Gut, 1964, **5**, 327

Studies on gastrin

K. S. LAI[1]

From the Department of Pharmacology, University of Edinburgh Medical School, and the Gastrointestinal Unit and the Teaching and Research Centre, Western General Hospital, Edinburgh

EDITORIAL SYNOPSIS These three papers present studies on gastrin. The first paper describes a method of biological assay using the rat. The second paper demonstrates that the highest concentration of gastrin-like activity occurs in the antral mucosa, with a clear gradient of concentration of activity down the gut. However, it is to be noted that the total amount of extractable activity is greatest in the duodenum, although the concentration there is less than in the antrum. No activity was detected in the pancreas. The third paper studies the contents of gastrin-like activity in patients with duodenal ulcer and demonstrates higher figures when stenosis is present. Patients with benign gastric ulcer and carcinomata showed results equal to or greater than in those with the average uncomplicated duodenal ulcer. It was noted that two patients with dilated antra both had very low total gastrin-like activity.

There was no correlation between total activity and maximal histamine-stimulated output of acid. There was, however, a positive correlation between the insulin-stimulated acid secretion and the total gastrin-like activity in the cases of uncomplicated duodenal ulcers.

The clinical studies are still tentative in view of the several variables present, but it seems likely that they will in due course clarify the role of gastrin in the ulcer problem.

Part I A method of biological assay of gastrin[2]

Various methods have been devised for assessing the biological activity of gastrin extracts, employing anaesthetized cats (Edkins, 1906; Komarov, 1942; Munch-Petersen, Rönnow, and Uvnäs, 1944) and conscious dogs (Keeton and Koch, 1915; Komarov, 1942; Gregory and Tracy, 1961, and others). These methods are at best semi-quantitative, since no accurate means have been provided for assessing variation in responses in different animals. Jalling and Jorpes (1947) first studied dose-response relationships in the assay of gastrin extracts and suggested using groups of animals to overcome individual variations in responses. These concepts were incorporated in Ferguson's (1950) study on gastrin activity in human gastric mucosal extracts. In 1961, Uvnäs and Emäs published a method of bioassay of gastrin with statistical control, using histamine as the reference standard. Doses of either substance were given intravenously over 15 minutes, and the responses were estimated as the total acid (in mEq.) secreted in one hour from the start of the gastrin infusion. The principle of the assay method lay in bracketing two identical doses of gastrin with two graded doses of histamine; and certain strictly defined criteria were to be satisfied before any assay was considered valid. The method fulfilled most of the essential requirements of modern bioassay techniques, but the fact that histamine and gastrin are different substances which probably act differently on the parietal cell would nullify the validity of the comparison (Gaddum, 1959).

Harper, Blair, and Reed (1962) independently devised a method for gastrin assay using the anaesthetized cat. A continuous basal acid gastric secretion was induced by injecting 0·5-1 mg. of a 'standard' gastrin every 15 minutes. The unknown to be assayed was given to replace one of the standard doses, and the HCl output in the subsequent 30 minutes was compared with the expected amount,

[1]In receipt of a scholarship awarded by the Commonwealth Scholarship Commission in the United Kingdom under the Commonwealth Scholarship and Fellowship Plan. Present address: University Department of Medicine, Queen Mary Hospital, Hong Kong.
[2]This paper contains part of the material from a Ph.D. thesis to the University of Edinburgh, October 1962.

had there been no substitution. A second gastrin preparation was assayed on 27 occasions in seven cats and its activity found to be 75% (S.E. ±1·97%) of the standard. For cross reference, the arbitrary standard was assayed against histamine as the unknown, given under specified conditions. Since pure gastrin was not available then, the use of an arbitrary standard with a composition similar to that of the test preparation must be acceptable on an empirical basis. It is obvious that eventually all arbitrary standards must be assayed against the purified hormone as the final reference standard.

Ghosh (1956) and Ghosh and Schild (1958) developed a method of assaying gastric secretory stimulants and depressants which depended on perfusion of the cavity of the rat stomach and measurement of the *p*H of the perfusate. The method was not designed specifically for assaying gastrin, but has been modified and used for that purpose in the present work.

