Effect of Cortisone on Ethionine-Induced Pancreatitis in the Rat. (25110)

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Beneficial results observed following cortisone in treatment of pancreatitis in man have led to its recommendation as a therapeutic adjunct in this disease(1,2). On the other hand, pancreatic lesions have been produced in rabbits by cortisone(3,4) and similar changes have been noted at post-mortem in humans receiving ACTH or cortisone(5). Also a decrease in pancreatic exocrine secretion following ACTH and adrenocortical steroids in man has been described(6). A study of cortisone in experimentally produced pancreatitis, therefore, seemed warranted. Furthermore, pancreatic pathology induced by ethionine is thought to result from alterations in methionine metabolism(7,11) and the effects of cortisone in protein metabolism gave particular interest to its use in ethionine pancreatitis.

Methods. Female rats of Wistar strain were utilized. All were allowed a Purina Chow diet consisting of 5% fat, 23% protein, 44% carbohydrate, 6% fiber and 9% ash, as desired, throughout the experiment. Cortisone and ethionine were injected into the peritoneal cavity. All animals were weighed at beginning and at termination of experiment. When rats were sacrificed and the viscera inspected, microscopic sections were prepared of pancreas, liver and stomach. Pathologic changes were arbitrarily graded from 1 to 3, according to severity. Initially, the effect of cortisone alone was studied. Five rats, averaging 200 g in weight, received 2 mg of cortisone daily for 7 days and were sacrificed on the eighth. Two similar groups of 5 rats each received 7 and 15 mg of cortisone and sacrificed on eighth day. In evaluation of ethionine and cortisone, 10 rats averaging 168 g received 0.6 mg/g of body weight of ethionine daily for 7 days and sacrificed on eighth. A second group of rats of same weight were given the same amount of ethionine, and in addition each rat received 7 mg of cortisone daily and sacrificed on eighth day. A third experiment was carried out similar in all ways to the 7 day evaluation of ethionine and ethionine plus cortisone, except that rats were sacrificed on third day. In the 2 day evaluation, the group given ethionine plus cortisone consisted of 9 rats.

Results. Rats receiving cortisone only continued in good health during the injections. Gain in weight during the 7 day period averaged 9 g/rat. There was no correlation of weight gain with dosage of cortisone used. There was considerable variation in amount of weight gained and some rats at each cortisone dosage level either failed to gain or lost weight during the experiment. Except for more darkly staining basophilic cytoplasm in the acinar cells, no alterations in pancreatic structure could be noted on microscopic section. Gross or microscopic abnormalities were also

TABLE I. Pathologic Changes in Rats Receiving Ethionine and Ethionine-Cortisone for 7 Days.

Animal No.	Drug	Microscopic pathology graded 1-3: Pancreas	Liver	Stomach	Avg wt loss, g
1	Ethionine, .6 mg/g	2	3	0	71
2	"	3	1	0	67
3	"	3	1	Ulcer	66
4*					
5*					
6*					
7	"	3	3	0	39
8†		3	2	Ulcer	51
9	"	3	1	0	65
10	"	3	2	Gastritis	49
11	*Idem* + cortisone, 7 mg daily	3	3	Ulcer	43
12†		3	2	Gastritis	56
13	"	3	3	Ulcer	58
14†		2	1	"	57
15	"	3	1	"	47
16	"	1	1	"	61
17†		3	1	Gastritis	59
18†		3	2	Ulcer	53
19	"	3	2	0	36
20	"	3	1	Ulcer	53

* Died on 3rd day. † Died on 8th day.

