J. Physiol. (1968), **198**, *pp.* 613–626
With 11 *text-figures*
Printed in Great Britain

ASSAY OF GASTRIN BY MEANS OF ITS GASTRIC ACID STIMULATING ACTIVITY

BY E. L. BLAIR, R. M. KEENLYSIDE, D. J. NEWELL, J. D. REED AND DIANA D. RICHARDSON*

From the Departments of Physiology and Medical Statistics, University of Newcastle upon Tyne, Newcastle upon Tyne

(*Received* 20 *May* 1968)

SUMMARY

1. A method is described for the bio-assay of gastrin activity in the anaesthetized cat, based on the acid stimulating activity of gastrin.

2. A 'gastrin' of unknown potency can be assayed in terms of a stable Standard Gastrin Extract or of Synthetic Human Gastrin I.

3. The sensitivity of the method is such that it will detect quantities of Synthetic Human Gastrin I at least as small as those at present described as detectable by radioimmunological techniques.

4. The precision of the assay is such that it falls in the category that has been described as very precise and well suited to clinical studies.

INTRODUCTION

The need for a method of gastrin assay has been illustrated by the publication of three new techniques within recent years (Uvnäs & Emås, 1961; Blair, Harper & Reed, 1962; Lai, 1964). The present paper describes in detail a modified, improved method based on that of Blair *et al.* (1962) previously communicated to the Physiological Society in a preliminary form. This method uses the anaesthetized cat and overcomes the problems which Uvnäs & Emås (1961) encountered in this animal preparation. Assay of an unknown 'gastrin' is performed by comparing its gastric acid stimulating activity with that of a reference Standard Gastrin Extract (Blair, Harper, Lake, Reed & Scratcherd, 1961), which itself has been assayed against Synthetic Human Gastrin I (Imperial Chemical Industries Ltd.).

METHODS

Animal preparation. Cats of either sex over 1·0 kg body wt. are used. Solid food is withheld for 24 hr and milk is allowed up to 16 hr before the start of the experiment. Anaesthesia is induced with ether and maintained by a single I.V. injection of chloralose (80 mg/kg body

* Present address: Department of Physiology, University of Bristol.

wt.). This will usually result in satisfactory anaesthesia for 8 hr but an experiment may be prolonged by the administration of open ether.

A rubber tube (i.d. 6 mm, o.d. 10 mm) is inserted into the stomach through an incision in the oesophagus in the neck. A tape ligature is tied around the pylorus, care being taken not to interfere with the blood supply to the stomach. The pancreatic and bile ducts are ligated. Both vagi are cut in the neck and the splanchnic nerves are cut extraperitoneally.

The animals are left post-operatively for at least 30 min before the experiment is continued. Rectal temperature is maintained at $38 \pm 1°$ C.

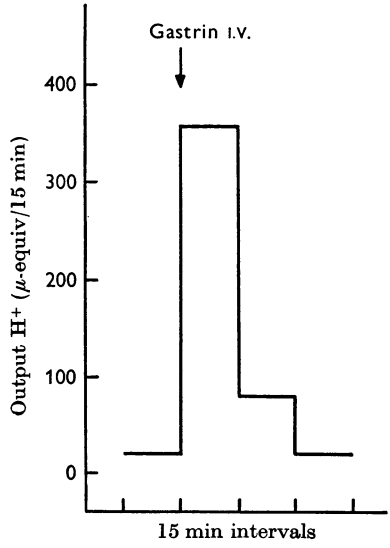

Fig. 1. A characteristic gastric acid secretory response following the intravenous injection of a dose of gastrin (dissolved in 1·0 ml. saline and injected over 1 min) when the maximum rate of response is < 400 μ-equiv H^+ in 15 min.

Determination of acid secretion. Gastric secretions are washed out of the stomach at 15 min intervals. Fifty millilitres of glycine solution (0·3 osmoles/l. and approximately pH 6·2) are instilled into the stomach at the start of each 15 min collection period and are siphoned off after 13 min. Between the 13th and 15th min, a further 50 ml. of glycine solution are run into and out of the stomach. The two gastric washings are combined and the hydrogen ion content is determined by electrometric titration back to the initial pH of the glycine solution with 0·01 N-NaOH. Results are expressed as μ-equiv H^+ output in 15 min.

Gastrin stimulation. Each dose of gastrin was injected intravenously in saline (0·9 g/100 ml.). The volume injected was not critical but most commonly was 1 ml. The injection was always completed in 3 min and most commonly in 1 min. For the purposes of accurate assay, the doses are arranged so that the rate of acid secretion in 15 min does not exceed 400 μ-equiv H^+. The pattern of the acid secretory response to a single dose of gastrin administered in this way is illustrated in Fig. 1. The secretory response is completed in two 15 min periods and is made up of a larger response in the first of these two periods and a smaller response in the second. When such doses of gastrin are injected at the beginning of successive 15 min periods, the total output of acid in a 15 min period is the combined result of the initial 15 min response to the gastrin dose injected at the start of the period and the second 15 min response to the gastrin dose injected at the start of the previous 15 min period (Fig. 2). The accuracy of assay is decreased if a secretory response exceeds 400 μ-

equiv/15 min, because in these circumstances the secretory response to a single gastrin dose may last appreciably longer than 30 min.