THE PERFUSED RAT STOMACH PREPARATION

This is based mainly on the work of Ghosh (1956) and Ghosh and Schild (1958) with modifications. Male albino rats of the Wistar strain were used in all the experiments. Their body weights ranged from 180 g. to 320 g. (mean 252 g.). Sugar cubes were given in place of the usual rat food for 48 hours before the animals were used. This avoided completely starving the rats while still providing a reasonably clean stomach at the time of the experiment.

ANAESTHESIA

General anaesthesia was induced by giving urethane in 25% solution (w/v), the usual dose being 0·7 ml./100 g. body weight of the rat. Rats varied in their sensitivity towards urethane. In order to achieve a similar level of anaesthesia for all rats, the following scheme was used. Half the calculated dose was given intraperitoneally; after five minutes the animal was usually drowsy, with only corneal and pain withdrawal reflexes present. This indicated that the dose was correct, and the remainder was then injected subcutaneously in two sites. If spontaneous movement of limbs, blinking, etc., persisted five minutes after the intraperitoneal injection, the dose was considered too small and 0·2 ml. was added subcutaneously. If pain and corneal reflexes were absent after the first injection, 0·2 ml. was subtracted from the remainder of the dose. The rats were usually fully under anaesthesia in about 45 minutes. By then the respiration was slow (about 60/minute) and mainly or wholly abdominal. Thoracic type of respiration was a bad sign and invariably meant a dying animal.

OPERATIVE PROCEDURE

The rat was placed on a rat operating stand and its body temperature was kept at 34 ±0·5° C. by a thermostatically controlled heating system (Ghosh and Schild, 1958). A tracheotomy was performed and the respiratory passages kept clear. The abdomen was then opened by a transverse incision, which provided a better exposure than a midline longitudinal incision. The duodenum was brought to the surface. Three ligatures were passed round the pyloro-duodenal junction as close to the gut wall as possible to avoid inclusion of any blood vessel. The first ligature served for traction. A polythene cannula was inserted via a duodenostomy through the pylorus into the stomach and doubly ligated in position.

A soft rubber urethral catheter (external diameter 2 mm.) was passed down the oesophagus until the tip just lay in the ruminal portion of the stomach. The approximate length required was equivalent to that between the teeth and the xiphoid process. A ligature was then placed round the oesophagus at the neck. The stomach was next mobilized from its bed and delivered through the abdominal wound, as far as the ligaments would allow without undue stretching. This was achieved mainly by gentle traction on the relatively avascular ruminal portion. Handling of the glandular portion was avoided, for such invariably resulted in trauma to the secreting mucosa with bleeding. The stomach was washed by a slow stream of tap water introduced via the oesophageal tube from a reservoir held 50 cm. above the rat. This was continued until the effluent was clear and all parts of the stomach, especially the ruminal portion, were empty and collapsed. The organ was returned into the abdomen and a moistened pad of cotton wool placed against the ruminal portion to keep it collapsed during the experiment.

The cavity of the stomach was perfused with 0·9% saline warmed to the body temperature of the rat and introduced via the oesophageal tube at a constant rate of 0·7 ml./minute (Fig.1). The gastric effluent was collected in 10-minute samples, which measured 7 ± 0·5 ml. in the majority of cases.

A fine polythene cannula was inserted into the right femoral vein and ligated in position, and a slow continuous basal infusion of 0·9% saline at 2 ml./hour was started using an electrically driven slow injection apparatus.

TITRATION

The 10-minute samples of gastric perfusate were titrated against N/100 NaOH with phenolphthalein as indicator. The NaOH solution was stored in a polythene reservoir connected directly to a 5 ml. microburette, the tip of which was narrowed by a polythene tube drawn out at one end so that the size of the drop was about 0·015 ml. The whole system was protected from atmospheric carbon dioxide by soda lime.

THE METHOD OF BIOLOGICAL ASSAY

THE BASAL SECRETION The anaesthetized rat produced a continuous basal acid gastric secretion measuring 1-2·5 μEq./10 minutes. It was usually constant but occasionally varied slightly (± 1 μEq./10 minutes) at intervals between doses of gastrin extracts.

