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Decreased Gastric Secretory Activity following Injection of Certain Maleimides, β-Mercaptoethylamine (or AET), or the combination of both types*

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We have been continuing a study[1] of the effects of certain substances on gastric secretion in the pylorus-ligated rat. Davenport *et al.*,[2] reported the inhibitory effects of *N*-ethylmaleimide and iodoacetamide on gastric secretion *in vitro*. More recently, Hollander[3] confirmed and amplified this observation *in vivo*: topical application of solutions of these substances to canine gastric mucosa resulted in secretory inhibition.

In this study we have investigated the inhibitory effect on gastric secretion, *in vivo*, of injected *N*-ethylmaleimide, *N*-2-fluorenylmaleimide (new substance, m.p. 187·5–188°, cor.), β-mercaptoethylamine hydrochloride† and the closely related 2-aminoethyl*iso*thiuronium bromide hydrobromide (AET). Since we found a decrease in secretory activity with each of these separately, we devised experiments seeking possible 'neutralizing' effects of the sulphydryl type compounds on subsequently injected maleimides. There appears to be such an effect.

Materials and Methods

Sprague-Dawley rats (250–300 g) fasted for 72 h and deprived of water just preceding the operation, were lightly anaesthetized with ether and the pylorus of each was ligated (Shay[4] preparation).

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compounds, each suspended in a finely divided con-
ıline (or in saline with added Tween 80), was injected
:ritoneal cavity. The controls were treated similarly
(or saline with added Tween 80). After 6 h the animals
ced and the stomachs removed, emptied and examined.
H, free and total acidity (by titration to a pH of 3·3
successively), and peptic activity[5] of the secretions
nined (see Tables).

I. Means, standard deviations from the means, and probabilities (p) calculated

roups	No. of animals	pH			Total vol. (ml)		
		mean	σ	p	mean	σ	p
aline at ligation	99	0·9	0·35		6·8	2·23	
	32	4·1	1·25	<0·01	0·8	0·38	<0·01
	20	3·1	1·09	<0·01	2·6	0·90	<0·01
; mg*†	15	3·4	1·31	<0·01	1·1	0·32	<0·01
. 400 mg*‖	21	1·8	0·65	<0·01	2·7	0·90	<0·01
rlmaleimide, 7 mg*†	11	3·0	0·74	<0·01	1·3	0·42	<0·01
. 400 mg*‖ and N-ethyl-	5	2·3	0·24	<0·01	1·5	0·39	<0·01
400 mg*‖ and N-2- ;*†	9	1·8	0·34	<0·01	1·6	0·60	<0·01

* Per kg rat body weight.
† Intraperitoneal injection at ligation.
‡ 2-Aminoethyl*isothiuronium* bromide hydrobromide.

iments where both a maleimide and a sulphydryl
(or the closely related AET) were used in the same
latter type was injected subcutaneously 20–40 min
Shay operation, and the maleimide was injected at

Results and Discussion

ıls with various levels of the four substances were run.
leimide, injected intraperitoneally, gave the greatest

inhibition of gastric secretion, without death of the
dosage of 7 mg per kg of rat body weight. The corr
dose for *N*-2-fluorenylmaleimide was 15 mg. The
approximately equivalent to the *N*-ethylmaleimide o
basis.

Although there can be no direct comparison, it is of
note that 'both male and female rats which ingested a
containing 0·042 per cent of the latter compound for eig

as levels of significance deviating from the mean of the control group (Fisher's t

Free acid, H+, meq/l.			Total acid, H+, meq/l.			Pepsin, mg/ml.			Peps
mean	σ	p	mean	σ	p	mean	σ	p	mean
73	19·3		119·0	16·1		7·01	1·43		49·77
0·04	2·4	<0·01	82·0	34·5	<0·01	12·48	4·42	<0·02	10·80
11·0	21·5	<0·01	78·0	25·7	<0·01	7·55	1·30	n.s.¶	18·99
5·0	7·8	<0·01	79·0	25·9	<0·01	—	—	—	—
35·0	27·1	<0·01	94·0	20·2	n.s.¶	14·40	1·30	<0·01	22·64
8·0	13·0	<0·01	81·0	16·2	<0·01	10·96	2·28	<0·01	13·11
14·0	6·2	<0·01	83·0	8·87	<0·01	—	—	—	—
26·0	12·4	<0·01	97·0	8·90	<0·01	—	—	—	—

§ Subcutaneous injection 40 min before ligation.
‖ Subcutaneous injection 20 min before ligation.
¶ Not significant.

showed no gross gastric lesions. The general health a
of these animals was comparable to that of control
same diet without the compound'*.