Method of assay. A record of a typical assay experiment is illustrated in Fig. 3. A known dose of a Standard Gastrin Extract is injected every 15 min, except on those occasions when the gastrin, the activity of which is under investigation (the 'unknown'), is substituted. No substitution with an 'unknown' is made until after the third or fourth injection of the standard.

The activity of the dose of the 'unknown' is assessed by comparing the total acid secreted in the 30 min period following its injection with the amount of acid which it is predicted would have been secreted in that period had the unknown not been substituted for the

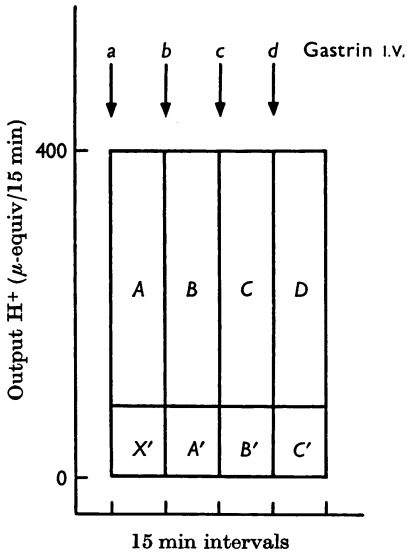

Fig. 2. A diagram illustrating the presumed gastric acid secretory pattern to the I.V. injection of the same dose of gastrin at 15 min intervals (gastrin dose adjusted to produce a rate of acid secretion no greater than 400 μ-equiv H^+/15 min). Responses *AA'*, *BB'* and *CC'* represent the responses to gastrin doses *a*, *b* and *c* respectively. *X'* represents the residual response to a dose of gastrin administered 15 min before the commencement of the record. A residual response *D'* would be expected to occur in the 15 min after this record was completed.

standard gastrin. The way in which this assessment is made can be described with the aid of Fig. 4 which represents a portion of the experimental record shown in Fig. 3 (periods 8–11 inclusive). The total acid secreted in the 30 min period following the injection of the 'unknown' (the assay $\frac{1}{2}$ hr) is represented by the area within the heavy line in periods 9 and 10 in Fig. 3, i.e. 0·128 + 0·114 = 0·242 m-equiv H^+. The amount of acid which, it is predicted, would have been secreted during this assay $\frac{1}{2}$ hr had there been no substitution of a dose of the 'unknown' gastrin for a dose of the standard gastrin during this period (the predicted response), is assessed by linear interpolation between the outputs of H^+ occurring in the 15 min periods 8 and 11 on either side of this assay $\frac{1}{2}$ hr (the shaded area in periods 9 and 10 in Fig. 4), i.e. 0·108 + 0·108 = 0·216 m-equiv H^+. The actual response in the assay $\frac{1}{2}$ hr can be expressed as a percentage of the predicted response (the % assay response) and in this instance is 112 %.

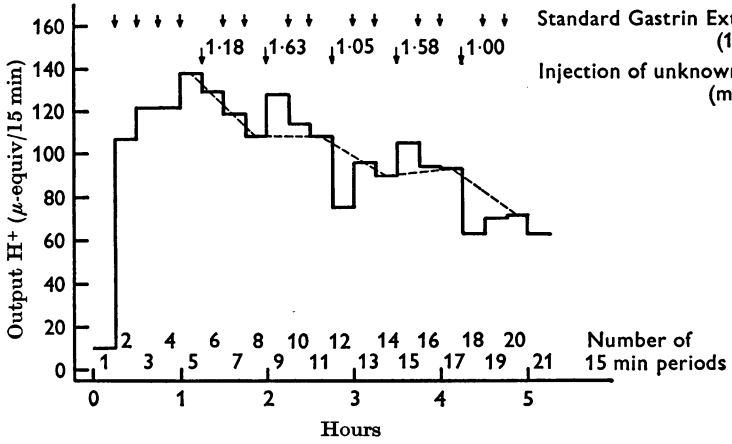

Fig. 3. Record of a typical gastrin assay experiment. The same dose of a standard gastrin is injected at the beginning of every 15 min period except in the first period (spontaneous secretion) and those periods (6, 9, 12, 15 and 18) when gastrin, the activity of which is being investigated (the 'unknown'), is substituted for the standard. The continuous lines indicate the observed secretory responses throughout the experiment and the interrupted lines indicate the predicted size of the secretory responses during each assay ½ hr had there been no substitution by the 'unknown' for the standard.

