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THE HALF-LIFE OF EXOGENOUS GASTRIN IN THE CIRCULATION

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SUMMARY

1. A method of gastrin bio-assay is described which can be used on as little as 30 ng synthetic human gastrin I at a minimum concentration of 2·5 ng/ml.

2. Pentagastrin or synthetic human gastrin I added to cat plasma can be stored on ice or at 4° C, for periods up to 27 hr without apparent loss of gastrin activity.

3. Between 1½ and 13 min after the rapid I.V. injection of pentagastrin in the anaesthetized cat and between 1½ and 15 min after the injection cf synthetic human gastrin I, there is a rapid reduction of the gastrin concentration in the arterial plasma. The data relating $\log_{10}$ gastrin concentration in arterial plasma with time can be fitted by a single term.

4. Studies *in vitro* show that over the periods of time involved in the *in vivo* studies, both pentagastrin and synthetic human gastrin I are stable in cat plasma at 37° C in concentrations which occurred in the circulating plasma.

5. The half-life of pentagastrin in the circulating arterial plasma of the anaesthetized cat is 1·50 min (S.E. ± 0·08) and the half-life of synthetic human gastrin I is 2·65 min (S.E. ± 0·09).

INTRODUCTION

The present paper describes an investigation in anaesthetized cats of the half-life in the circulation of administered gastrin (Synthetic Human Gastrin I‡, S.H.G.I.) and pentagastrin§ (N-tert-butyloxycarbonyl-β-Ala. Try. Met. Asp. Phe—NH_2). Gastrin activity in the plasma was measured

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§ Peptavlon, purchased from Imperial Chemical Industries Ltd.

at intervals following instantaneous intravenous injection of gastrin or pentagastrin. Plasma gastrin activity was measured by a modification of the method of Blair, Keenlyside, Newell, Reed & Richardson (1968).

METHODS

Cats of either sex over 1·0 kg body weight were used. Solid food was withheld for 48 hr and milk allowed up to 16 hr before the start of an experiment. Anaesthesia was induced with ether and maintained by a single I.V. injection of chloralose (80 mg/kg body weight). Anaesthesia may be prolonged by the administration of open ether. A rubber tube (i.d. 6 mm, o.d. 10 mm) was inserted into the stomach through an incision in the oesophagus in the neck. A tape ligature was tied around the pylorus, care being taken not to interfere with the blood supply to the stomach. The pancreatic and bile ducts were ligated. Both vagi were cut in the neck and the splanchnic nerves were cut extraperitoneally. The rectal temperature was maintained at 38° C ± 1° C. Intravenous injections were given through an indwelling cannula in a saphenous vein.

Collection of gastric secretion. Gastric secretions were washed out of the stomach at precisely timed intervals; 50 ml. of a 0·3 osmolar mannitol solution at 38° C was instilled into the stomach at the start of each 15 min collection period and was siphoned off 1½ min before the end of the collection. A further 50 ml. mannitol was then run in and out of the stomach during the 1½ min before the start of the next collection period. The two gastric washings were combined and the secreted hydrogen ion determined by electrometric titration to pH 6·0 with 0·001 N-NaOH and deduction of a very small control value representing the amount of NaOH required to titrate the mannitol solution alone to pH 6·0.

Collection of blood. In those animals in which the half-lives of the gastrin peptides were studied, blood was collected from an arterial cannula ('Portex', transparent vinyl NT/3,5 H95) inserted into one of the carotid or femoral arteries. When blood was being collected, approx. 0·5 ml. was first discarded to clear the dead space and the blood for investigation was run into heparinized tubes (10 u. heparin/ml.). Between collections the cannula was filled with heparinized saline (10 u. heparin/ml. 0·9 % NaCl) and was occluded distally by a clamp applied to a sleeve of silicone rubber tubing placed on the free end of the cannula. The blood was centrifuged at 3000 rev/min for 15 min within 30 min of its collection and the plasma removed. The plasma was stored on ice and either assayed on the day of collection, or on the following day after it had been stored overnight at 4° C. Volumes of 1–20 ml. of blood were collected and immediately replaced by an equal amount of blood infused into the saphenous vein by a roller pump from a reservoir at 37° C. Blood for replacement which contained no detectable gastrin activity was previously collected from other cats, heparinized (10 u./ml.) and stored at 4° C before its use.