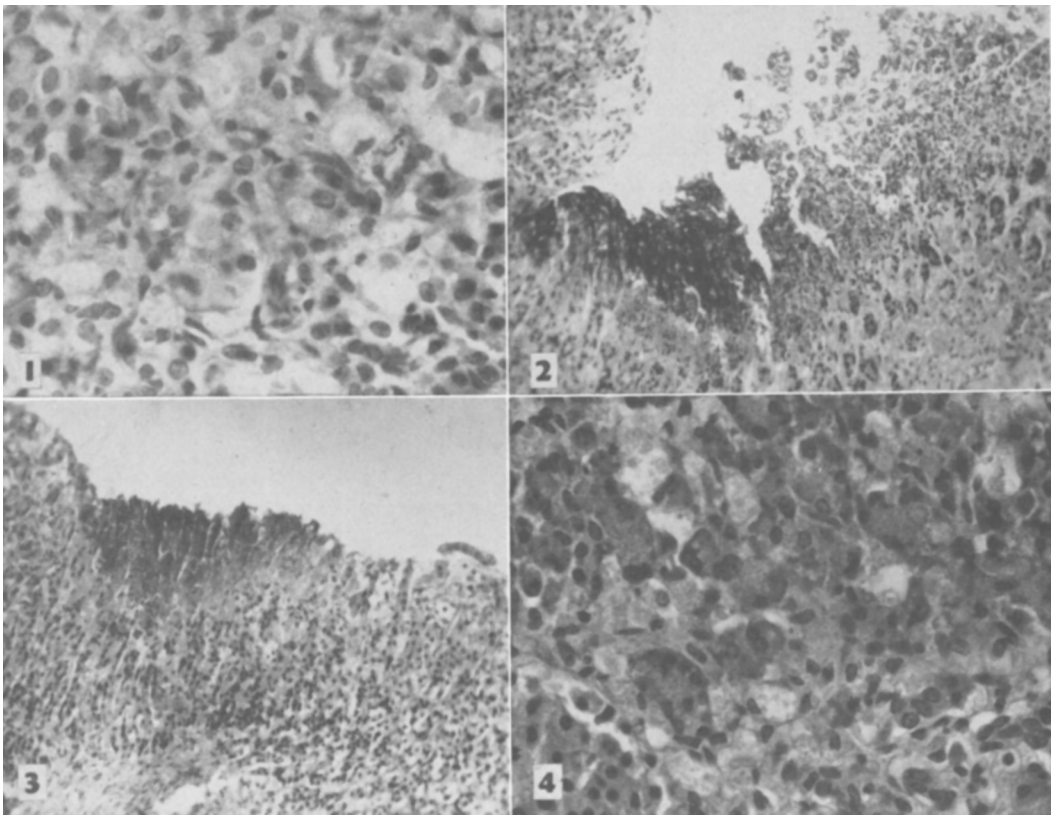

FIG. 1. Grade III pancreatic lesion.
FIG. 2. Gastric ulcer in rat receiving ethionine and cortisone for 7 days.
FIG. 3. Gastritis in rat receiving ethionine and cortisone.
FIG. 4. Grade I pancreatic lesion in rat receiving ethionine and cortisone for 2 days.

absent in livers and stomachs of these animals.

Results in rats receiving ethionine only and ethionine plus cortisone for 7 day period appear in Table I. Animals 4, 5 and 6 died on third day and autolysis of tissue made evaluation of histologic changes impossible. Rats 8, 12, 14, 17 and 18 died on eighth day; *i.e.*, the day they would have been sacrificed. Gastric ulceration with hemorrhage was found in each. An average weight loss of 58.5 g/rat over the 7 day period was noted in those receiving ethionine only. Those receiving ethionine and cortisone lost 52.3 g/rat. All rats appeared ill by third day; their fur was ruffled and stained, their eyes were red, their food intake was markedly reduced, and they showed little spontaneous activity. The hepatic and pancreatic lesions seemed identical both in character and severity in rats receiving ethionine and those receiving ethionine and cortisone. Pathologic changes which occurred in the pancreas are demonstrated in Fig. 1, which shows a lesion considered Grade III in severity. The pancreas showed marked disturbance of architectural pattern. Normal acini were rare and the acinar cells showed loss of basophilic cytoplasm. Proliferation of young fibroblastic tissue and infiltration with mononuclear cells were prominent in each section. Pancreatic blood vessels appeared dilated and congested but intra-pancreatic hemorrhage was minimal. Vacuolization of varying degree was present. Liver pathology consisted of congestion and periportal infiltration with mononuclear cells. Fibroblastic proliferation of mild degree was found in the periportal area in some sections. Hepatic cell necrosis or fatty infiltration was conspicuously absent.

The gastric mucosa was somewhat less than normal in thickness in all rats. A hemor-

TABLE II. Pathologic Changes in Rats Receiving Ethionine and Ethionine-Cortisone for 2 Days.

Animal No.	Drug	Microscopic pathology graded 1-3: Pancreas	Liver	Stomach	Avg wt loss, g
	Ethionine,				
1	.6 mg/g	1	0	0	2
2	"	1	0	0	6
3	"	1	0	0	0
4	"	1	0	0	12
5	"	1	0	0	0
6	"	1	0	0	4
7	"	1	0	0	1
8	"	1	0	0	5
9	"	1	0	0	7
10	"	1	0	0	7
				Avg wt loss	4.4
	Idem + cortisone, 7 mg				
11	daily	1	0	0	3
12	"	1	0	0	6
13	"	1	0	0	11
14	"	1	0	0	1
15	"	1	0	0	8
16	"	1	0	0	5
17	"	1	0	0	5
18	"	1	0	0	1
19	"	1			1
				Avg wt loss	4.1

rhagic gastritis or shallow gastric ulceration was found in 3 of 7 rats given ethionine only, and in 9 of the 10 rats receiving cortisone in addition. Figs. 3 and 4 are illustrations of the gastritis and gastric ulcers found. The ulcers were small, discrete, often multiple and occurred in both squamous and glandular portions of the stomach. All animals with such gastric lesions had black guaiac-positive material in the stomach and small bowel. The 5 animals dying of effects of drugs had gastric lesions. Four of these had been given cortisone and ethionine, and one only ethionine.