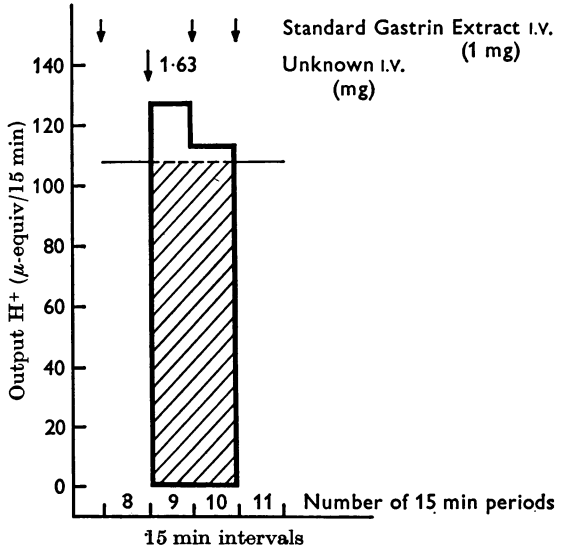

Fig. 4. A larger scale representation of periods 8, 9, 10 and 11 of the assay experiment illustrated in Fig. 3. The area within the heavy lines in periods 9 and 10 shows the observed secretory response in the assay ½ hr following the injection of 1·63 mg of the unknown gastrin. The response which it is predicted would have occurred at this time, had there been no substitution of the 'unknown' for the standard at the beginning of period 9, is indicated by the hatched area.

The % assay response calculated in this way is used throughout the present work to measure the response to an 'unknown'. It is clear, however, that this is not a response in the commonly accepted sense in that it is not the value for the amount of secreted hydrogen ion. There is, however, a linear relationship between the dose of an 'unknown' and the % assay response if this is kept within $\pm 25\%$ of the predicted response. This has been shown for two different doses of the 'background' standard by carrying out experiments in which differing doses of the standard gastrin itself are used as the 'unknown' (Fig. 5*A*, *B*). Provided therefore that an 'unknown' has the same dose–response relationship as that of the standard gastrin, the linear dose–response regressions calculated from the data in Fig. 5*A*, *B* can be used to estimate the activity of an 'unknown' in terms of the standard gastrin when 1·0 mg or 0·3 mg respectively of the standard is the background stimulus. For example, the assay value for the dose of the 'unknown' illustrated in Fig. 4, which gave a response of 112 %, is equivalent to 1·37 mg of the Standard Gastrin Extract as determined from the appropriate calculated dose–response regression line in Fig. 5*A*. As in this instance 1·63 mg of the 'unknown' was injected, it follows that 1 mg of this 'unknown' is equivalent to 0·84 mg of the Standard Gastrin Extract. If a response in the assay ½ hr falls outside the range $\pm 25\%$ of the predicted response, an approximate assessment of the activity of the 'unknown' can be made from a linear extrapolation of the dose–response lines illustrated in Fig. 5. Such a result, although unsatisfactory for accurate assay, provides a guide to a suitable dose for future assay.

When the data in Fig. 5 are calculated with the doses of the 'unknown' expressed not as absolute values but as percentages of the background dose of the standard (Fig. 6), it is clear that there is no significant difference in the relationship between the dose expressed in this way and the percentage assay response whether the background dose of the standard is 1·0 mg or 0·3 mg. These data are derived from experiments covering a wide range of rates of acid secretion varying from 10 to 400 μ-equiv H^+/15 min. It is therefore reasonable to conclude that when assays are conducted in this secretory range with any known dose of the standard gastrin as the background, the dose of an unknown can be determined in terms of the Standard Gastrin Extract by referring to a single regression line derived from all the data which go to make the two separate regression lines in Fig. 6. The regression line ($r = 0{\cdot}876$; $P < 0{\cdot}001$, $m = 0{\cdot}302$; $c = 71{\cdot}1$) is valid for all values of Y (the % assay response) between 75 and 125 %.

The estimation in Fig. 4 was chosen as the simplest example to illustrate the method of assay because there is no significant alteration in the sensitivity of the animal to the background gastrin during the period of assay. More commonly, assay has to be performed during a period of increasing or decreasing sensitivity (Fig. 3). The same method is, however, used to predict the response which would have occurred during the assay ½ hr had there been no substitution of an 'unknown' for the standard; it is assumed that there is a linear alteration in sensitivity of the animal between the 15 min period immediately preceding and the 15 min period immediately following the assay ½ hr. The activity of an 'unknown' is therefore assessed in precisely the same way during a period of changing sensitivity as when there is no change in sensitivity during the period of assay (Fig. 7). When the response of 83·5 % is referred to the dose–response line in Fig. 5*A* it is found to be equivalent to one which results from the injection of 0·4 mg of the standard gastrin, i.e. in this instance 1·0 mg of the 'unknown' is equivalent to 0·4 mg of the Standard Gastrin Extract. The justification for determining the predicted response by linear interpolation lies in the accuracy of the assay technique when this procedure is used. Occasionally, an attempt at assay may be invalidated by a change in direction of sensitivity during the course of an estimation. When such a change occurs it is usually readily apparent and is confirmed if in the assay ½ hr the difference between the amount of acid secreted in a 15 min period and the amount which, it was predicted, would have been secreted had there been no substitution of the 'unknown' for the standard in the same period, is greater in the second than in the first 15 min period of the assay.

