Brit. J. Pharmacol. (1961), **17**, 41–50.

THE EFFECT OF ATROPINE, PROPANTHELINE AND POLDINE ON THE VAGALLY STIMULATED GASTRIC MOTILITY AND THE HISTAMINE-STIMULATED ACID GASTRIC SECRETION IN THE RAT

BY

P. N. AARSEN AND J. VAN NOORDWIJK

From the Pharmacotherapeutic Laboratory, University of Amsterdam

(*Received March* 6, 1961)

Histamine-induced acid gastric secretion in the anaesthetized rat was not diminished by poldine in a dose which reduced vagally stimulated gastric contractions by approximately 75%. A dose of atropine, twice as large as the dose which reduced gastric contractions by 75%, had no apparent effect on the histamine-stimulated acid gastric secretion up to 2 hr after the injection. Only when more than 40 times as much atropine was injected did a slight inhibition of the acid secretion occur in 80 to 120 min. Propantheline, in a dose which inhibited gastric contractions by approximately 75%, slightly diminished acid secretion in 40 to 80 min. This effect was not increased by a further dose of propantheline. It was concluded that, in so far as any inhibition of acid gastric secretion had occurred, this could not be interpreted as an anti-muscarine or a direct toxic effect, but rather as an indirect effect possibly due to interference with the blood flow through the stomach wall.

Atropine, propantheline and poldine are used clinically to depress gastric secretion. Their effect is generally explained in terms of blockade of the muscarine effect of locally released acetylcholine. In high dosage these compounds have a transient ganglion-blocking action (Bainbridge & Brown, 1960); they may also be feebly antihistaminic (Acred, Atkins, Bainbridge, Brown, Quinton & Turner, 1957). Nevertheless it is unlikely that their apparent clinical usefulness in depressing gastric secretion depends on these effects: more powerful antihistamines such as mepyramine do not depress gastric secretion. Janowitz & Hollander (1956) found in the dog that atropine sulphate in doses of 0.2 and 0.4 mg/kg reduced the rate of secretion in vagally denervated gastric pouches which were stimulated by histamine: the acidity, however, remained relatively constant and independent of the secretory rate. They also showed that inhibition increased with increasing doses of atropine and also with time, being greatest in the third hour after its injection. Although atropine sulphate in a dose of 0.4 mg/kg produced toxic effects, the authors were unable to explain the effect of this dose on secretion solely as a toxic manifestation of the drug, since inhibition developed gradually. The same slow onset of inhibition occurred even with the lowest dose.

In previous experiments with rats we have shown that phloxin acts rapidly to inhibit acid gastric secretion (van Noordwijk & Aarsen, 1954). In contrast with atropine, it had no effect on gastric motility or peptic activity (Aarsen, 1959). We

were therefore interested to study the effect on the histamine-stimulated acid gastric secretion in this species of atropine and atropine-like drugs applied in doses which reduced or abolished the motility of the stomach. The required doses were found by recording the effect of these drugs on gastric contractions induced by vagal stimulation.

METHODS

Female rats of 200 to 250 g were used in all experiments. The animals were starved during the preceding night, and were anaesthetized by a single intramuscular injection of 0.6 ml./100 g of 25% solution of urethane.

Operative technique. This was the same for recording the gastric contractions and the acid secretion, except that for the former the right vagus nerve in the neck was exposed, ligated and cut so that the peripheral end could be stimulated. The trachea and the left jugular vein were exposed and cannulated. The abdomen was opened through a transverse incision just below the costal arch; both epigastric arteries were ligated. Then the oesophagus was exposed at the cardia and the ventral vagus trunk lying on the oesophagus was dissected. A polythene tube of 14 cm length and 2 mm external diameter was passed into the stomach via the oesophagus and fixed at the cardia excluding the vagus trunk. A polythene cannula of 5 mm external diameter and bevelled at the tip was introduced into the stomach through an incision in the duodenum and fixed by a ligature round the pylorus. Directly behind the bevelled tip this cannula had two lateral holes to prevent recess formation around this end protruding in the antrum. Before closing the abdominal wall the stomach was rinsed by perfusing it swiftly with pre-warmed physiological saline (N/4,000 sodium hydroxide was used in the experiments for recording acid gastric secretion). At the same time gentle massage was applied to the stomach.