Assay of gastrin activity. This was by a modified form of the bio-assay method of Blair *et al.* (1968) that has proved to be more sensitive and precise. A 'background' dose (*Xs*) of a standard gastrin extract, pentagastrin or synthetic human gastrin I is injected at 15 min intervals except when an unknown for assay is substituted. At the start of the experiment and immediately prior to the commencement of assay of an unknown, the background standard gastrin is injected at three successive 15 min intervals. An initial estimate (*VXu*) of the activity of an unknown (*Xu*) is made by comparing the total amount of acid secreted in the 15 min following its injection with the amount which it is predicted would have been secreted in that period had the unknown not been substituted for the 'background' dose and assuming a linear

relationship between dose and response. The predicted secretory rate is assessed by linear interpolation between the outputs of H^+ (Hs_1 and Hs_2) occurring in the 15 min periods following the injection of the background standard (doses Xs_1 and Xs_2 respectively) on either side of the assay 15 min (dashed line, Fig. 1). The background doses of gastrin extract (50 μg), pentagastrin (20–40 ng) and synthetic human gastrin I (20–40 ng) are chosen so that the acid secretory responses to all injected stimulants are expected to be complete in 15 min.

The final estimate of the activity of an unknown is determined by studying the responses to two proportions of it (Xu and a proportion y of Xu). The dose Xu is

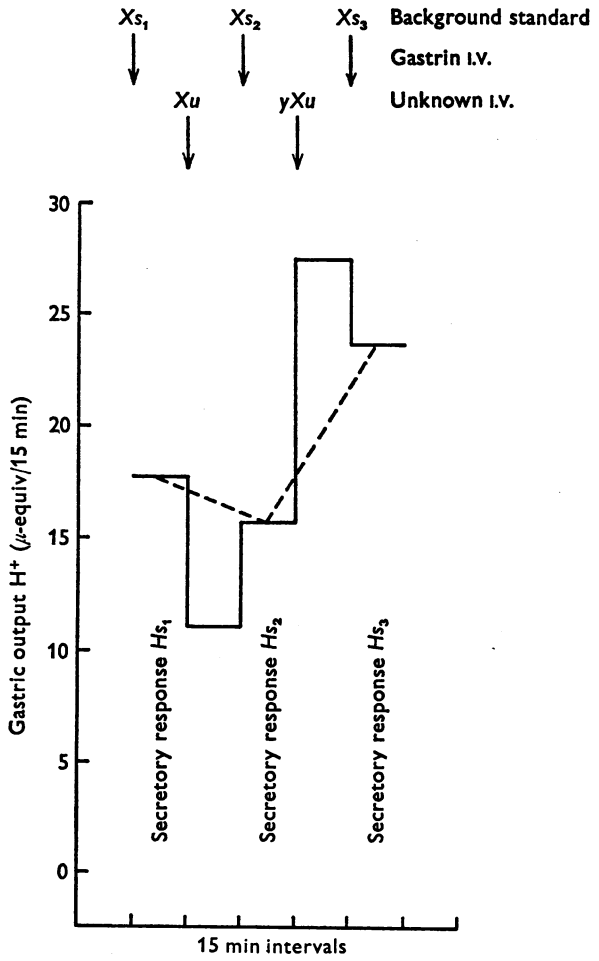

Fig. 1. Record of a typical gastrin assay. Xs_1, Xs_2 and Xs_3 are similar doses (50 μg) of the background standard gastrin. Xu is a dose of the 'unknown' which produces a response less than that to the background standard and yXu is a proportion y of Xu which produces a response greater than that to the background. The responses Hs_1, Hs_2 and Hs_3 are the responses following doses Xs_1, Xs_2 and Xs_3 respectively.