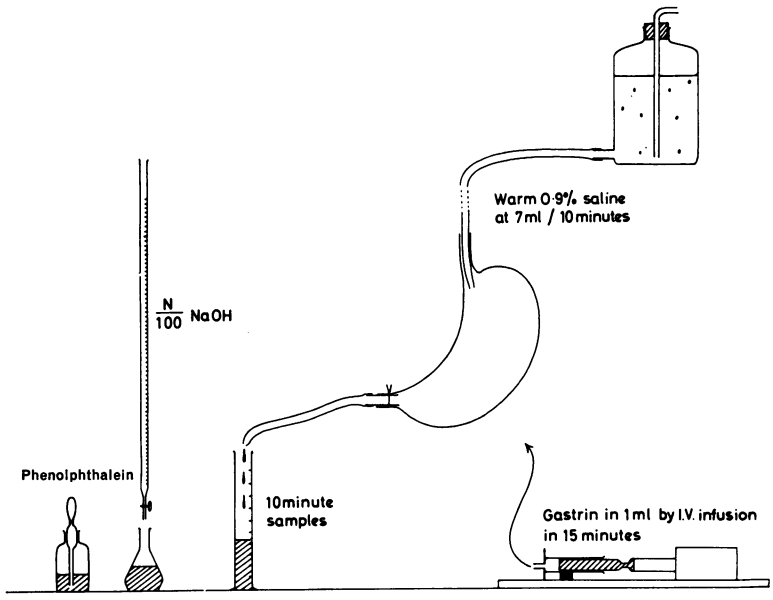

FIG. 1. *Simplified diagram of the rat preparation.*

MEASUREMENT OF EFFECT All doses of gastrin extracts were contained in 1 ml. of normal saline at *p*H 7 and injected intravenously over a period of 15 minutes. Studies on the patterns of response with repeated identical doses revealed that the best criterion for assessment of response under the conditions of the experiment was the 'mean rate of acid secretion', expressed in μEq./10 minutes and defined as:

$$\frac{\text{Total acid output} - \text{estimated basal acid output (in } \mu\text{Eq.)}}{\text{Duration of response (in periods of 10 minutes)}}$$

where total acid output = acid output from baseline (*i.e.*, resting rate of secretion) to baseline.

Estimated basal acid output = basal rate of acid secretion × duration of response

Basal rate of acid secretion = $\frac{1}{2}$ (mean rate of secretion before the dose + mean rate of secretion after the dose)

Each mean rate is based on three consecutive similar readings differing by less than 0·2 μEq./10 minutes.

Duration of response = period between start of response to a dose and the next baseline reading

An example of the calculation is given in Figure 2.

This method of calculation takes into consideration minor changes in the baseline. For the purpose of the assay, however, a 'resting' secretion which persisted at twice the original basal level (or higher) for more than 60 minutes was taken as evidence of accumulation of the injected material, and the assay of subsequent doses was therefore considered inaccurate.

DOSE-RESPONSE RELATIONSHIP

SENSITIVITY Rats varied in their sensitivity to gastrin. Differences of up to eight-fold have been noticed, though most of them fell within the range of four-fold.

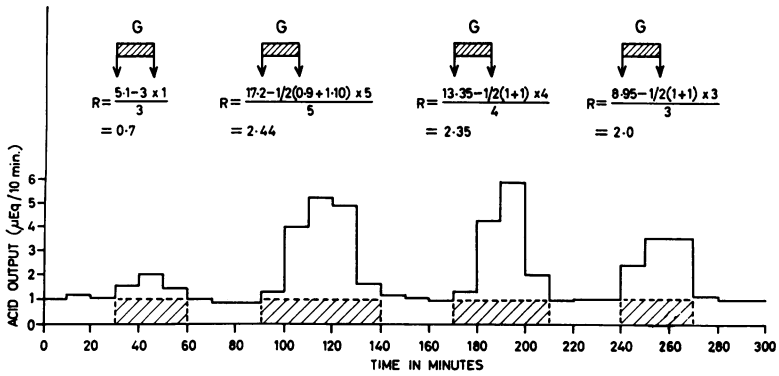

FIG. 2. *The measurement of effect. Male rat, 280 g.*
G = 'crude gastrin' (stage 1 material) 0·5 mg./100 g. body weight in 1 ml. given by intravenous infusion over 15 minutes.
R = estimated 'mean rate of acid secretion'.
The response to the first dose was disregarded.

