Brit. J. Pharmacol. (1954), **9**, 329.

DOSE-RESPONSE CURVES FOR THE EFFECT OF HISTAMINE ON ACID GASTRIC SECRETION IN MAN

BY

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(RECEIVED APRIL 1, 1954)

The effect of intravenous infusions of histamine on gastric secretion has recently been studied quantitatively in the cat (Wood, 1948) and in the dog (Öbrink, 1948; Hanson, Grossman, and Ivy, 1948). These authors observed that the effect of the drug increased with the dose, and that by choosing a suitable range of doses it was possible to obtain data which could be used to construct dose-response curves.

Little is known about the effect of histamine infusions on gastric secretion in man. According to McElin and Horton (1946), the threshold dose is less than 30 ng./kg./min.; Hanson *et al.* (1948) found it to be as low as 4 ng./kg./min. Although the dose for a maximal effect has not been determined, Hanson *et al.* produced a flow of 285 ml. of juice./hr. with an infusion rate of 300 ng./kg./min. Our aim was to study the secretory behaviour of the human stomach in response to prolonged continuous intravenous infusions of histamine, and to determine the relationship between dose and response. At the same time we attempted to gain further information on the fate of histamine in the body by estimating it in samples of various body fluids, including the gastric juice. This part of the work has been reported elsewhere (Adam, Card, Riddell, Roberts, and Strong, 1954).

Our data were obtained from three healthy men designated as W.I.C., J.A.S., and A.A.G., each of whom received a series of intravenous infusions of histamine. Estimates of the rates of acid secretion were plotted against rates of infusion of the drug. Each infusion was thus a self-contained experiment and could be represented by a point on a graph. By applying statistical methods to the results it was possible to fit various parameters of the dose-response curves.

METHODS

Intubation of the Stomach and Collection of the Juice.—The subject omitted breakfast. A few drops of local anaesthetic (Amethocaine HCl) were instilled into the nose and a Ryle's tube was passed by this route into the stomach. The position of the tip was ascertained radioscopically and the tube manipulated until the tip lay on the left border of the vertebral column. The subject then lay on his left side, and the stomach contents were aspirated by syringe. This was sometimes made easier by washing the stomach out with warm water. The Ryle's tube was then connected to a source of continuous suction not exceeding 40 mm. Hg. The gastric juice flowed into a 50-ml. boiling tube, which was replaced every ten minutes by a clean tube. At slow rates of secretion the juice tended to be thick and mucinous and occasionally blocked the tube. The injection of a small quantity of warm water easily cleared the tube, and a correction was subsequently applied to the volume of the sample. Saliva was collected by expectoration. The duodenal juice was not withdrawn.

Titrations.—The volume of the sample of gastric juice was measured and the free and total acid were determined by titration against 0.1N-NaOH, using Töpfer's reagent and phenolphthalein as internal indicators. The concentration of acid was expressed in m.equiv. HCl/l. The product of the concentration of total acid and the volume of the sample in litres gave an estimate of the output of acid in m.equiv. HCl.

Infusions.—Histamine acid phosphate was supplied by British Drug Houses Ltd. All the values for histamine quoted in this paper are expressed in terms of the base, on the assumption that it represents 36.16% of the salt by weight. On the day before the experiment a solution of histamine was prepared in 0.9% NaCl from a stock solution and sterilized by heat. The final dilution in saline was made immediately before the infusion, which was given into a forearm vein from a 50-ml. syringe. The plunger of the syringe was propelled by an electric motor which ran at constant speed. The mean delivery rate from the syringe was 32.2 ± 0.3 ml./hr. A length of polythene tubing and a metal adaptor connected the syringe to the needle (15 S.W.G.) in the vein. Most of the infusions were given at weekly intervals.

Estimate of the Basal Output of Acid.—A solution of 0.9% NaCl was infused for about an hour and the total acid titrated in the juice collected during this period was taken to represent the basal output of acid, which was expressed in m.equiv. HCl/hr. This basal output was assumed to continue at the same rate during the period of the histamine infusion.

Estimate of the Response to Histamine.—At the end of an hour the basal secretion was usually steady and the saline was replaced by the histamine solution. The output of acid usually rose to a maximum within the first hour and a half, fell slightly, and thereafter remained more or less steady during the second and third hours of the infusion. The output of acid during these last two hours only, when corrected for the basal output, was taken to represent the response to histamine and this was expressed in m.equiv. HCl/hr.