arranged to be such as to produce a secretory response less than that to the background dose and the dose yXu to produce a response greater than that to the background dose. These responses are kept within $\pm 75\%$ of the response to the standard and the values for y are greater than 1 but no greater than 5. The two proportions of the unknown are tested one after the other and an initial estimated value ($VyXu$) is determined for yXu in the same way that VXu is determined for Xu. In these experiments, not all of the H^+ secreted in a 15 min period is necessarily the result of the injected stimulus alone and other factors such as non-stimulated (basal) acid secretion also contribute. This results in errors in calculating the activity of an unknown when the dose of the unknown (Xu or yXu) differs from that of the standard (Xs). These errors will be expected to increase as the dose of the unknown departs from that of the standard, i.e.

$$VXu - Xu \propto Xs - Xu,$$

and

$$VyXu - yXu \propto Xs - yXu.$$

In the calculation of the final assay value it is therefore assumed that there is a constant error K per unit difference of the dose of an unknown from that of the standard. From this it follows that as:

$$VXu - Xu \propto Xs - Xu,$$

therefore

$$VXu = Xu + K_1(Xs - Xu) \tag{1}$$

and as

$$VyXu - yXu \propto Xs - yXu,$$

therefore

$$VyXu = yXu + K_2(Xs - yXu). \tag{2}$$

In the assay method of Blair *et al.* (1968), a single correction factor was applied to all responses in all animals, whereas in the present method of assay it is assumed only that K remains constant during the assay of two proportions of an unknown immediately after one another, i.e. that $K_1 = K_2$ in eqns. (1) and (2). Under these circumstances, it is possible to solve for K in eqns. (1) and (2)

$$K = \frac{yVXu - VyXu}{Xs(y-1)}. \tag{3}$$

It is then possible to calculate Xu and yXu by substituting for K in eqns. (1) and (2).

This method of calculation can be illustrated by referring to the example in Fig. 1. The responses in this particular assay were as follows:

Hs_1, 15 min H^+ output following $Xs_1 = 17{\cdot}88$ μ-equiv H^+;
the 15 min H^+ output following $Xu = 11{\cdot}00$ μ-equiv H^+;
Hs_2, the 15 min H^+ output following $Xs_2 = 15{\cdot}60$ μ-equiv H^+;
the 15 min H^+ output following yXu ($y = 3{\cdot}0$) $= 27{\cdot}40$ μ-equiv H^+;
Hs_3, the 15 min H^+ output following $Xs_3 = 23{\cdot}61$ μ-equiv H^+.

From these results the following calculations are made:

(*a*) Initial estimate of value for Xu

$$VXu = \frac{11{\cdot}00}{\frac{17{\cdot}88 + 15{\cdot}60}{2}} \times 50 = 32{\cdot}8.$$

(*b*) Initial estimate of value for yXu

$$VyXu = \frac{27{\cdot}40}{\frac{15{\cdot}60 + 23{\cdot}61}{2}} \times 50 = 69{\cdot}8.$$

(*c*) Error per unit difference in dose between 'unknown' and standard

$$K = \frac{(32{\cdot}8 \times 3) - 69{\cdot}8}{50\,(3-1)} = 0{\cdot}286.$$

(*d*) Assay value for Xu (obtained by substitution in eqn. 1)

$$\equiv 25{\cdot}9\ \mu g \text{ gastrin extract}$$

(*e*) Assay value for yXu (obtained by substitution in eqn. (2) or more simply by multiplying the value for Xu by the value for y)

$$= 77{\cdot}7\ \mu g \text{ gastrin extract.}$$

The quantities of gastrin extract used for Xu and yXu in this instance were 25 and 75 μg respectively.