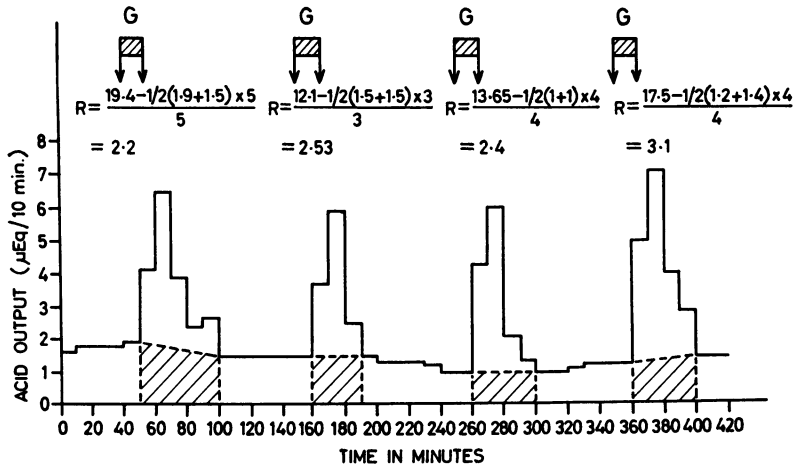

FIG. 3. *Reproducibility of effect with identical doses.*
Male rat, 260 g.
G = 'crude gastrin' (stage I material) 0·5 mg./100 g. infusion over 15 minutes.
R = estimated 'mean rate of acid secretion'.
The response to the first dose has been omitted. Responses to the second, third, and fourth doses were comparable, but that to the fifth was increased.

RESPONSE TO REPEATED IDENTICAL DOSES Equal doses (0·5 mg./100 g. body weight) of 'crude gastrin' (stage 1, Gregory and Tracy, 1961) were given to the same rat. Response to the first dose was irregular and usually smaller than the rest. This was true for histamine (Ghosh, 1956). When subsequent doses were given in rapid succession, *i.e.*, a second dose given as soon as the response to the previous one ceased, increasing rates of secretion were invariably observed, commencing with the third or fourth dose. Prolonged waiting in between doses delayed but did not prevent this tendency of increasing responses, which eventually supervened with the fourth or fifth dose in any one animal.

To overcome this interference of one dose with another, the following rules were observed:—

The effect of the first dose was disregarded, though it was useful in giving a rough guide to the sensitivity of the rat.

After each dose, an interval was allowed which was equal to the period of response to that dose.

The size of the dose was limited so that the peak acid output/10 minutes with any dose did not exceed 10 μEq.

With these precautions, the estimated 'mean rates of acid secretion' for the second, third, and fourth, and occasionally the fifth dose were closely similar (Fig. 3). In the circumstances of the experiment then,

TABLE I

REPEATED IDENTICAL DOSES OF CRUDE GASTRIN (STAGE I MATERIAL)

	Dose Order			
Rat No.	*Second*	*Third*	*Fourth*	*Block Total*
1	0·65	1·04	1·68	3·37
2	0·85	1·50	1·20	3·55
3	1·23	1·60	1·80	4·63
4	1·20	0·60	1·00	2·80
5	2·20	2·53	2·40	7·13
6	1·64	1·42	1·42	4·48
Column total	7·77	8·69	9·50	5.96
Mean	1·295	1·45	1·58	

Each dose = 0·5 mg./100 g. body weight of rat

TABLE OF ANALYSIS OF VARIANCE

Source of Variance	*d.f.*	*Sum of Squares*	*Variance*	*Variance Ratio*	*P*
Between columns	2	0·249	0·125	1·23	>0·2
Between blocks	5	3·941	0·788	8·34	0·01-0·001
Error	10	0·944	0·094		
Total	17	5·134			

For differences between the mean responses to the second and fourth doses:

$$t = \frac{\bar{X}^1 - \bar{X}_2}{S\bar{X}^1 - \bar{X}_2} = 0{\cdot}051 \text{ d. f. } = 10 \quad P > 0{\cdot}5$$

the number of doses to be assayed in each animal had to be limited to three.