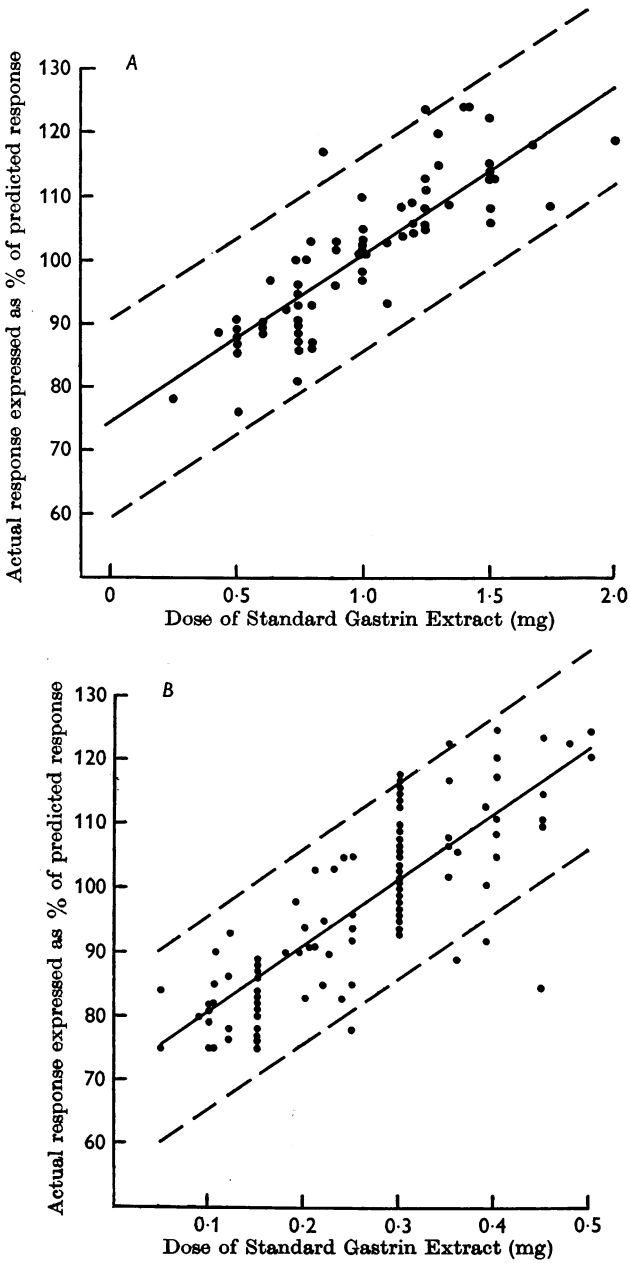

Fig. 5. Illustrating the relationship between the dose of an 'unknown' gastrin (abscissa) and the actual acid secretory response it produces expressed as a percentage of the predicted response had there been no substitution for the standard (ordinate). (*A*) sixty-nine observations from fifteen animals: $r = 0{\cdot}803$, $P < 0{\cdot}001$, $m = 0{\cdot}029$, ———— $c = 71{\cdot}36$, —— —— 95 % confidence limits about y. (*B*) 124 observations from forty-six animals: $r = 0{\cdot}810$, $P < 0{\cdot}001$, $m = 0{\cdot}103$, ———— $c = 70{\cdot}4$, —— —— 95 % confidence limits about y. The 'unknowns' were varying doses of the standard gastrin and the background doses of standard gastrin were respectively 1·0 mg in (*A*) and 0·3 mg in (*B*). The continuous lines are the calculated dose–response regression lines and the interrupted lines are their 95 % confidence limits about y.

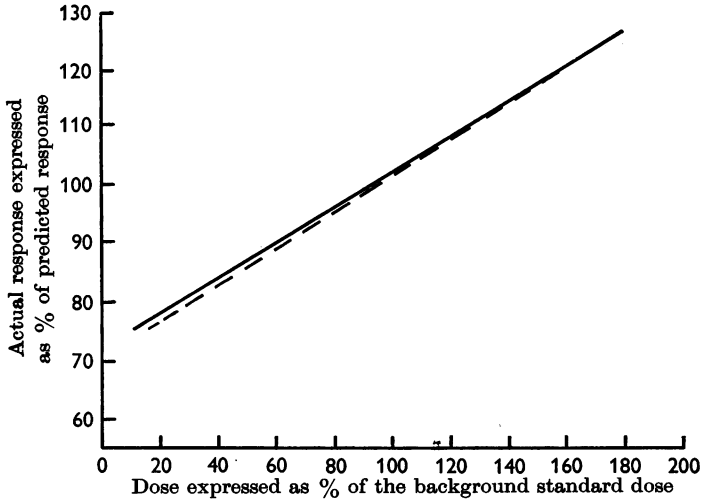

Fig. 6. Illustrating the calculated regression lines for the data in Fig. 5*A* and 5*B* when the doses of the 'unknown' are expressed as a percentage of the background dose of standard gastrin. ———— Dose of background standard 1·0 mg, $m = 0{\cdot}296$, $c = 71{\cdot}36$ ($P < 0{\cdot}001$). — — — Dose of background standard 0·3 mg, $m = 0{\cdot}309$, $c = 70{\cdot}37$ ($P < 0{\cdot}001$).

