excretion of ornithine, cystine, arginine, and lysine, which was found to be about 1:1:2:4, is quite similar from one subject to the next despite the haphazard nature of the case material and the absence of any dietary control. The results suggest the hypothesis that cystinuria involves an enzymatic defect which affects simultaneously at some site or sites (probably the kidney) some phase of the metabolism of arginine, lysine, ornithine, and the sulfur amino acids, and perhaps also isoleucine and taurine.

It is a pleasure to acknowledge the expert laboratory assistance of Mrs. Gertrude C. Carey.

1. Garrod, A. E., *Inborn Errors of Metabolism*, 2nd edition, Henry Frowde and Hodder and Stoughton, London, 1923.

2. Yeh, H. L., Frankl, W., Dunn, M. S., Parker, P., Hughes, B., and Gyorgy, P., *Am. J. Med. Sci.*, 1947, v214, 507.

3. Dent, C. E., *Partition Chromatography*, Biochemical Society Symposium No. 3, London, 1948.

4. Dent, C. E., and Rose, G. A., *Quart. J. Med.*, 1951, v20, 205.

5. Moore, S., and Stein, W. H., *J. Biol. Chem.*, 1951, v192, 663.

6. Stein, W. H., and Moore, S., *J. Biol. Chem.*, 1951, v190, 103.

7. Brand, E., Cahill, G. F., and Harris, M. M., *J. Biol. Chem.*, 1935, v109, 69.

8. Lewis, H. B., Brown, B. H., and White, F. R., *J. Biol. Chem.*, 1936, v114, 171.

9. Rachele, J. R., Reed, L. J., Kidwai, A. R., Ferger, M. F., and du Vigneaud, V., *J Biol. Chem.*, 1950, v185, 817.

Received October 22, 1951. P.S.E.B.M., 1951, v78.

The Screening of Antiulcer Drugs by a Quantal Procedure. (19190)

RAYMOND L. CAHEN AND KRISTEN M. TVEDE. (Introduced by Louis S. Goodman.)
From the Maltbie Laboratories, Morristown, N. J.

The pyloric ligation method of Shay(1) which has won universal acceptance has made it possible to solve many problems of the pathogenesis of gastric ulcer(2-4). The Shay rat, however, has been used only occasionally for evaluating the antiulcer effect of drugs: antacids(2,3), Thephorin(6), tetraethylammonium(7), phenothiazine derivatives(8), and urinary extracts(4,9).

Moreover, studies of the activity of atropine have been quite contradictory. Protection has been noted with 250 mg/kg subcutaneously (2); 10 mg kg(6); 1.5-3 mg/kg(10); whereas, no protection has been observed with 100 mg kg(8). A systematic investigation of compounds reported clinically effective in peptic ulcer therefore seems warranted. For that purpose a quantal procedure has been devised.

Material. Twelve drugs were tested. A. Anticholinergic agents: 1. atropine (sulfate) USP; 2. trihexyphenidyl, *Artane*. B. Pure ganglionic blocking agents: 1. pentamethonium bromide; 2. hexamethonium bromide; 3. tetraethylammonium bromide; 4. tetraethylammonium chloride. C. Ganglionic blocking-anticholinergic agents: 1. B-diethylaminoethyl-xanthene-9-carboxylate methobromide, Methantheline Bromide, *Banthine;* 2. 10 - (B - diethylaminoethyl) - phenothiazine (hydrochloride) *Diparcol*. D. Antihistaminic agents: 1. tripelennamine hydrochloride USP, *Pyribenzamine;* 2. diphenhydramine hydrochloride USP, *Benadryl;* 3. phenindamine hydrogen tartrate, *Thephorin;* 4. Chlorothen Citrate, *Tagathen*. 580 male albino Wistar rats maintained on a diet of Wayne "Rat Blox" were used; those weighing 180-250 g were starved for 72 hours before the ligation, while younger rats weighing 125-180 g were starved for 48 hours only. *Method.* The original Shay rat method(1) was used. However, whereas previous authors used as criteria the decrease of gastric acidity and volume, together with the pepsin concentration and a system of scoring the severity of ulcerations, the test selected in our assays concerns only the appearance of ulcerative lesions in the rumen of the rat ob-

TABLE I. Average Protective Dose of Drugs Against Ulcer Formation in the Shay Rat. Single subcutaneous administration; rats 125-250 g; sacrificed 17-19 hr after ligation.