RESULTS

Accuracy of the assay method

This was tested by making fifty-three estimations in fifteen cats with gastrin extract, synthetic human gastrin I or pentagastrin as the background Xs, when known quantities of the respective stimulants were used as 'unknowns'. Thirty-one observations were made in eleven cats when 50 μg gastrin extract was used as Xs (2·5 μg gastrin extract $\equiv$ 1 ng S.H.G.I.); fourteen observations were made on three cats with 20–40 ng synthetic human gastrin I as Xs and eight observations in one cat when 20 ng pentagastrin was Xs. The range of doses of the 'unknowns' was 10–90 μg gastrin extract, 6–80 ng synthetic human gastrin I and 10–30 ng pentagastrin. Over this range, the $\log_{10}$ dose expressed as a percentage of the background dose was directly proportional to the acid secretory response occurring in the 15 min following the injection of the unknown, expressed as a percentage of the response which it is predicted would have occurred to the background dose in this same 15 min period (values VXu and $VyXu$). The results are illustrated in Fig. 2 and the regression line fitted to these data provides a dose–response line common to all of the assays in all of the animals. The index of precision (Loraine & Bell, 1966) for these log dose–response data is 0·19.

Greater precision in assay is, however, achieved if instead of assessing the activity of an unknown by reference to such a dose–response line, the assessment is made with aid of a correction factor (K) derived from analysis of the responses to two proportions of the unknown obtained one after the other. The results obtained when the activities of the unknowns are estimated in this way are illustrated in Fig. 3.

Storage and assay of gastrin in cat plasma

This was studied by adding pentagastrin or synthetic human gastrin I to arterial plasma freshly collected from five fasting, anaesthetized cats.

The plasma samples were stored on ice or at 4° C in a refrigerator for varying intervals until they were assayed for their gastrin activity. The gastrins were added in concentrations varying between 4 and 40 ng/ml. and the samples were stored for up to 27 hr. It is clear from Fig. 4 that the stability of the gastrins under these conditions was not affected by their concentration in the plasma. The mean percentage recovery for synthetic

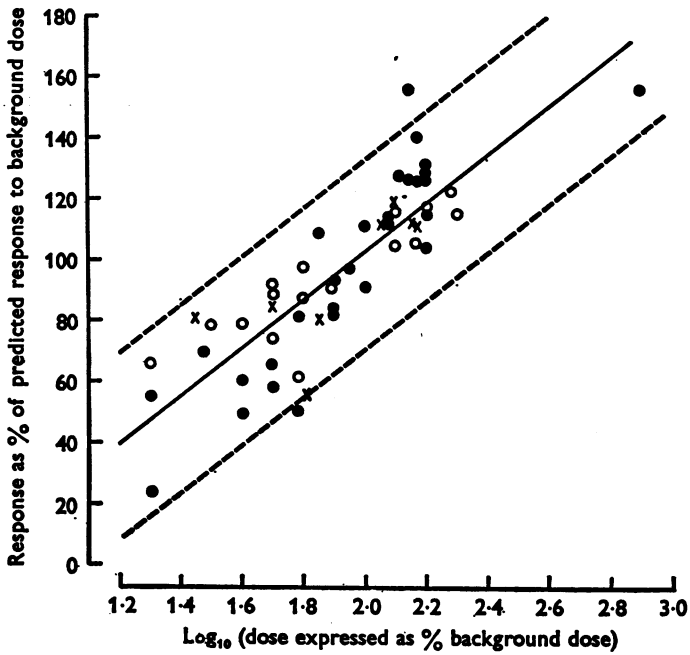

Fig. 2. Illustrating the relationship between the acid secretory responses in the 15 min period following the injection of each unknown (expressed as % of the calculated secretory response it is predicted would have occurred to the background dose in that same period) and the $\log_{10}$ dose expressed as a % of the background dose. Results with gastrin extract (●), S.H.G.I. (○), and pentagastrin (×) ($r = 0{\cdot}8515$; $P < 0{\cdot}001$). The slope of the regression ($m = 78{\cdot}04$) is indicated by the continuous line and the interrupted lines represent the 95% confidence limits about the y values ($\pm 30{\cdot}1$).

