and Farber[32] found that the pancreas of the ethionine-treated rat contained significantly more protein, amylase, and total proteolytic enzyme activities and acid soluble nitrogen than the pancreases of control animals. In these experiments, ethionine was given intraperitoneally in a single large dose, and determinations were made after 7 and 24 hours. In all of the experiments in

which the serum amylase was elevated, the degree of pancreatic damage described was much greater than in the animals used in the present experiments. This increase in serum amylase has been explained as being due to increased permeability of cells[16] or to breakdown of the barrier between pancreatic acini and the blood vessels because of pancreatic acinar damage.[3,33]

In experiments where ethionine administration was continued, there was an eventual and significant fall to subnormal levels of serum amylase values. Bollag and Gallico[2] found a marked reduction in the level of amylase in pancreatic tissue following ethionine administration. Wiberg and Tuba[11] gave rats intraperitoneal injections of 100 mg. of ethionine daily for 14 days. At the end of 14 days, the serum amylase values were approximately 50 per cent of those of the control animals, while the tissue levels in the pancreas were approximately 1.5 per cent of the control animals. Others found there was a decrease in the total amount of pancreatic juice[34] as well as in the output of pancreatic amylase[35] in ethionine-induced pancreatitis in dogs. In other experiments, there was a return to normal levels of plasma amylase after ethionine administration had been discontinued.[5,6] In this connection it is of interest that Younathan and Frieden[36] demonstrated inhibition of synthesis of pancreatic amylase *in vitro* by ethionine and other amino acid analogues.

In general, the results obtained by others are in agreement with the present data. The experiments reported here were designed to determine if the plasma amylase levels would reflect pancreatic injury and then return to normal when regeneration of the pancreas occurred during ethionine administration. Therefore, ethionine was administered in older animals in quantities calculated to produce significant pancreatic damage but not to inflict damage incompatible with life for an extended period. It appears, then, that plasma amylase levels are significantly lowered during ethionine administration and remain at low levels so long as the animals receive ethionine, even though there is histologic evidence of pancreatic regeneration as well as regeneration of the liver and a concomitant increase of body weight.

Summary

Male rats were fed 0.5 per cent ethionine. The animals were sacrificed at intervals ranging from 8 hours to 60 days. The histologic changes in the pancreas, liver, and salivary glands were evaluated. Plasma amylase determinations were made throughout the experiments.

There was a significant decrease in plasma amylase values 20 hours after the start of the experiment. The fall in plasma amylase values

paralleled the appearance of degenerative changes in the acinar cells of the pancreas.

Regeneration of the acinar cells of the pancreas was first noted on the 18th experimental day and became more pronounced as the experiments continued. However, this process was not accompanied by a rise in plasma amylase levels.

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