Results

The effects of a series of infusions with increasing doses, taken from the data of W.I.C., are shown graphically in Fig. 1. The acid output values plotted at 20-min. intervals were obtained as the sum of two consecutive 10-min. samples. This has the effect of smoothing out variations between samples which sometimes occurred from faulty collection. Dilution by saliva would not alter the total output of acid, and the occasional regurgitation of duodenal juice appeared to produce little effect—though the extent of its interference is not known. It is certain that some saliva was swallowed, because the total concentration of chloride in some samples of gastric juice was as low as 80 m.equiv./l. Ihre (1939) removed the saliva by continuous suction and rarely obtained values for chloride in gastric juice below 120 m.equiv./l. The possible effect of the non-parietal component of the gastric juice will be discussed later.

Table I contains the results for the three subjects. The experiments are numbered according to the magnitude of the dose. Except with W.I.C., the infusions were not given in any particular order. The result of the infusion given to W.I.C. (2/1/50) three days after a previous experiment was so anomalous that it has been excluded from the calculations. The experiments on A.A.G. were begun in the summer months, but could not be completed; they were resumed in the following winter. The results for the two periods are given separately.

It is evident, in each set of results, that the response increases with the dose. When the response is plotted against log-dose, the points fall on a roughly sigmoid curve.

Fitting Theoretical Dose-Response Curves.—Unfortunately, it is not possible to infer the mechanism of the secretion process from the form of the dose-response curve. Many different models could be proposed, each leading to a curve consistent with the observed points.

One possible mechanism would be a population of secretory units each giving an all-or-none response when stimulated by histamine, but differing in threshold sensitivity to histamine according to some defined frequency distribution. If the sensitivity to log-dose follows the normal distribution, as so often happens with pharmacological responses, the well-known symmetrical sigmoid normal integral curve would be obtained. There would be three adjustable parameters—a vertical scale parameter which can conveniently be taken as H, the asymptotically maximal response at very high concentration; a location parameter which may be identified with m, the ED50 or

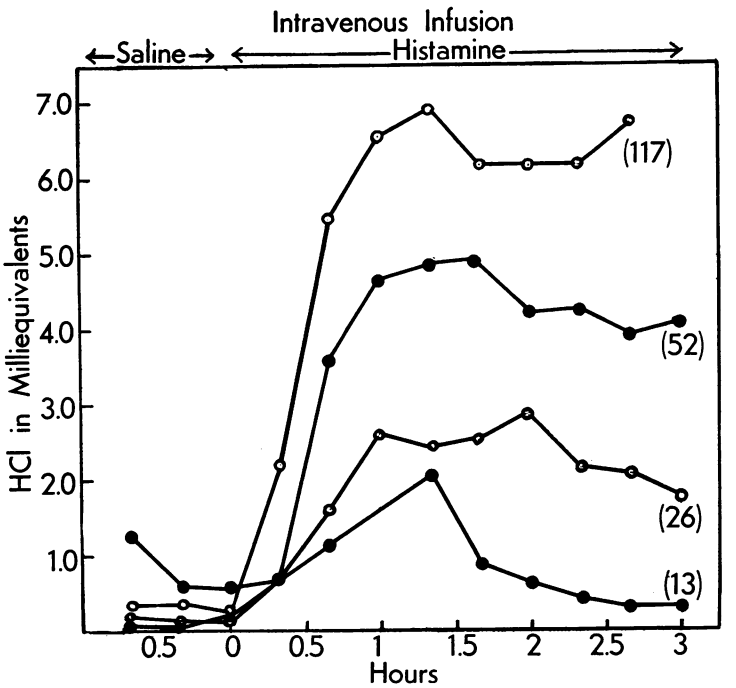

FIG. 1.—Subject W.I.C. Intravenous infusions of histamine. Ordinate: total HCl in m.equiv. present in juice formed during 20-min. intervals. Abscissa: time in hr. Brackets: dose of histamine expressed in ng./kg./min. (see Table I).