The results of experiments on six rats are shown in Table I. There is no significant difference between the means of the 'mean rates of acid secretion' of the three doses. The response to the first dose in each rat has been omitted.

RESPONSES TO GRADED DOSES Experiments were carried out with 'purified gastrin' (stage III material by the method of Gregory and Tracy, 1961, freeze-dried). Doses were given at three levels, A, B, and C, graded on a logarithmic scale so that C was double B and four times A. Any error arising from bias in dose order was controlled by giving them in random order to a group of six rats so that all possible dose sequences were encountered within the group. The same rules as those for studying responses to identical doses were observed. The results are represented graphically in Fig. 4, and analysed in Table II. The dose-response relationship within the tested range was approximately linear in all six rats, and the group analysed as a whole showed highly significant regression and little evidence of deviation from linearity. It also confirmed considerable variations in responses between animals.

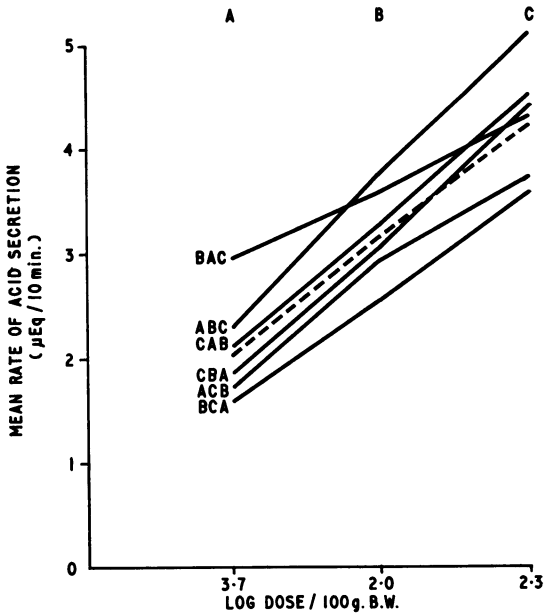

FIG. 4. *Response to graded doses.*
Dose A = 5·5 μg./100 g. body weight of rat.
Dose B = 11·0 μg./100 g. body weight of rat.
Dose C = 22·0 μg./100 g. body weight of rat.
Dotted line = mean of the responses

THE BALANCED INCOMPLETE BLOCK DESIGN FOR BIOASSAY

In the design of a method of biological assay, it is desirable to arrive at one which, on analysis of the data, would provide information concerning (*a*) the significance of linear regression and of deviation from parallelism of the dose-response curves of the standard and the test preparation; (*b*) variability due to differences between blocks (rats) and between different positions in each block (order of administration of treatments), and the consequent effect on the potency estimate; and (*c*) the error of the assay, and hence the fiducial limits of the estimate.

The simplest method answering to these requirements is the four-point assay with construction of

TABLE II

DOSE-RESPONSE RELATIONSHIP WITH GRADED DOSES OF PURIFIED GASTRIN (BATCH B, FREEZE-DRIED)

Rat No. and Dose Order	*A*	*B*	*C*	*Block Total*
1 A, C, B	1·70	2·90	3·74	8·34
2 B, A, C	2·93	3·57	4·36	10·86
3 C, A, B	2·10	3·30	4·51	9·91
4 B, C, A	1·60	2·56	3·63	7·79
5 C, B, A	1·85	3·10	4·43	9·38
6 A, B, C	2·30	3·68	5·11	11·09
Column total	12·48	19·11	25·78	57·37
Mean	2·08	3·185	4·3	

TABLE OF ANALYSIS OF VARIANCE

Source of Variance	*d.f.*	*Sum of Squares*	*Variance*	*Variance Ratio*	*P*
Regression	1	14·7408	14·7408	233·2	<0·001
Deviation from regression	1	0·0001	0·0001	0·0016	N.S.
Between columns	2	14·7409	7·37	116	<0·001
Between blocks	5	2·936	0·587	92·8	<0·001
Error	10	0·632	0·0632		
Total	17	18·3086			

4 × 4 Latin square designs. However, the strict limitation of three doses to each rat excluded this possibility. The difficulty was overcome by applying the balanced incomplete block design of Youden (1937, 1940). The statistics of the design and its application to this problem have been worked out by Colquhoun (1963).