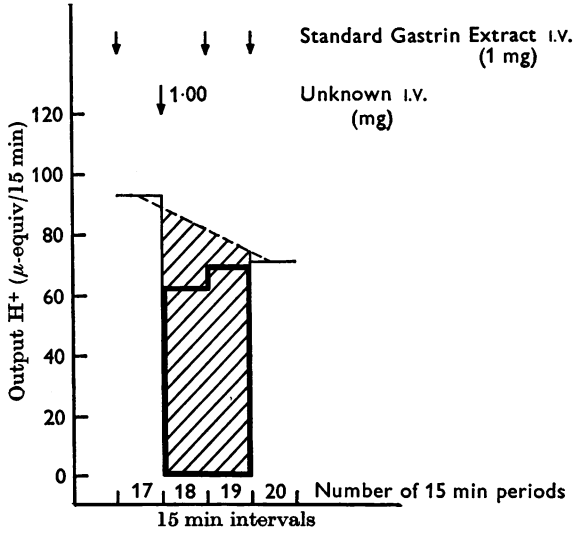

Fig. 7. A larger scale representation of periods 17, 18, 19 and 20 of the assay experiment illustrated in Fig. 3. The area within the heavy lines in periods 18 and 19 shows the observed secretory response in the assay ½ hr following the injection of 1·0 mg of the unknown gastrin. The response which, it is predicted, would have occurred in this time had there been no substitution of the unknown for the standard at the beginning of period 18 is indicated by the hatched area. The observed secretory response is 83·5 % of the predicted response in this instance.

RESULTS

Accuracy of the assay technique. The standard gastrin was used as both the 'background' and the 'unknown' and assayed against itself in order to determine the accuracy of the assay method.

The results obtained when the standard gastrin was repeatedly assayed against itself in this way are shown in Fig. 5*A*, *B*. The 95% confidence limits about the y variable for a single observation are given by

$$y \pm ts\sqrt{\{1+(1/n)+(x-\bar{x})^2/\Sigma(x-\bar{x})^2\}},$$

where t is read with $(n-2)$ degrees of freedom and $P = 0{\cdot}05$; and s is the residual standard deviation in y about the regression line. The respective 95% confidence limits for these two dose–response relationships are illustrated in Fig. 5*A*, *B*.

In the application of the method it is necessary to predict the x value and its 95% confidence limits for an observed value of y. For a single observation these limits are given by

$$\frac{y-c}{m} \pm ts_1$$

where:

m is the slope of the regression line relating x and y,

c is the intercept of the regression line on the y-axis,

t is read with $(n-2)$ degrees of freedom and $P = 0{\cdot}05$,

s_1 is the standard error of x and is determined as follows:

$$s_1 = \frac{s}{m}\sqrt{\left[1+\frac{1}{n}+\left(\frac{y-\bar{y}}{m}\right)^2\left(\frac{1}{\Sigma(x-\bar{x})^2}\right)\right]}.$$

The 95% confidence limits about the x-axis for more than one observation (k observations) are given by

$$\frac{\bar{y}-c}{m} \pm ts_k,$$

where s_k, the standard error for k observations of x, is determined as follows:

$$s_k = \frac{s}{m}\sqrt{\left[\frac{1}{k}+\frac{1}{n}+\left(\frac{\bar{Y}-\bar{y}}{m}\right)^2\left(\frac{1}{\Sigma(\bar{x}-\bar{x})^2}\right)\right]},$$

where $\bar{Y}$ is the mean value of y for k observations.

If, for example, 0·300 mg of the standard gastrin is injected as the 'unknown' in an assay in which the dose of the 'background' standard is 0·300 mg there is a 95% probability that a single estimation will give a result which lies between 0·148 and 0·452 mg of standard gastrin. If the estimation is repeated 6 times then there is a 95% probability that the mean assay result will be between 0·226 and 0·374 mg and if repeated

12 times there is a 95% probability that this mean value will be between 0·254 and 0·346 mg. The number of estimations which are made in respect of a particular unknown can be chosen according to the degree of accuracy required in the final result. The preliminary procedures in the preparation of the assay animal are completed in about 1 hr and thereafter it is possible to complete one estimation every 45 min. It is possible therefore to make twelve estimates in a reasonable working day.

The reliability of an assay technique can also be judged from the Index of Precision (Loraine & Bell, 1966). In assays which depend on graded effects this index is calculated from log dose–response data; the standard deviation of the responses about the regression (s) is divided by the slope of the regression (m). This has been calculated for those experiments in which the standard was assayed against itself with both 300 and 1000 μg of the Standard Gastrin Extract as the background. Data relating to the linear portion of the $\log_{10}$ dose–response relationship were used; this meant exclusion of data from only one set of results, viz. data obtained when doses of less than 100 μg of Standard Gastrin Extract were assayed with 300 μg of the standard as the background stimulus (3 out of 293 observations). The Index of Precision in those experiments in which 300 μg of Standard Gastrin Extract was used as the background stimulus was $8{\cdot}1/57{\cdot}35 = 0{\cdot}14$ and with 1000 μg of Standard Gastrin Extract as the background stimulus it was $6{\cdot}7/58{\cdot}69 = 0{\cdot}11$.