TABLE I

EFFECT OF INTRAVENOUS INFUSIONS OF HISTAMINE ON ACID GASTRIC SECRETION IN THREE HEALTHY MEN

Exp.	Date	Dose ng./kg./min.	Estimate of Total Acid Output m.equiv. HCl				Response m.equiv. HCl/hr. ½(2nd + 3rd hr.)— Basal Value
			Basal m.equiv./hr.	During Infusion 1st hr.	2nd hr.	3rd hr.	
	Subject W.I.C. ♂. Height 74 in. Weight 82 kg.						
1	26/6/50	0	1·04	0·98	0·28	0·66	
2	7/11/49	13·0	0·77	3·35	3·89	1·01	1·78
3	4/7/50	13·7	1·14	1·03	1·37	2·17	0·63
4	12/11/49	26·1	1·93	6·01	7·29	5·89	4·66
5	19/11/49	29·1	3·07	8·31	9·89	9·15	6·45
6	3/12/49	52·2	0·36	8·79	13·38	12·23	12·74
7	31/12/49	63·0	1·09	11·44	15·87	14·87	14·28
8	2/1/50	88·0	0·76	5·41	13·53	12·90	12·45
9	10/12/49	117·0	0·86	14·19	19·25	18·60	18·06
	Subject J.A.S. ♂. Height 74 in. Weight 75 kg.						
1	20/3/50	0	0·47	0·56	1·39	0·31	
2	27/3/50	3·6	0·81	1·20	1·76	1·84	0·99
3	13/3/50	8·0	0·60	0·58	2·06	3·99	2·42
4	17/8/50	12·1	0·36	4·22	9·11	5·32	6·85
5	6/3/50	16·0	0·36	3·33	7·54	9·79	8·30
6	12/8/50	21·3	0·56	10·47	17·32	22·32	19·26
7	17/4/50	28·9	1·38	8·68	22·67	24·16	22·03
8	27/2/50	48·0	0·35	15·85	29·85	26·78	27·96
9	24/4/50	72·0	0·72	19·55	31·90	35·73	32·09
10	5/8/50	114·0	0·61	19·24	35·67	35·62	35·03
	Subject A.A.G. ♂. Height 69 in. Weight 73 kg.						
	(Summer)						
1	5/6/50	0	2·22	2·34	3·98	1·01	0·27
2	15/5/50	7·4	2·74	3·49	5·40	5·27	2·60
3	8/5/50	14·9	3·98	11·89	9·53	12·90	7·23
4	29/5/50	29·6	5·90	16·80	15·03	14·93	9·08
5	12/6/50	59·2	3·09	13·87	15·58	18·66	14·03
	(Winter)						
1	3/2/51	7·6	0·30	1·20	0·71	6·64	3·37
2	27/1/51	12·5	3·38	10·09	14·39	10·63	9·13
3	20/1/51	23·5	4·16	13·57	20·21	19·14	14·51
4	13/1/51	47·0	3·10	19·36	20·66	20·05	10·93
5	6/1/51	94·0	1·65	15·21	26·57	25·77	24·57

dose eliciting half the maximal response; and a horizontal scale parameter which may be taken as b, the reciprocal of the standard deviation of the tolerance distribution in log-dose units.

An alternative model would be a population of identical secretory units, S, which are active only when forming an addition compound with a definite number, *a*, of molecules of the drug D, according to the reversible chemical equation

$$S + aD \rightleftharpoons SD_a,$$

where only the compound on the right is able to effect secretion. Intermediate compounds with fewer than *a* molecules of the drug are assumed to be unstable. If the concentration of this complex is R and the concentration of the drug is C, it follows from the law of mass action that

$$R = \frac{HC^a}{K^a + C^a}$$

where R is proportional to the response, H is the asymptotic maximum approached by R as C becomes very large, *a* is the number of histamine molecules uniting with one secreting unit to give the active complex, and K is the value of D at which R is $\frac{1}{2}H$. If R is plotted against log C, the formula gives the symmetrical sigmoid curve known as the logistic, which is almost identical in form with the normal integral curve, and is also fully specified by three parameters. Two of these, H and m, have exactly the same meaning for the two curves; for the logistic, $m = \log K$. The scale parameter of the logistic, $B = a \log_e 10$, is analogous to, but will differ in magnitude from, the horizontal scale parameter of the normal curve, and will supply an estimate of *a*, on the assumption that the addition compound model is true.

Formulae of both types were fitted to each of the four series of observations. The fitting was performed by the method of maximum likelihood, which here consists in minimizing the sum of squares of residual deviations of the observed values. The computational methods used, which were simpler than those described by Finney (1947), will be published elsewhere. Table II gives the maximum likelihood estimates of the various parameters with their standard errors, or fiducial limits when these are more appropriate indicators of precision. The table also gives the standard error of estimate, this being the standard deviation of the residuals around the curve. This is a good measurement of closeness of fit.