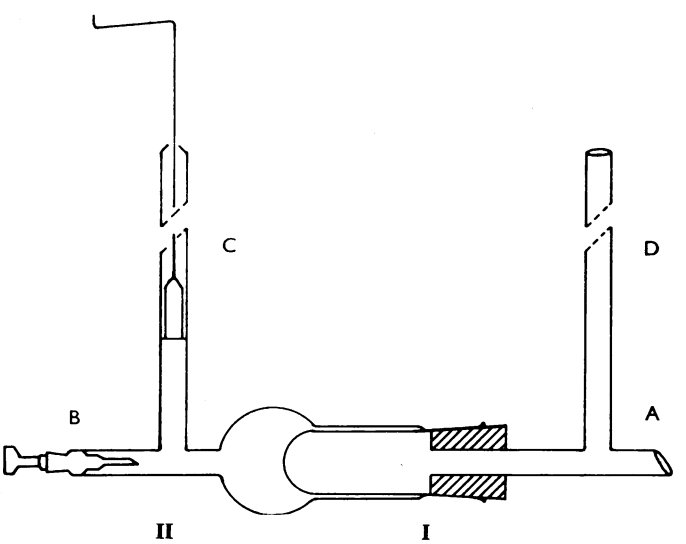

Fig. 1. Modified tambour used for recording of gastric contractions in the rat. The gastric cannula was attached to side-arm A of the compartment I. The compartments I and II were separated from each other by a thin rubber membrane (finger cot). Compartment I was filled with physiological saline; compartment II with a saturated solution of sodium sulphate. The volume in this second compartment could be changed through the side-arm B. The vertical glass tube D served as a manometer. A thin glass rod bent at the tip served as a writer. This was attached to the light polythene float in the glass tube C.

Recording of gastric contractions. These were recorded with a modified tambour (Fig. 1). The part connected to the gastric cannula (A) was completely filled with physiological saline at a pressure of 8 cm of water. The part which included a thin rubber membrane (finger cot) was filled with a saturated solution of sodium sulphate. Linear recording of pressures in the range from 4 to 15 cm of water could be obtained by changing the vol. of sodium sulphate in the second compartment through the side arm (B). A thin glass rod bent at the tip was connected to the light polythene float in the vertical glass tube (C), and served as a writer. The vertical glass tube (D) served as a manometer; the stomach was adjusted to the zero level of this manometer. At the beginning of the experiment the pressure in the compartment (I) connected to the saline-filled stomach was adjusted to 8 cm of water by adding saline via the manometer (D). The peripheral end of the cut right vagus nerve was stimulated by stainless steel electrodes applied to the nerve only during the periods of stimulation. The electrodes were connected to an electronic stimulator via an interruptor so that the nerve was stimulated for 5 sec periods, at 15 sec intervals. The square wave pulses had a duration of 5 msec at a frequency of 10/sec. The current was adjusted until the contractions became regular for this frequency and duration of stimulation; in most experiments the current was about 0.04 mA.

Recording of acid gastric secretion. The method of Ghosh & Schild (1958) for the continuous recording of the total acid secretion was used. However, we did not open the stomach to remove food debris. This method is based on continuous perfusion of the stomach with N/4,000 sodium hydroxide and recording of the *p*H of the effluent. This method makes it possible to test the effect of several successive intravenous doses of histamine in the course of one experiment lasting several hr. Repeated administration of the same dose of histamine usually produces constant effects. In nearly all experiments the constant dose of histamine base was 100 μg. For some rats, however, this dose was too large or too small; in these animals we used 50 or 200 μg of histamine for consecutive stimulation of the acid gastric secretion. All intravenous injections of histamine, except the first, were given when the gastric effluent had reached *p*H 6. The responses are expressed in terms of the lowest *p*H value measured after the injection of the drug.

Anti-acetylcholine action in vitro. A 6 to 7 cm segment of terminal ileum of guinea-pigs weighing 250 to 300 g was suspended in 15 ml. oxygenated Tyrode's solution from which magnesium chloride had been omitted. The temperature was kept at 35° C. A dose cycle of 2 min with 10 sec contact was used. The experiments were carried out as described by Schild (1947). Three doses of acetylcholine alone and three double doses of acetylcholine in the presence of a constant dose of antagonist were administered in random order. This series was repeated 4 times on each preparation. The antagonist was added 60 sec before acetylcholine. Regression-lines were fitted to the results of these assays according to the method of least squares (Emmens, 1948).