human gastrin I was 97·3% (S.D. ±15·2%) and for pentagastrin it was 108·5% (S.D. ±19·8%). There was no correlation between the percentage recovery and the duration of storage up to 27 hr (S.H.G.I. $r = 0{\cdot}3359$, $P > 0{\cdot}1$; pentagastrin, $r = 0{\cdot}3031$, $P > 0{\cdot}1$). When, however, in similar experiments, synthetic human gastrin was stored in plasma for longer periods, there was evidence for increasing loss of gastrin activity with increasing duration of storage. There is a significant negative correlation

between the gastrin activity remaining and storage times between 40 and 174 hr ($r = 0{\cdot}9461$; $P < 0{\cdot}001$).

Effect of incubation at 37° C on gastrin in cat plasma

This was studied by adding pentagastrin or synthetic human gastrin I to freshly collected arterial plasma to give concentrations of 200 ng/ml. and 320 ng/ml. respectively. Blood was collected from two fasting anaesthetized cats and immediately centrifuged. Plasma from one of the cats

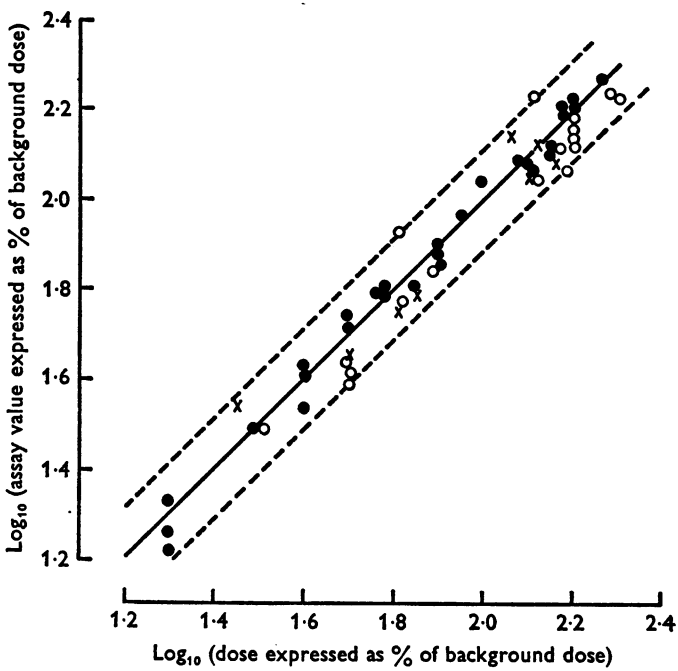

Fig. 3. Illustrating the relationship between $\log_{10}$ assay values of 'unknowns' expressed as a % of the background dose and $\log_{10}$ actual doses expressed as a % of the background dose. Results with gastrin extract (●), S.H.G.I. (○) and pentagastrin (×) ($r = 0{\cdot}9822$; $P < 0{\cdot}001$). The slope of the regression ($m = 0{\cdot}9806$) is indicated by the continuous line and the interrupted lines represent the 95% confidence limits about the y values ($\pm 0{\cdot}107$).

was used for the experiments with pentagastrin and plasma from the other cat was used for the experiments with synthetic human gastrin I. Aliquots of 8–10 ml. of the plasmas were set aside for the determination of their endogenous gastrin activity. The remainder of the plasma was in each instance brought to 37° C before the exogenous gastrins were added. The time which elapsed from the collection of the blood to the addition of pentagastrin or synthetic human gastrin I was less than 30 min. Immedi-

ately after the gastrin was added, the plasma was returned to the incubator at 37° C. At intervals of 5, 10, 15, 20 and 30 min after the addition of the gastrins, aliquots of the plasmas were removed from the incubation mixture. The plasma samples were stored on ice before the assay. All of the assays were completed within 5¼ hr of the addition of pentagastrin or synthetic human gastrin I to the plasma. The mean recovery from the