There are many possible balanced incomplete block designs, but the pattern employed in this assay method had 12 treatments (doses) in each block, *i.e.*, group of four rats. An example is shown in Table III. Each of the four rats received three treatments, which were so distributed that each column, *i.e.*, the four first, second, or third doses in the four rats (see Table IIIa), was formed by a complete replicate and that no treatment was repeated in any rat. Table IIIb shows the same

TABLE III

AN EXAMPLE OF A BALANCED INCOMPLETE BLOCK DESIGN

(a)

Rat No.	*Dose Order*		
	First	*Second*	*Third*
1	HS	LS	HT
2	HT	LT	LS
3	LS	HS	LT
4	LT	HT	HS

(b)

Rat No.	*Dose*			
	HS	*LS*	*HT*	*LT*
1	x	x	x	
2		x	x	x
3	x	x		x
4	x		x	x

HS = High dose of the standard
LS = Low dose of the standard
HT = High dose of the test preparation (unknown)
LT = Low dose of the test preparation

block in which the doses have been re-arranged according to their size. It can be seen again that each block consists of three complete replicates of two graded doses, *i.e.*, a 'high' and a 'low' dose, each of the standard and the test preparation; the 'high' doses were double the 'low' ones, and their sizes were adjusted so that the magnitude of the corresponding responses was similar. Statistical analysis of results obtained gave all the necessary information as listed at the beginning of this section. The smallest 'balanced' block comprised four rats, but greater accuracy of estimation could be obtained with more blocks and pooling of the results.

FIDUCIAL LIMITS OF THE ESTIMATE

In order to determine the error of the assay method, the same 'purified gastrin' (stage III material by the method of Gregory and Tracy (1961), freeze-dried) in different known concentrations was used as both standard and test preparation and assayed against itself. Three blocks of four rats each were used, and the results analysed in single blocks, in combination of two blocks and all three blocks together. All the fiducial limits of the estimates included the true potency. For single blocks of four rats, the fiducial limit ranged from about ± 15% to about ± 20%. When two blocks were combined, it was about ± 10% to about ± 20%. With 12 rats (three blocks), it was down to ± 12%. The 'mean rates of acid secretion' for each block are shown in Table IV. For the derivations of the equations, the reader is referred to Colquhoun's work (Colquhoun, 1963).

DISCUSSION

The pattern of response limited the assessment of response to the only suitable measurement of 'mean rate of acid secretion'. A number of others, including the peak secretory rate and the total acid output to a dose with or without a specified time limit, have been found unsatisfactory.

The study of dose-response relationships using the purified preparation of gastrin has led to the conclusion that the freeze-dried stage III product by the method of Gregory and Tracy (1961) would form a suitable arbitrary standard for the assay of gastrin activity in the rat preparation.

The balanced incomplete block design of Youden (1937, 1940) has hitherto been largely applied to agricultural problems, but its use in the present assay has been demanded by the restriction of the number of treatments in each block. The design of the block and the statistical analysis of data, however, yielded more information than would have been obtainable from other designs with the same amount of data. The fiducial limit of the assay improved with more blocks, so that the fiducial range of 12 rats was about half of that with four rats. When balancing economy of time with gain in accuracy of the assay, however, it was decided to use single blocks of four rats for each assay.