The following salts of the 3 drugs were used: atropine sulphate, propantheline bromide (Pro-Banthine) and poldine methylmethosulphate (Nactate). The doses given below are expressed in terms of the salts.

RESULTS

Effect on gastric contractions. Two experiments were performed in which the vagus nerve was stimulated repeatedly as described above for periods of at least 2 hr. Each block of stimuli resulting in 15 contractions took 5 min; between every two successive blocks there was a resting interval of 5 min. As shown in Fig. 2, which is representative of both experiments, the response was relatively constant for at least 2 hr. As a quantitative parameter of the response we took the mean height of the contractions of a block, omitting the first and the last contraction. The experiment (see Fig. 2) consisted of 14 of such blocks: the mean height was 2.3 ± 0.2 cm (s.d.). In another experiment with 7 blocks of contractions the mean was 4.8 ± 0.4 cm (s.d.). After two control blocks of contractions, a dose of

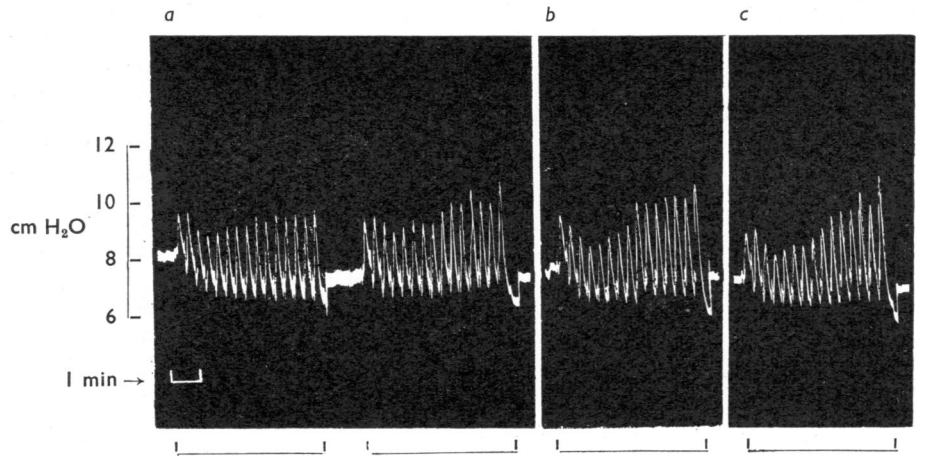

Fig. 2. Records of the vagally stimulated gastric contractions in the rat. For about 140 min the vagus nerve was stimulated repeatedly as follows: 5 min periods of stimulation (indicated by the horizontal brackets) were alternated with 5 min periods of rest, during which the kymograph was stopped. Each block of stimuli resulted in 15 contractions. *a*, The first and second block of contractions. The tone decreased only during the recording of the first block. *b*, Block of contractions after 1 hr.; *c*, after 2 hr.

the antagonist was injected in the course of the third block (Fig. 3), and stimulation was continued for 5 min after the injection. The inhibitory effect of the dose was taken as the height of the contractions obtained at the point of maximum inhibition: the height was then expressed as a percentage of the mean height of the control contractions. Because of the cumulative effect of the antagonist it was not possible to obtain more than one estimate in a single experiment. The second dose of atropine (4 μg) produced a more powerful inhibition than the first which was given 90 min earlier. At the time of the second dose the contractions had recovered their original height (Fig. 3).