β-Mercaptoethylamine hydrochloride gave the gr
sistent inhibition (but not as marked as with the mal
400 mg per kg body weight either when injected intra
at ligation or subcutaneously 20–40 min before liga

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tions of 75 per cent (or even 50 per cent) of the above dose frequently gave noticeable inhibition, but results were inconsistent. The corresponding level for AET was much less on a molar basis. Even at 250 mg per kg body weight, the inhibition was as consistent and as marked as with β-mercaptoethylamine at 400 mg. Intraperitoneal injection, at ligation, of AET at twice the usual level proved lethal.

When β-mercaptoethylamine or AET was given subcutaneously 40 min before ligation and the maleimide intraperitoneally directly following ligation, a moderate 'neutralization' of the secretory inhibition, expected with the maleimide alone, was observed (see Table II). In these experiments less toxic effect was encountered

Table II. Comparison of means of groups having single injections of the maleimide or the sulphydryl compound with the groups having both substances injected

Rat groups	pH		Ml Total volume		Free H meq/l.	
	means	p‡	means	p‡	means	p‡
N-Ethylmaleimide 7 mg.* at ligation	4·1	<0·01	0·8	<0·02	0·04	<0·01
β-Mercaptoethylamine, 400 mg† subcutaneously before ligation and N-ethylmaleimide 7 mg* at ligation	2·3	—	1·5	—	14·0	—
β-Mercaptoethylamine 400 mg† before ligation	1·8	<0·02	2·7	<0·01	35·0	<0·01

* Per kg of rat body weight, intraperitoneally.
† Per kg of rat body weight, subcutaneously.
‡ Probability calculated as levels of significance in the deviation of the middle group from each group with a single injection.

when 40 min had elapsed between the first injection and the operation than when only 20 min had elapsed. In effect, injection of the thiol compound (of AET) alone inhibited secretion moderately, injection of the maleimide alone caused marked inhibition, but when administered successively as described there was less effect than with the maleimides alone and slightly more than with the sulphydryl type alone. It remains to be seen whether the sulphydryl compound (or AET, as such or altered after injection), having spread throughout the body in the time before

ligation, reacted directly with the maleimide as the latter was absorbed or successfully blocked sites were involved in the gastric secretory mechanism, thus largely preventing the more effective maleimide from blocking those sites. The observation of the inhibition of the oxidation of succinate by AET;[6] the inactivation of a component in the succinoxidase system by 2,3-dimercapto-1-propanol,[7] perhaps by disulphide formation; possible participation of this enzyme system in gastric acid secretion,[8] and the fact that succinic dehydrogenase contains —SH groups, essential for its activity,[9] would all seem to be related to these present experiments. The report of Moussatché and Prouvost-Danon[10] that succinate increases the amount of histamine liberated in certain experiments, whereas sulphydryl blocking agents inhibit the anaphylactic reaction, is also of interest.

A cancellation of effect was of course noted when equimolar amounts of a maleimide and the sulphydryl compound were injected one after the other intraperitoneally. This presumably resulted from reaction of the two substances together at the site of injection.

All of the compounds at their optimum dosage protected the animals from ulceration both as to percentage incidence and extent. Comparison of the test animals with controls, with respect to ulceration, was similar to the comparison noted with calcium lignin sulphonate.[1]

Summary. Gastric secretion is markedly inhibited in the Shay rat by a single small injection (about 10 mg per kg rat body weight) of certain *N*-substituted maleimides or, less markedly, by single injections of β-mercaptoethylamine hydrochloride (about 400 mg per kg rat body weight) or 2-aminoethyl*iso*thiuronium bromide hydrobromide (AET). Injections of either of the latter two substances prior to the Shay operations, followed by injection of the maleimide, give results indicating that the sulphydryl compound prevents the maleimide from exerting its full effect. Tables are presented summarizing data for pH, acidity, volume, and pepsin of the gastric secretions of treated animals and controls.

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