Drug	No. rats	ED_{50}, mg/kg	19/20 confidence limits	P	Slope	λ	LD_{50}
Control	78						
Atropine (SO_4)	53	11	7.3–16.5	.91	2.8	.35	
Artane	34	70	39–125	1	2.6	.38	
Diparcol	12	>200					200
Banthine	44	5.3	3.6–7.9	.97	2.9	.34	
Pentamethonium	12	>200					
Hexamethonium	18	>200					200
Tetraethylammonium (Br.)	6	>200					200
" (Cl.)	18	>200					<200
Pyribenzamine	26	> 75					75*
Benadryl	23	>300					400
Thephorin	51	98	75–125		1.3		*
Tagathen	20	>150					

* Convulsion at 100 mg/kg.

served under a 10x binocular microscope. In order to modify the graded response procedure previously used into a quantal one, the 50% protective dose (ED_{50}) was determined. The present method involves treating groups of Shay rats with graded doses of antiulcer compounds and evaluating the probit of each group which fails to show ulcers following pyloric ligation. From the data so obtained a regression line is constructed and the ED_{50} evaluated and compared with the ED_{50} of atropine used as standard. In designing such a quantal response, the selection of the period of ligation which gives 100% ulcer in controls is essential. In preliminary experiments the 17-19 hours period selected by Shay for adult rats (180-250 g) was used. The period was decreased to 10 hours in a second group of experiments.

Results. 1. *Adult rats injected subcutaneously at the time of ligation and sacrificed 17-19 hours later.* Table I summarizes the results in Shay rats of the average protective dose (ED_{50}); the 19/20 confidence limits calculated according to Litchfield and Wilcoxon; the probability factor (P) according to Fisher; the slope of the curve; the standard deviation of the logarithms of the individual effective dose, and the reciprocal of the slope (λ). The comparison between the dose inducing side effects or toxic symptoms and the protective dose listed in Table I gives some information about the specificity of action of some drugs. Of interest is the increased toxicity of Pyribenzamine on the Shay rat as compared with data of the literature (11) and of our own experiments on normal rats.* Fig. 1 shows a linear relationship between the probit of protected rats and the logarithm of the dose of atropine, Banthine, Artane, and Thephorin. Neither antihistamine compounds such as Benadryl, Pyribenzamine, and Tagathen nor ganglionic blocking agents such as pentamethonium, hexamethonium, and tetraethylammonium were active unless doses causing side effects or toxic effects were used.

2. *Young rats injected subcutaneously both at the time of ligation and 5 hours later and sacrificed 10 hours after ligation.* Because of the ineffectiveness in the Shay rat of pentamethonium, hexamethonium, and tetraethylammonium, which are reported clinically active, it seemed important to study comparatively another method involving a shorter duration of ligation (10 hours) and using 2 injections, one at the time of ligation and the other 5 hours later. Preliminary experiments, however, showed that in such conditions in order to obtain 100% ulcers in controls, younger animals (50-100 g) should be used. This confirms the data of Shay who found that in 180 g rats ulcers were noted in only 72% of the controls. The results summarized in Table II show a significant increase in the

* Ether seems to potentiate the toxic effects of Pyribenzamine. 100 mg/kg Pyribenzamine subcutaneously induces 12/18 mortalities in unoperated male rats anesthetized with ether *versus* 2/18 in controls.