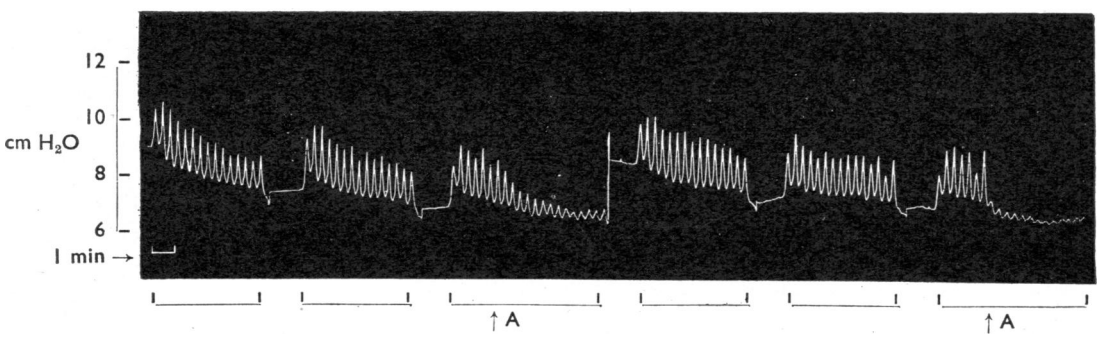

Fig. 3. The effect of two doses of 4 μg atropine (A) given intravenously with an interval of 90 min on the vagally stimulated gastric contractions. After two preceding control blocks of contractions the dose of atropine was injected in the course of the third. The second dose of atropine caused a stronger inhibition than the first made 90 min before, in spite of the fact that the preceding contractions had recovered their original height. The horizontal brackets indicate the periods of stimulation; the kymograph was stopped in the intervening 5 min rest periods.

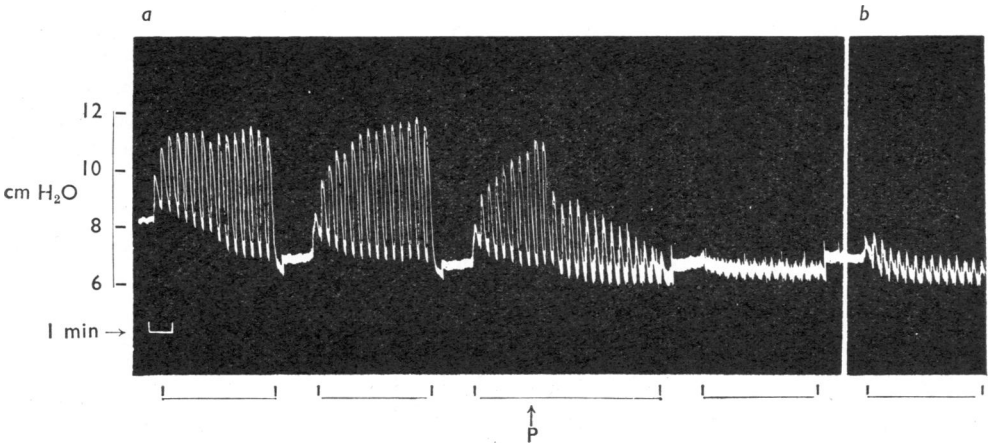

Fig. 4. Records of gastric contractions following vagal stimulation. *a*, The effect of 2 μg poldine (P) given intravenously. *b*, After a period of about 2 hr the inhibitory influence of poldine was still effective. The horizontal brackets indicate the periods of stimulation; the kymograph was stopped in the intervening 5 min rest periods.

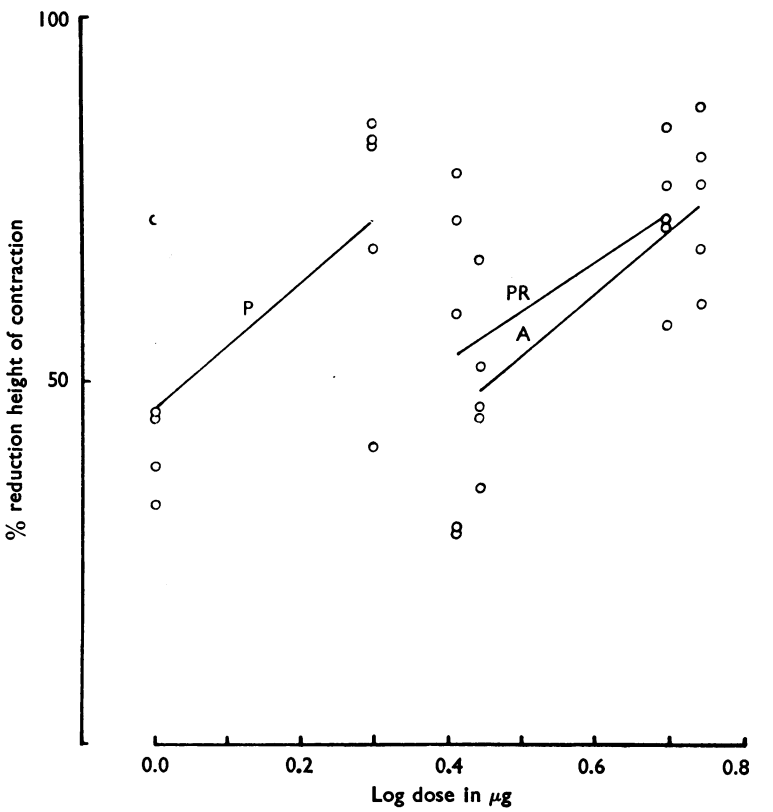

Fig. 5. The effects of atropine (A), propantheline (PR) and poldine (P) on the vagally stimulated gastric contractions in the rat.