Assay of Synthetic Human Gastrin I. A preparation of Synthetic Human Gastrin I (Beacham, Bentley, Gregory, Kenner, MacLeod & Sheppard, 1966) prepared by Imperial Chemical Industries Ltd. was also assayed as an 'unknown'. Fourteen estimations were made with Synthetic Human Gastrin I in five animals when the background gastrin dose was 300 μg of Standard Gastrin Extract (Fig. 8*A*) and eighteen estimations were made in four animals when the background dose was 1000 μg of Standard Gastrin Extract (Fig. 8*B*).

In order to be able to express the activity of Synthetic Human Gastrin I in terms of the Standard Gastrin Extract it is necessary to ensure that their dose–response relationships are parallel. Evidence for parallelism was sought by comparing the linear portion of the respective $\log_{10}$ dose–response curves for the two substances (Finney, 1952) when 300 μg and 1000 μg respectively of the Standard Gastrin Extract were used as the background in the assays.

When Synthetic Human Gastrin I and Standard Gastrin Extract were assayed against the same background dose of the standard, no significant difference was found between the slopes of their respective dose–response lines (Fig. 9*A*, $t = 0{\cdot}297$ and $P = 0{\cdot}7$; Fig. 9*B*, $t = 0{\cdot}517$ and $P = 0{\cdot}6$).

Once it was established that there is no significant difference between the

slopes of the regression lines compared in Fig. 9*A* and *B*, two parallel lines were fitted by the method shown in Quenouille (1950), to each of the two sets of data (Fig. 10*A*, *B*). The relative potency of the two substances

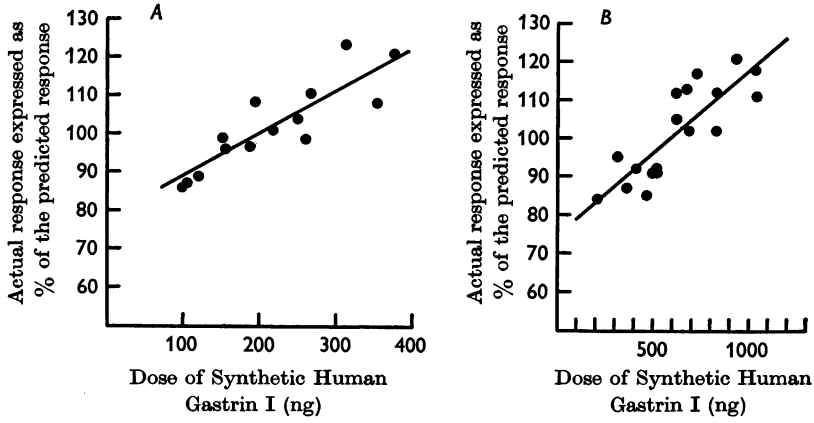

Fig. 8. Illustrating the relationship between the dose of Synthetic Human Gastrin I (abscissa) and the actual acid secretory response it produces expressed as a percentage of the predicted response had there been no substitution for the background standard gastrin (ordinate). Results with a background dose of standard gastrin of (*A*) 300 μg: $r = 0{\cdot}878$, $P < 0{\cdot}001$, $m = 0{\cdot}110$, $c = 78{\cdot}26$. (*B*) 1000 μg: $r = 0{\cdot}959$, $P < 0{\cdot}001$, $m = 0{\cdot}043$, $c = 74{\cdot}80$.

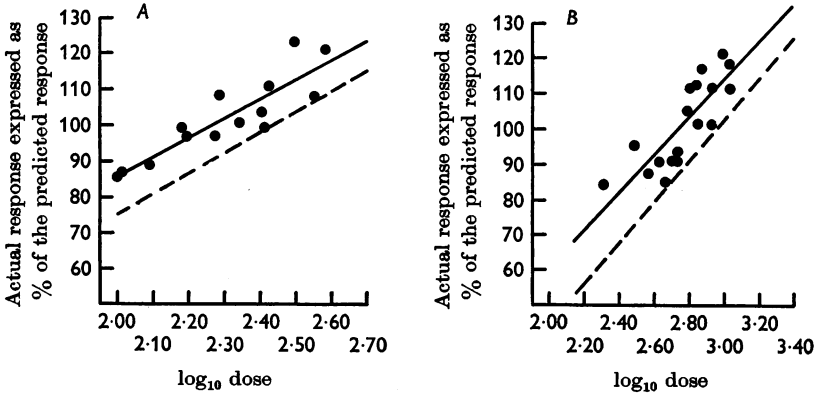

Fig. 9. Comparison of the slopes of the calculated dose–response regression lines for Synthetic Human Gastrin I (SHG I) and the Standard Gastrin Extract (*A*) with 300 μg background dose of standard gastrin: Regression lines: — — Standard Gastrin Extract, $m = 57{\cdot}35$, $c = -39{\cdot}46$. ———— Synthetic Human Gastrin I, $m = 51{\cdot}16$, $c = -21{\cdot}39$. ● Individual observations SHG I. (*B*) with 1000 μg background dose of standard gastrin: Regression lines: — — Standard Gastrin Extract, $m = 58{\cdot}69$, $c = -73{\cdot}2$. ———— Synthetic Human Gastrin I, $m = 53{\cdot}49$, $c = -46{\cdot}07$. ● Individual observations SHG I.