SUMMARY

A method of biological assay of gastrin using the anaesthetized rat is described. The 'mean rates of acid secretion' in response to graded intravenous doses of gastrin extracts were estimated and the effects of test preparations thus compared with those of an arbitrary standard according to the pattern of a balanced incomplete block design. The results were analysed statistically. The fiducial limits of

TABLE IV

RESULTS OF THREE BALANCED INCOMPLETE BLOCKS IN ASSAY OF PURIFIED GASTRIN (BATCH B, FREEZE-DRIED)

	Dose Order			
Rat No.	*First*	*Second*	*Third*	*Block Total*
Block No. 1				
1	LS = 2·53	HS = 4·11	LT = 2·17	8·81
2	LT = 1·96	HT = 3·54	LS = 3·15	8·65
3	HS = 4·79	LT = 2·30	HT = 4·24	11·33
4	HT = 3.67	LS = 3·14	HS = 4·67	11·48
Column total	12·95	13·09	14·23	40·27

	Dose Order			
Rat No.	*First*	*Second*	*Third*	*Block Total*
Block No. 2				
5	HS = 3·70	LS = 2·60	LT = 2·7	9·00
6	HT = 3·24	LT = 2·46	LS = 2·77	8·47
7	LS = 1·70	HT = 2·63	HS = 3·34	7·67
8	LT = 1·30	HS = 2·60	HT = 2·0	5·90
Column total	9·94	10·29	10·81	31·04

	Dose Order			
Rat No.	*First*	*Second*	*Third*	*Block Total*
Block No. 3				
9	HS = 2·19	LS = 0·98	HT = 1·70	4·87
10	LT = 1·57	HS = 3·13	LS = 1·85	6·55
11	LS = 1·15	HT = 2·27	LT = 0·73	4·15
12	HT = 2·57	LT = 1·68	HS = 3·00	7·25
Column total	7·48	8·06	7·28	22·82

the estimate is within $\pm$ 20% ($P = 0.05$) when four rats were used for each assay.

REFERENCES

Colquhoun, D. (1963). Balanced incomplete block designs in biological assay illustrated by the assay of gastrin using a Youden square. *Brit. J. Pharmacol.*, **21**, 67-77.

Edkins, J. S. (1906). The chemical mechanism of gastric secretion. *J. Physiol. (Lond.)*, **34**, 133-144.

Ferguson, D. J. (1950). Studies on gastrin from human stomachs. *Surg. Forum*, **1**, 84-88.

Gaddum, J. H. (1959). *Pharmacology*, 5th ed. p. 510. Oxford University Press, London.

Ghosh, M. N. (1956). Pharmacological studies of inhibition of gastric secretion. Ph.D. Thesis, University of London.

——, and Schild, H. O. (1958). Continuous recording of acid gastric secretion in the rat. *Brit. J. Pharmacol.*, **13**, 54-61.

Gregory, R. A., and Tracy, H. J. (1961). The preparation and properties of gastrin. *J. Physiol. (Lond.)*, **156**, 523-543.

Harper, A. A., Blair, E. L., and Reed, J. D. (1962). *Proc. int. Union physiol. Sci.*, 22nd Congress, Leyden, Vol. 1, pp. 334-337.

Jalling, O., and Jorpes, J. E. (1947). On the biological assay of gastrin. *Acta physiol. scand.*, **13**, 231-237.

Keeton, R. W., and Koch, F. C. (1915). The distribution of gastrin in the body. *Amer. J. Physiol.*, **37**, 481-504.

Komarov, S. A. (1942). Studies on gastrin. I. Methods of isolation of a specific gastric secretagogue from the pyloric mucous membrane and its chemical properties. *Rev. canad. Biol.*, **1**, 191-205.

Munch-Petersen, J., Rönnow, G., and Uvnäs, B. (1944). Further studies on the gastric secretory excitant from the pyloric mucosa. *Acta physiol. scand.*, **7**, 289-302.

Uvnäs, B., and Emås, S. (1961). A method for biologic assay of gastrin. *Gastroenterology*, **40**, 644-648.

Youden, W. J. (1937). Use of incomplete block replications in estimating tobacco-mosaic virus. *Contr. Boyce Thompson Inst.*, **8**, 41-48.

—— (1940). Experimental designs to increase accuracy of greenhouse studies. *Ibid.*, **11**, 219-228.