From the values of the parameters of the probability integral curve it was possible to derive the equations for the regression of the response in probits (Y) on log-dose (X).

W.I.C. $Y = 3.41X - 0.452$
J.A.S. $Y = 3.19X + 0.687$
A.A.G. $Y = 2.2X + 1.909$
$Y = 1.92X + 2.396$

TABLE II

ESTIMATES OF PARAMETERS OF DOSE-RESPONSE CURVES

Subject	Probability Integral Curve				Logistic Curve			
	H±S.E.	ED50	Fiducial Limits	b±S.E.	H±S.E.	ED50	Fiducial Limits	a±S.E.
W.I.C.	19·1±1·18	39·7	26·7–59·3	3·41±0·39	19·3±0·85	40·1	29·0–53·4	2·44±0·24
J.A.S.	34·6±2·54	22·5	8·9–57·1	3·19±0·62	34·9±2·40	22·5	9·3–54·6	2·30±0·45
A.A.G. (S)	17·4±11·1	(25·4)	0– ∞	2·2 ±2·7	17·4±9·3	(20·4)	0– ∞	(1·5 ±1·8)
A.A.G.(W)	28.4±4·1	(22·7)	0– ∞	1·92±0·51	26·8±6·2	(22·9)	0– ∞	(1·35±2·75)

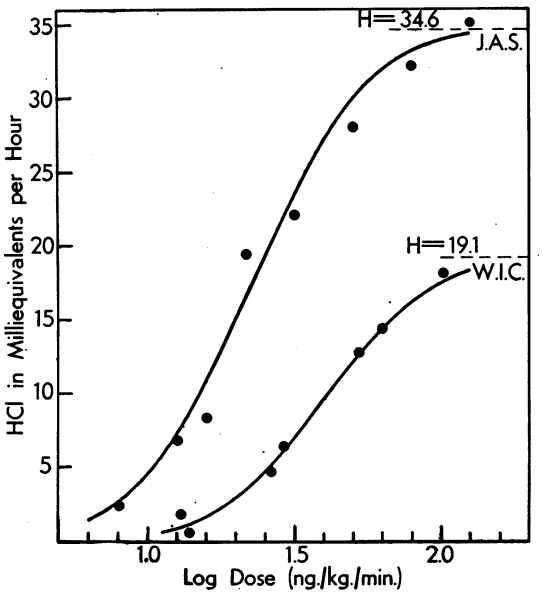

FIG. 2.—Dose-response curves for subjects J.A.S. and W.I.C. Ordinate: rate of secretion of HCl in m.equiv./hr. Abscissa: log dose in ng./kg./min. H is calculated from the data and is the estimate of the secretory maximum.

Values for Y were then calculated for a range of values of X. The probits were converted into percentages from probit tables and plotted as a percentage of H, the maximum value, on a linear-log scale.

A similar manipulation was carried out on the parameters of the logistic functions to derive the equations for the regression of the response in logits (Y) on log-dose (X).

W.I.C. $Y=5.62X-8.99$
J.A.S. $Y=5.29X-7.16$
A.A.G. $Y=3.45X-4.52$
$Y=3.11X-4.22$

Values for Y were then calculated for a range of values of X. The logits were converted into percentages from logit tables and plotted as percentages of H, the maximum value, on a linear-log scale. The probability integral curves for two subjects (W.I.C. and J.A.S.) are shown in Fig. 2. The superimposed curves of the probability integral curve and the logistic are almost identical.

Estimate of the Parietal Secretion.—Since the estimate of the response to histamine is the output of total acid from the stomach, it may be asked how far the observed values represent the response of the parietal cells alone. There is evidence for the view that gastric juice consists of a mixture of parietal and non-parietal secretions. These secretions have not yet been obtained pure, but their probable composition has been calculated from various data (Hollander, 1938; Gray and Bucher, 1941). More recently, these earlier results have received support from the careful analysis by Fisher and Hunt (1950) of Ihre's data on human gastric secretion. These authors concluded that the most probable values for the composition of parietal secretion were 160 m.equiv./l. hydrion and 10 m.equiv./l. neutral chloride, and that the non-parietal secretion of iso-osmotic strength contained 125 m.equiv./l. neutral chloride and 45 m.equiv./l. bicarbonate. Hence, if the volume and acidity of a sample of gastric juice are known, an estimate of the volume of the parietal secretion can be calculated on the basis of these figures:

Let V be the volume of juice in litres,
and x be the volume of parietal juice in litres,
and y be the volume of non-parietal juice in litres.
Then $V=x+y$ .....(1)
If A is the amount of acid in the juice in m.equiv., then
$A=160x-45y$ .....(2)

Estimates for the volume of parietal and non-parietal secretion have been calculated in this way from the data of subject J.A.S. (Table I) and have been fitted to the logistic function as already described (Fig. 3). It will be seen that the values for the parietal secretion fit the curve reasonably well, but the values for the non-parietal secretion ($\times 5$)

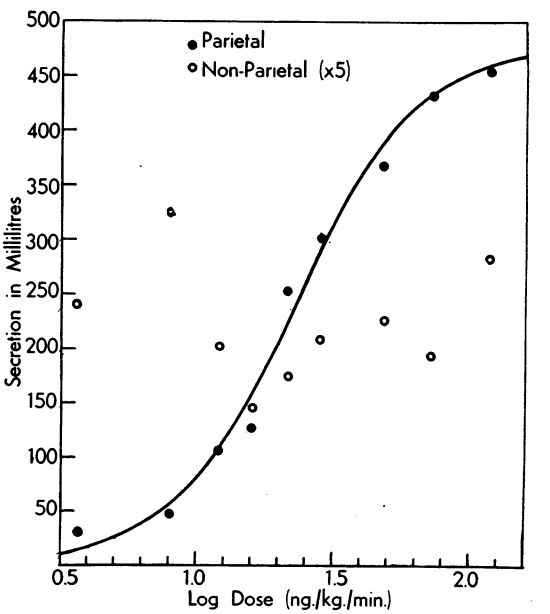

FIG. 3.—Subject J.A.S. Calculated volume of parietal and non-parietal secretion (ml.) in response to log dose in ng./kg./min. The values for the non-parietal secretion have been multiplied by 5 to bring them on to the scale of the ordinate.

bear no relation to the dose of histamine infused. All our values for the response could have been expressed in terms of the volumes of parietal juice, but, with our present data, this procedure seemed to offer no practical advantage.

Discussion

The secretory response to histamine was evident within a few minutes of starting the infusion, but did not reach a maximal value until after 60 to 80 minutes. This delay, which appeared to be independent of the rate of the infusion, may have been due to a slow cumulation of free histamine in the tissues. Teorell (1933, 1937a, b) was the first to consider how the concentration of free histamine in the blood and tissues might vary with the rate of infusion. He assumed that the speed of cumulation depended upon the difference between the rate of entry of the drug and the rate of its disappearance from the blood. When the rates became equal cumulation ceased; the concentration then remained steady and was proportional to the rate of infusion. Öbrink (1948) has since obtained evidence in support of this hypothesis. Our curves for single infusions show that, after the output of acid has reached a maximum, it tends to fall slightly during the second hour and then continues more or less steadily. These changes in acid output may mean that the plasma histamine falls slightly, or that the parietal cells become less sensitive during the course of the infusion.

In man the parietal cells of the stomach appear to be more sensitive to histamine than any other cells in the body (Weiss, Robb, and Ellis, 1932). The mean threshold infusion rate was 10 ng./kg./min. (range (3) 3.6–13.0), which confirms the values obtained by Hanson *et al.* (1948). When the rate of infusion was less than 30 ng./kg./min. the only detectable clinical effect appeared to be stimulation of gastric secretion. This suggests that histamine could, under physiological conditions, pass continuously into the plasma and attain a concentration which would stimulate secretion without necessarily producing overt symptoms such as headache and flushing (Kahlson, 1948). The following argument supports this view. Healthy men (Roberts and Adam, 1950) and children and infants (Adam and Mitchell, 1953) excrete free histamine in the urine. When histamine is given by slow intravenous infusion the effect of the infusion on the urinary excretion of free histamine and on the output of gastric HCl is roughly proportional to the dose (Adam, Card, Riddell, Roberts, and Strong, 1954). This may be taken as indirect evidence that free histamine in the urine derives from the plasma and not from the renal tract. The proportion that appeared in the urine as free histamine was about 1% of the dose, which suggests that the normal excretion for adults (approx. 20 μg./24 hr.) represents a similar proportion of histamine that may be continuously passing into the blood stream from various sources. Calculation shows that this quantity would be about 2,000 μg./24 hr., or, roughly, 20 ng./kg./min. for a 70-kg. man, which is within the range of infusion rates used. This suggests the conclusion that free histamine in the circulating blood may help to maintain the basal acid secretion. Although direct evidence for this hypothesis is lacking, it seems that human blood plasma contains a minute quantity of histamine in the free and active form. By ion exchange chromatography it is possible to separate a fraction with pharmacological properties similar to those of histamine. The histamine equivalent of this fraction has varied from <1 to 2 ng./ml. plasma (Adam, Hardwick, and Spencer, unpublished).