is a function of the distances between the two lines, in each instance. The distance (M) between the two lines on the log scale is calculated from

$$M = (\bar{x}_1 - \bar{x}_2) - \frac{\bar{y}_1 - \bar{y}_2}{m}$$

and the antilogarithm of this value gives the relative potency (R) of the two materials. The 95% confidence limits of M can be calculated by the method given in Finney (1952, p. 114), from which the confidence limits for R are found by taking antilogarithms (Table 1).

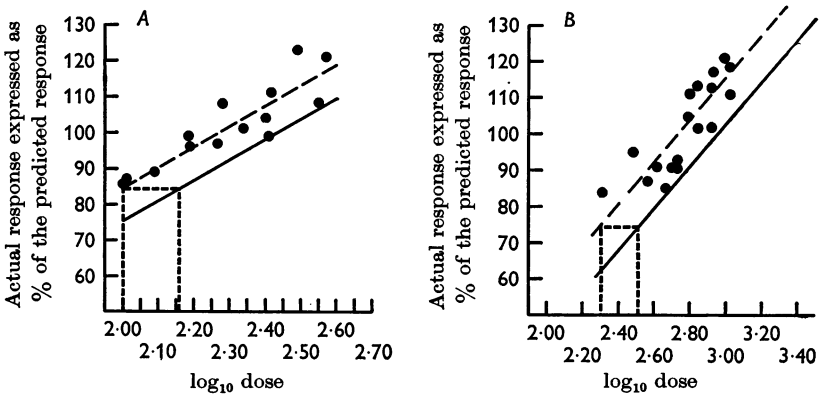

Fig. 10. Illustrating the determination of the relative potencies of Standard Human Gastrin I (SHG I) and Standard Gastrin Extract after fitting parallel lines (Quenouille, 1950) to the dose–response data. (*A*) with 300 μg background dose of standard gastrin: 100 ng SHG I ≡ 147 μg Standard Gastrin Extract. — — Synthetic Human Gastrin I. ———— Standard Gastrin Extract. (*B*) with 1000 μg background dose of standard gastrin: 200 ng SHG I ≡ 330 μg Standard Gastrin Extract: — — Synthetic Human Gastrin I. ———— Standard Gastrin Extract.

TABLE 1.

Substances compared	Relative potency of Synthetic Human Gastrin I: Standard Gastrin Extract	95% confidence limits (P = 0·05)
Synthetic Human Gastrin I and Standard Gastrin Extract		
A. 300 μg background standard gastrin	1470:1	1230:1 1760:1
B. 1000 μg background standard gastrin	1648:1	1420:1 1900:1

There is no significant difference between the two assay values for the relative potency of Synthetic Human Gastrin I, when it is determined with 300 or 1000 μg of Standard Gastrin Extract as background. A single estimate of the relative potency of these two substances has been derived by combining the results obtained when Synthetic Human Gastrin I was

assayed against 300 and 1000 μg respectively of the Standard Gastrin Extract. The quantities of Standard Gastrin Extract which will produce the same percentage responses as those obtained with the different doses of Synthetic Human Gastrin I (Fig. 8*A*, *B*) can be determined by reference to the appropriate dose–response regressions for the Standard Gastrin Extract illustrated in Fig. 5*A*, *B*. The results which are obtained when the

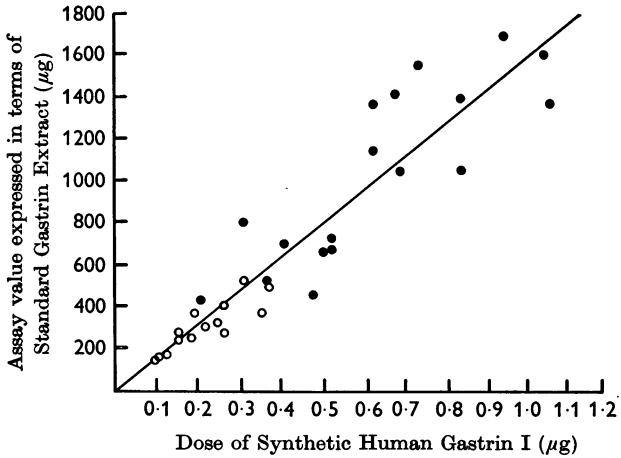

Fig. 11. Illustrating the calculated regression between the dose of Standard Human Gastrin I and its assay value expressed in terms of Standard Gastrin Extract (from the same data as Fig. 8*A* and *B*). ○ experiments in which the background dose of Standard Gastrin Extract was 300 μg. ● Experiments in which the background dose of Standard Gastrin Extract was 1000 μg. $r = 0{\cdot}933$, $P < 0{\cdot}001$, $m = 1596$, $c = -1{\cdot}701$.

activity of Synthetic Human Gastrin I is expressed in terms of Standard Gastrin Extract in this way is illustrated in Fig. 11. It is concluded from the calculated regression line for these data, that weight for weight, Synthetic Human Gastrin Extract is 1594 times more active than the Standard Gastrin Extract in its ability to stimulate gastric acid secretion. The 95% confidence limits of the estimate ($P = 0{\cdot}05$) are $\pm$ 204.