The duration of effect of approximately equivalent doses of atropine, propantheline and poldine was found to increase in this order. Fig. 4 shows that after a period of about 2 hr a dose of 2 μg of poldine was still effective. Fig. 5 shows the log-dose-effect lines obtained for the three drugs investigated. The difference between the slopes of these lines was not significant at the 10% level. The slopes of the lines for atropine, propantheline and poldine were 82.48, 63.09 and 85.18, respectively. The activity-ratios of these drugs based on the doses giving a 50% reduction in height of contraction, as determined from the regression-lines, are given in Table 1.

TABLE 1

RATIOS FOR SPASMOLYTIC AND ANTI-ACETYLCHOLINE ACTIVITIES OF ATROPINE, PROPANTHELINE AND POLDINE

In brackets activities according to Acred *et al.* (1957)

Drugs	Spasmolytic activity on gastric contractions	Anti-acetylcholine activity on isolated guinea-pig ileum
Atropine	1·00	1·00
Propantheline	1·27	1·22 (2·09)
Poldine	2·60	1·00 (1·02)

Anti-acetylcholine activity. The anti-acetylcholine activity of propantheline and poldine was compared with that of atropine on the isolated guinea-pig ileum. The results are expressed as dose ratios and are given in Table 1.

TABLE 2

THE EFFECT OF ATROPINE ON THE HISTAMINE-STIMULATED ACID GASTRIC SECRETION

Roman numerals indicate the successive stimulations of acid secretion by a constant dose of histamine before and after atropine

Rat no.	Drug injected	Control estimation (lowest *p*H measured)			Estimations after atropine (difference between lowest *p*H measured and mean of control *p*H)		
		I	II	Mean	I	II	III
1	Atropine,	4·33	4·04	4·18	−0·06	+0·14	+0·08
2	11·2 μg	4·36	4·50	4·43	+0·05	+0·37	+0·43
3		4·88	4·73	4·80	−0·67	−0·12	−0·66
4		4·28	4·53	4·40	+0·36	+0·42	−0·22
5		3·73	3·77	3·75	−0·07	0·00	—
Mean					−0·07	+0·16	−0·09
6	Atropine,	4·22	3·78	4·00	−0·38	−0·13	+0·04
7	500 μg	3·82	4·12	3·97	−0·04	+0·23	+0·42
8		4·56	4·38	4·47	−0·22	−0·53	+0·35
9		5·03	4·63	4·83	−0·09	+0·14	+0·47
10		3·90	4·01	3·95	+0·29	+0·52	—
11		4·53	4·67	4·60	+0·64	+0·74	+0·90
12		4·16	4·45	4·30	+0·18	+0·40	+0·53
13		4·35	4·08	4·21	+0·02	+0·05	—
14		5·28	5·17	5·22	−0·26	−0·23	−0·07
Mean					+0·01	+0·16	+0·37

Acid gastric secretion. Starting after two control determinations (the response to the first dose of histamine in each case was neglected), the dose of the drug under test was injected intravenously about 3 min before the constant dose of histamine. Table 2 shows the results of experiments on the effect of atropine on acid secretion stimulated by successive, constant doses of histamine. Friedman's ranking test (Friedman, 1937) was applied to results obtained before and after administration of 11.2 μg of atropine (twice the dose which inhibited gastric contractions by 75%). This dose of atropine did not produce a significant ($P>0.05$) effect on acid secretion. Likewise, atropine in a dose of 500 μg was without effect on the acid secretory response to the first and second doses of histamine ; however, it did reduce ($P<0.05$) the effect of the third dose of histamine. The third dose was given at about 80 min after the administration of atropine.