Dose-Response Curves.—The remarkable consistency of the response, from experiments conducted over some months, made it possible to derive curves from the data. The mathematical treatment of the data has already been discussed. Since the data could be fitted equally well to functions representing two fundamentally different theories of the mode of action of histamine, it was not possible to distinguish between these two theories. Nevertheless, the dose-response curve may have a practical value, since it provides a reliable method of comparing the secretory responses of different stomachs or of the same stomach after various treatments.

The estimate of the secretory maximum (H) or the height parameter of the curve can, on either theory of secretion, be more easily interpreted than the values for the other parameters. The secretory maximum implies that all the acid-secreting cells of the stomach have been stimulated under the conditions of the experiment, and that each cell has responded maximally. The maximum, however, might depend not only on the mass of secretory cells but also on the rate at which each cell produces acid. In this sense the value for the maximum could vary under conditions which enhance or depress the rate of acid formation, but which do not alter the number of cells in action. The finding of Guiss and Stewart (1948), that there is a significant correlation between parietal cell counts in resected stomachs and the highest concentration of acid reached in a previous test meal, lends support to the conception that the secretory maximum may bear a definite relation to the mass of secretory

tissue thrown into activity. In the present experiments the value for J.A.S. (34.6 ± 2.5 m.equiv./HCl/hr.) was significantly greater than that for W.I.C. (19.1 ± 1.2 m.equiv./HCl/hr.). In both these subjects the rate of disappearance of the dose by excretion as free histamine in the urine was approximately the same (Adam *et al.*, 1954). This suggests that the concentration of free histamine in the plasma may have been about the same in the two subjects and that the higher result for J.A.S. was therefore due to a difference in the mass of secretory tissue. This has been referred to elsewhere as "secretory cell mass" (Card, 1952), though the term "secretory unit mass" might be preferable. The estimate of H for A.A.G. is different in summer and winter, but the difference is not statistically significant.

The ED50, or the estimate of the location parameter, is difficult to interpret because it may depend not only on the sensitivity of the cells to histamine but also on the rate of disappearance of histamine from the blood. Thus a low value could mean that the parietal cells were unusually sensitive or that the histamine disappeared at a slow rate; and, conversely, that at a high value the cells were relatively insensitive or the rate of disappearance was rapid. It is not possible on the present evidence to say which of these views is correct. Until dose can be related to plasma histamine concentration the meaning of the ED50 must remain uncertain.

The estimates of the horizontal scale parameter are also difficult to interpret. On the first theory the estimate of b might be taken as a measure of the homogeneity of the parietal cell population. The fact that values for b in the three subjects did not differ significantly would suggest that the sensitivities of the parietal cells were similarly distributed in each subject. On the second theory the estimate of the parameter, a, might be taken as the number of receptors of the secretory unit which can be occupied by histamine. If this number were constant throughout all secretory units, the estimate of this parameter would be a whole number. The data in Table II are consistent with, but do not prove, the supposition that $a=2$ in all subjects.

Since the validity of either theory remains unproved, further discussion of the location and horizontal scale parameters serves no useful purpose. Nevertheless, it would seem likely that the height parameter, H, is the chief, and perhaps the only, parameter necessary to define the gastric secretory output in response to histamine.

Summary

1. The effect of a series of intravenous infusions of histamine on the acid gastric secretion has been investigated in three healthy men. The threshold rates of infusion were 4, 9, and 13 ng./kg./min. Gastric secretion appeared to be the only clinical effect at these rates.

2. From the data it was possible to relate dose to response and to calculate curves representing theories of the mode of action of histamine on gastric secretion.

3. The curves were defined by three parameters; estimates of two of these were taken to represent the secretory maximum and the ED50 respectively.

4. It is suggested that the secretory maximum is the most important parameter for defining the secretory response to histamine.

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