DISCUSSION

It is likely that the study of gastrointestinal hormones will eventually benefit from the application of radioimmuno-assay techniques which have recently revolutionized the quantitative study of other polypeptide hormones. At the present time, however, although McGuigan (1967), and Stremple, Abramoff, van Oss, Wilson & Ellison (1967) have succeeded in producing immune antibodies to pure gastrin polypeptides, an immunoassay technique has not yet been characterized in detail. Furthermore,

while immuno-assay will determine quantitatively the existence of a polypeptide it does not establish the biological activity of the material it identifies. It is therefore important that immuno-assay and bio-assay techniques should if possible develop alongside one another. Ideally these methods should be equally sensitive and precise.

The smallest quantity of Synthetic Human Gastrin I assayed in the present work was 100 ng (50 p-moles). Although this is not the smallest amount of gastrin activity which can be detected by the method of bioassay (Blair & Wood, 1968), it is less than the minimum molar quantity of the gastrin peptides (60 p-moles) so far detected by McGuigan (1967) with a radioimmunological technique. The precision of radioimmuno-assay tends to be of a high order but there is at present no Index of Precision available for the immuno-assay of gastrin. The Index of Precision for the present bioassay was 0·14 when 300 μg of Standard Gastrin Extract was used as the background stimulus and 0·11 when 1000 μg of Standard Gastrin Extract was used as the background. It is possible to determine the gastrin activity of twelve 'unknowns' with this degree of precision in a 10 hr working day. Each reduction or increase of 45 min in experimental time reduces or increases by one the number of 'unknowns' that can be assayed. The precision of the assay of a single 'unknown' can be improved by its repeated assay. Loraine & Bell (1966) arbitrarily divide assay techniques into three groups according to their degree of precision. The best group is the one in which the Index of Precision is less than 0·2 and assays in this group are considered by these workers to be sufficiently precise to be well suited to clinical studies.

It is of considerable practical importance for day-to-day work on the bioassay of gastrin that it is possible to assay the gastric acid stimulating activity of the synthetic polypeptide, Synthetic Human Gastrin I, with a cheaply and readily prepared stable gastrin extract (Standard Gastrin Extract). When an 'unknown' is to be assayed it is necessary either to know that it behaves as gastrin or to establish that it has a dose–response curve for gastric acid secretion which parallels that for gastrin. If an 'unknown' does not meet these requirements it must be concluded that it is not gastrin; if it does meet these requirements however this does not necessarily establish that it is gastrin.

We would like to thank the M.R.C. for the award of a Scholarship for Training in Research Methods to Diana D. Richardson (née Wood). We would also like to indicate our appreciation of the help given by Mrs D. Weightman in the statistical calculations.

REFERENCES

BEACHAM, J., BENTLEY, P. H., GREGORY, R. A., KENNER, G. W., MACLEOD, J. K. & SHEPPARD, R. C. (1966). Synthesis of Human Gastrin I. *Nature, Lond.* **209**, 585–586.

BLAIR, E. L., HARPER, A. A., LAKE, H. J., REED, J. D. & SCRATCHERD, T. (1961). A simple method of preparing gastrin. *J. Physiol.* **156**, 11–13*P*.

BLAIR, E. L., HARPER, A. A. & REED, J. D. (1962). An assay technique for gastrin. *J. Physiol.* **163**, 47–48*P*.

BLAIR, E. L. & WOOD, DIANA, D. (1968). The estimation of gastrin activity in blood. *J. Physiol.* **194**, 44–45*P*.

FINNEY, D. J. (1952). *Statistical Method in Biological Assay*. London: Charles Griffin and Co. Ltd.

LAI, K. S. (1964). Studies on gastrin. Part I. A method of biological assay of gastrin. *Gut* **5**, 327–333.

LORAINE, J. A. & BELL, E. T. (1966). *Hormone Assays and their Clinical Application*. Edinburgh and London: E. and S. Livingstone Ltd.

MCGUIGAN, J. E. (1967). Antibodies to the carboxyl-terminal tetrapeptide of gastrin. *Gastroenterology* **53**, 697–705.

QUENOUILLE, M. H. (1950). *Introductory Statistics*. London: Butterworth-Springer.

STREMPLE, J. F., ABRAMOFF, P., VAN OSS, C. J., WILSON, S. D. & ELLISON, E. H. (1967). Antibodies to synthetic human gastrin I. *Lancet* **ii**, 1180–1182.

UVNÄS, B. & EMÅS, S. (1961). A method for biologic assay of gastrin. *Gastroenterology*, **40**, 644–648.