Table 3 gives the results of experiments on the effect of propantheline and of poldine on histamine-stimulated acid secretion. In nearly all these experiments two

TABLE 3

THE EFFECT OF PROPANTHELINE AND POLDINE ON THE HISTAMINE-STIMULATED ACID GASTRIC SECRETION

Roman numerals indicate the successive stimulations of acid secretion by a constant dose of histamine

Rat no.	Control estimations (lowest *p*H measured) I	II	Mean	Drug injected	Estimations after injection (difference between lowest *p*H measured and mean of control *p*H) I	II	Drug injected	Estimations after injection (difference between lowest *p*H measured and mean of control *p*H) I
15	3·92	4·18	4·05	Propanthel-	+0·34	−0·05	Propanthel-	−0·27
16	4·02	3·63	3·82	ine 5·2 μg	−0·05	+0·68	ine 10·4 μg	+0·02
17	3·73	3·78	3·75		+0·05	+0·37		+0·38
18	3·55	3·53	3·54		+0·24	+0·38		+0·38
19	4·50	4·40	4·45		−0·35	+0·21		—
Mean					−0·04	+0·31		+0·12
20	4·04	4·12	4·08	Poldine	+0·08	−0·01	Poldine	+0·09
21	3·62	3·47	3·54	2 μg	+0·48	+0·13	10 μg	+0·20
22	4·03	4·03	4·03		+0·08	+0·20		+0·10
23	3·77	3·65	3·71		+0·21	+0·22		+0·07
24	3·73	—	—		+0·31	—		+0·34
Mean					+0·23	+0·13		+0·16

doses of the compound under test were injected. The first dose of each compound was that which reduced gastric contractions by approximately 75%. The dose of propantheline was then increased by twofold and, of poldine, by fivefold. These increased doses constituted the second dose of each compound.

Application of Friedman's ranking test shows that propantheline did not diminish the acid secretory response to the first dose of histamine which was injected immediately after the dose of propantheline. However, when the second dose of

histamine was injected after the propantheline had been allowed to act for 40 min, the acid secretory response was found to be significantly reduced ($P<0.05$). A second double dose of propantheline had no further effect on the response. Poldine in doses of 2 and 10 μg had no significant effect ($P>0.05$) on the histamine-stimulated acid secretion.

DISCUSSION

It is still an open question whether atropine and atropine-like drugs have a specific inhibitory effect or not on histamine-stimulated acid gastric secretion. Code (1951) points out that the size of the dose of atropine does not appear to be related quantitatively to the effect, that *in vivo* atropine never seems to abolish the effect of histamine, particularly in man. Polland (1930) found that the output of acid may be reduced, but the changes were small. Sometimes none were noted (Lim, Matheson & Schlapp, 1923). Atkinson & Ivy (1938) reported that toxic effects may be present before significant inhibition occurs.

Many investigators have found that in dogs atropine has an inhibitory effect on acid gastric secretion stimulated by histamine. However, the experiments of Janowitz & Hollander (1956) in dogs demonstrated clearly that the effect of 0.1 mg/kg atropine was delayed, since it appeared one hr after administration of the dose; thereafter the effect increased with time. Only a toxic dose of 0.4 mg/kg caused a reduction of the acid output in the first hr.

In the present experiments on rats 2.84 μg atropine was required for a 50% inhibition of the gastric contractions induced by vagal stimulation. Propantheline was 1.27 times, and poldine 2.6 times, more active than atropine. Poldine is about twice as active *in vivo* as *in vitro*, as judged by its anti-acetylcholine effect on isolated guinea-pig ileum. Poldine has the same anti-acetylcholine activity as atropine, which confirms the results of Acred, Atkins, Bainbridge, Brown, Quinton & Turner (1957). These authors reported that the anti-acetylcholine activity of propantheline as measured on the cat blood pressure, pupil diameter and salivary flow was much less than the anti-acetylcholine activity of propantheline on the isolated guinea-pig ileum. Nevertheless, they obtained a higher activity ratio for propantheline/atropine than that found in the present experiments.

A dose of atropine, twice as large as the dose which reduced gastric contractions by 75%, had no apparent effect on the histamine-stimulated acid gastric secretion. The same result was obtained with poldine in a dose which inhibited gastric contractions by approximately 75%. In a similar experiment with propantheline (also given in a dose which inhibited gastric contractions by approximately 75%) no inhibition of acid secretion was found after the first subsequent dose of histamine; however, the response to the second dose of histamine was probably diminished ($P<0.05$). Gastric contractions were diminished within a few seconds of injecting the drug, and therefore well within the period of 3 min elapsing between the injection of the drug and the administration of histamine.

A very high dose of atropine (500 μg) caused a delayed inhibitory effect, and only the stimulating effect of the third dose of histamine, injected about 80 min after the

atropine, was significantly reduced. Delayed inhibition was also seen after propantheline. It was possible that the delayed inhibition produced by atropine and propantheline was due not to the drug itself but to some metabolite. Injection of a double dose would then be expected to cause the formation of more of this hypothetical metabolite, leading to an increased inhibitory effect. The results of the experiments with propantheline shown in Table 3 did not support this hypothesis.