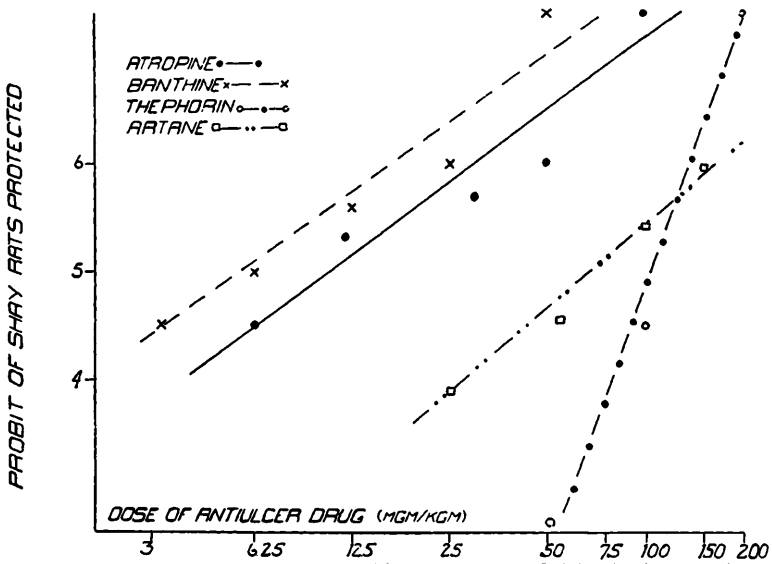

FIG. 1. Dose effect curve of atropine, Banthine, Artane and Thephorin. Abcissa: Dose of antiulcer (logarithmic scale). Ordinates: Probit of Shay rats protected against ulcers.

protection afforded by various drugs, especially short acting drugs: tetraethylammonium, hexamethonium, pentamethonium, and Diparcol.

3. *Adult rats submitted to oral administration and sacrificed 17-19 hours after ligation.* Because the oral route of administration is generally used for antiulcer compounds, comparative experiments were run following this route. Table III summarizes the effect of drugs given orally 1 hour before pyloric ligation, the animals being sacrificed 17-19 hours after ligation.

Discussion. The Shay rat has been found of value for the qualitative assay of antiulcer preparations, although its specificity has been debated(5). In order to give reproducible results, however, the procedure involves the laborious experimental effort of time-consuming tests and calculations. A modification into a quantal procedure is here described which is more rapid and more suitable for quantitative evaluations. The regression line when tested for linearity was found satisfactory and gave estimates of the potency of various clinically active drugs.

It was found essential to select 10 hours as the optimum time interval between the pyloric ligation and observation of the ulcerations. Table II shows a greater effect at 10 hours than 18 hours after injection. This result is of importance in the case of short acting drugs such as pentamethonium, hexamethonium, and tetraethylammonium. Of course, this interval period is not as practicable as 17 or 19 hours, which permits the running of experiments during the ordinary working schedule.

Contradictory results were reported concerning the antiulcer effect of histamine blocking agents. Our data indicate the inefficiency of Pyribenzamine, Benadryl, and Tagathen in the Shay rat and the slight activity of Thephorin, smaller than previously reported (6), observed by us only at a dose inducing central stimulation. This confirms the failure of these drugs to block histamine induced gastric secretion(12). Conversely, the effectiveness of atropinelike compounds in the Shay rat is in agreement with clinical experience.

Inasmuch as the ganglionic blocking action of Banthine is complicated by powerful anticholinergic effects, the specificity of its ganglionic site of action is questioned. Our results on the Shay rat reveal that Banthine falls in the class with atropine rather than with tetraethylammoninm; its ganglionic blocking effect is not, however, excluded.

Summary. (1) A modification of the pyloric ligation method of Shay into a *quantal*

TABLE II. Comparative Effect of Antiulcer Drugs According to Mode of Administration and Duration of Pyloric Ligation.