There was no correlation between weight loss and presence or severity of lesions. There appeared to be no correlation between severity of pancreatic lesions and presence of gastric lesions.

Results in animals receiving ethionine and ethionine plus cortisone for a 2 day period appear in Table II. All of these lived to termination of experiment. They appeared healthy and their appetite remained good throughout. An average weight loss of 4.3 g/rat during the 2 day period occurred. There was no significant difference in weight loss of those receiving ethionine from those receiving both drugs. Livers and stomachs appeared normal grossly and on microscopic examination. Pancreatic changes are shown in Fig. 4. These were considered Grade I, consisting of loss of basophilic cytoplasm and some disturbance in acinar arrangement. The pancreatic lesions were of equal severity in those receiving ethionine only and in those receiving ethionine and cortisone.

Discussion. The nature of metabolic abnormality produced by ethionine is not entirely clear. An inhibition by ethionine of the hepatic uptake of radioactive sulfur from labeled methionine has been demonstrated(10). In a similar study of pancreatic metabolism, an accelerated rate of methionine incorporation was found(11). In each case, the metabolic abnormality and the associated hepatic or pancreatic structural change were prevented by additional methionine. The overall catabolic effect of cortisone on nitrogen metabolism is accompanied by actual increase in liver protein and in serum protein(12). This result indicates that the alteration in protein metabolism produced by cortisone does not provide utilizable methionine in sufficient amounts to prevent development of ethionine lesions.

Death of the 3 ethionine-treated rats on third day of the planned 7 day experiment, is unexplained. If cortisone had a protective effect during the first days of ethionine administration which was dependent upon modification of the pancreatitis, some amelioration of the pancreatic lesion would have been expected in rats sacrificed on third day. No significant difference in severity could be found at this time. The anti-inflammatory effect of cortisone would not seem to be of great importance inasmuch as neutrophilic infiltration and edema were not prominent in ethionine lesions.

The high incidence of gastric lesions with hemorrhage and the probable part they played in the demise of 5 rats is of great interest. Alterations in gastric mucosa have been reported(13,14) after ethionine but ulcerations with hemorrhage have been infrequent in reports of ethionine-induced pancreatitis. Although the numbers are small, the 90% inci-

dence of gastric ulceration in those receiving cortisone as well as ethionine as compared to the less than 50% incidence in those given ethionine only, suggests that cortisone accelerates development of these lesions. Neither the mode of production of gastric lesions nor the mechanism by which cortisone influences their development, is apparent. The high rate of metabolic activity in the gastric mucosa as in the pancreas is presumably an important factor in explaining the vulnerability of these organs to injury by ethionine. Prolonged administration of adrenal corticoids(15) and hypothalamic stimulation(16) increased gastric acid and pepsin secretion, and cortisone may aggravate ulcer formation in this manner.

Summary. Typical pancreatic changes were induced by ethionine, which were not altered by addition of cortisone. The high incidence of gastric ulceration and hemorrhage found with ethionine alone was doubled in rats receiving cortisone also.

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Relationship Between Inactivation of Poliovirus by Phenol and Appearance of Ribonuclease-Labile Infectivity.* (25111)

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Gierer and Schramm(1) found that infectivity remaining after treatment of tobacco mosaic virus with phenol could be destroyed with ribonuclease in contrast to the stability to ribonuclease of original untreated virus. Similar results were subsequently reported for Mengo encephalitis virus(2), poliovirus(3,4, 5), West Nile encephalitis virus(3), Eastern equine encephalomyelitis virus(6), and Semliki Forest virus(7). In the above studies aqueous virus preparations were serially extracted with phenol. Our results of an investigation of some factors involved in use of phenol for obtaining ribonuclease-labile infectivity from poliovirus are given below.

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Materials and methods. Polioviruses. Wild type virus of the Brunhilde strain (antigenic type 1) and of the Akron strain (type 1) and the cr^i mutant(8) of Akron were used. *Reagents.* Solutions of phenol saturated with water at 0° were prepared; these were 8.03 M phenol (a) by titration with standard NaOH and (b) by quantitative organic hydroxyl determination(9). Solutions of phenol were also prepared by dissolving a weighed amount of phenol, dried over P_2O_5, to volume in high-cystine (0.20 mM L-cystine) altered Eagle's