The fact that a second double dose of propantheline failed to increase the delayed inhibition suggested that the drug was not exerting a direct toxic effect.

Hence we concluded that the delayed inhibition was neither a specific anti-muscarine effect nor a manifestation of a direct toxic effect of atropine or propantheline.

Since the doses of atropine (or propantheline) and histamine were injected at regular intervals, it is possible that the non-specific suppressing effect on the acid secretion arose from the combined administration of these agents. It is known that both affect the blood vessels, and, moreover, Thompson & Vane (1953) have reported that a decrease of the gastric blood flow caused a decrease of the acid gastric secretion. Nordgren & Öbrink (1957) suggested that any substance that interferes in either direction with the blood flow in the gastric mucosa would alter the normal relationship between the volume output and acidity. They thought that the action of atropine on the acidity regulation is dependent on its effect on the blood flow through the gastric mucosa.

Experiments which permit the study of the combined effects of atropine and histamine on the gastric blood flow might therefore be helpful in elucidating this non-specific action on the acid secretion.

We are grateful to Professor S. E. de Jongh, University of Leiden, for his critical comments on this paper. We wish to thank Mrs H. Jansen-Otten and Mr R. Charles for carrying out a part of the experiments described.

The poldine was kindly supplied by Beecham Laboratories through the medium of their agent in the Netherlands, Establissementen R. Barberot N.V.

REFERENCES

AARSEN, P. N. (1959). Preventie van maagzuursecretie en experimentele maagzweren door floxine. Thesis, University of Amsterdam.

ACRED, P., ATKINS, E. M., BAINBRIDGE, J. G., BROWN, D. M., QUINTON, R. M. & TURNER, D. H. (1957). Pharmacological properties of esters of 1-alkyl-2-hydroxyalkylpyrrolidine and their quaternary derivatives. *Brit. J. Pharmacol.*, **12**, 447–452.

ATKINSON, A. J. & IVY, A. C. (1938). Studies on the control of gastric secretion. I, Drugs acting on the autonomic sympathetic system. II, Drugs acting as central emetics. *Amer. J. dig. Dis.*, **4**, 811–816.

BAINBRIDGE, J. G. & BROWN, D. M. (1960). Ganglion-blocking properties of atropine-like drugs. *Brit. J. Pharmacol.*, **15**, 147–151.

CODE, C. F. (1951). The inhibition of gastric secretion: a review. *Pharmacol. Rev.*, **3**, 59–106.

EMMENS, C. W. (1948). *Principles of Biological Assay*, 1st ed. London: Chapman and Hall.

FRIEDMAN, M. (1937). The use of ranks to avoid the assumption of normality implicit in the analysis of variance. *J. Amer. stat. Ass.*, **32**, 675–701.

GHOSH, M. N. & SCHILD, H. O. (1958). Continuous recording of acid gastric secretion in the rat. *Brit. J. Pharmacol.*, **13**, 54–61.

JANOWITZ, H. D. & HOLLANDER, F. (1956). Effect of atropine on histamine-stimulated gastric secretion in the dog. *Amer. J. Physiol.*, **186**, 373–376.

LIM, R. K., MATHESON, A. R. & SCHLAPP, W. (1923). Observations on the human gastro-duodenal secretions with special reference to the action of histamine. *Quart. J. exp. Physiol.*, **13**, 361–391.

NOORDWIJK, J. VAN & AARSEN, P. N. (1954). Acid gastric secretion in the rat and its inhibition by phloxin. *Brit. J. Pharmacol.*, **9**, 253–259.

NORDGREN, B. & ÖBRINK, K. J. (1957). The effect of atropine on the acidity of a denervated gastric pouch. *Acta physiol. scand.*, **40**, 297–304.

POLLAND, W. S. (1930). The effect of atropine upon gastric secretion after histamine stimulation. *J. clin. Invest.*, **9**, 319–332.

SCHILD, H. O. (1947). *p*A, A new scale for the measurement of drug antagonism. *Brit. J. Pharmacol.*, **2**, 189–206.

THOMPSON, J. E. & VANE, J. R. (1953). Gastric secretion induced by histamine and its relationship to the rate of blood flow. *J. Physiol. (Lond.)*, **121**, 433–444.