Drug	Total dose, mg/kg	Single inj., 17-19 hr ligation, % protected	No. rats	Double inj., 10 hr ligation, % protected	No. rats
Control			78		30
Atropine (SO_4)	22	72	53	100	6
Artane	100	66	34	100	6
Banthine	10.6	75	44	100	6
Pentamethonium	100	33	12	88	12
Hexamethonium	200	0	18	100	12
Tetraethylammonium (Br.)	100	0	6	50	12
" (Cl.)	100	0	18	66	18
Diparcol	200	0	12	100	6

TABLE III. Average Protective Dose of Drugs Against Ulcer Formation in the Shay Rat. Oral administration; rats 125-250 g; sacrificed 17-19 hr after ligation.

Drug	No. rats	ED_{50}, mg/kg	19/20 confidence limits	P	Slope	λ	LD_{50}
Control	36						
Atropine (SO_4)	29	70	47–105	.8	2.2	.45	
Banthine	18	140	110–255	1	1.6	.63	<400†
Thephorin	18	140	110–255	.5	1.5	.67	400*

* Convulsions at 100-400 mg. † Hemorrhage in the glandular area.

procedure is described. A linear relationship exists between the logarithm of the doses of antiulcer compound and the probits of Shay rats failing to show gastric ulcerations. (2) Anticholinergic agents such as atropine, Artane, and Banthine administered subcutaneously and orally show unequivocal protection against ulcerative lesions of Shay rats. (3) Short acting *pure* ganglionic blocking agents: hexamethonium, pentamethonium, tetraethylammonium afford significant protection only following a short duration of pyloric ligation and by using a fractionated injection. (4) No antihistamine agent seems to be effective against ulcer in the Shay rat. (5) The Shay rat is a valuable tool for the screening of antiulcer drugs.

Grateful acknowledgement is given to Dr. A. C. Bratton. Jr. (Parke, Davis and Co.), Dr. E. de Beer, (The Wellcome Research Laboratories), Dr. W. Hambourger (G. D. Searle & Co.) Dr. R. Mayer (Ciba Pharmaceutical Products), Dr. J. M. Ruegsegger (Lederle Laboratories), Dr. E. Sevringhaus (Hoffmann-La Roche), Dr. C. Winter (Merck & Co.) for generous supplies of compounds used in this investigation.

1. Shay, H., Komarov, S. A., Fels, S. S., Meranze, D., Gruenstein, M., Siplet, H., *Gastroenterology,* 1945, v5, 43.

2. Komarov, S. A., Shay, H., Siplet, H., Gruenstein, M., *Brit. Jour. Pharm. and Chemotherapy,* 1950, v5, 1.

3. Shay, H., Komarov, S. A., Siplet, H., Gruenstein, M., *Am. J. Dig. Diseases,* 1946, v14, 99.

4. Risely, E. A., Raymond, U. B., Barnes, R. H., *Am. J. Physiol.,* 1947, v150, 754

5. Madden, R. J., Ramsburg, H. H., Hundley, J. M., *Gastroenterology,* 1951, v18, 119.

6. Lehman, G., Stefko, P. L., *J. Lab. and Clin. Med.,* 1949, v34, 3.

7. Little, J. M., Ogle, B. C., Yeagley, J. D., Cayer, D., *Science,* 1947, v106, 448.

8. Bovet, D. and Marotta, M., *Arch. Int. Pharmacodyn.,* 1951, v86, 177.

9. Wick, A. N., Irish, A. J., Pauls, F., MacKay, E. M., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 40.

10. Personal communication with Dr. Harry Shay.

11. Mayer, R. L., Hays, H. W., Brousseau, D., Mathieson, D., Rennick, B., Yonkman, F. F., *J. Lab. and Clin. Med.,* 1946, v31, 7.

12. Loew, E. R. and Chickering, O., PROC. SOC. EXP. BIOL. AND MED., 1941, v48, 65.

Received October 23, 1951. P.S.E.B.M., 1951, v78.