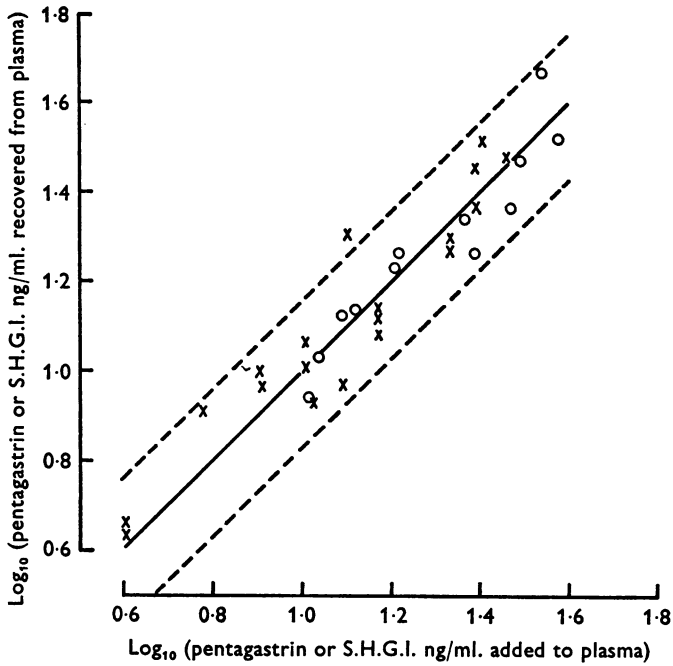

Fig. 4. Illustrating the recovery of added pentagastrin and S.H.G.I. from fresh cat plasma after storage on ice or at 4° C for varying intervals up to 27 hr. Results with S.H.G.I. (○) and pentagastrin (×) (r = 0·9469; $P < 0·001$). The slope of the regression (m = 0·9205) is indicated by the continuous line and the interrupted lines represent the 95% confidence limits about the y values (± 0·166).

five aliquots of the plasma sample to which pentagastrin was added was 91·8 S.E. ± 2·6% and from the five aliquots of the plasma sample to which synthetic human gastrin I was added it was 105 S.E. ± 1·4%. There was no correlation between the recovery values and the duration of incubation at 37° C ($P > 0·1$).

Half-life of pentagastrin and synthetic human gastrin I in the circulating plasma of cats

This was studied following the instantaneous injection of pentagastrin or synthetic human gastrin I into the saphenous vein of fasting, anaes-

thetized cats. These doses were immediately washed into the circulation with 1 ml. saline (0·9 g NaCl/100 ml.). Samples of arterial blood were collected 1½ min after the injection of gastrin and at least two other samples were collected at approximately 3, 6, 9, 12 or 15 min after the injection. The exact times of collection were noted. About 3 sec before a blood collection, the clamp on the distal end of the arterial cannula was removed and the dead space of the cannula was cleared and 0·5 ml. blood run to waste. Collection of the blood samples took 3–60 sec depending on the volume of blood to be collected and the nominal time of collection was recorded as the time half-way through the collection period. Blood was collected up to 13 min after the injection of pentagastrin and up to 15 min after the injection of synthetic human gastrin I. Approximately 2 ml. blood was collected at 1½ and 3 min. At 6 min, 3 ml. blood was removed in the synthetic human gastrin I experiments and 6 ml. in the pentagastrin experiments; at 9 min the volumes were 5 and 15 ml. respectively; at 12 and 13 min they were 7 and 20 ml., and 15 ml. blood was collected for the 15 min sample in the synthetic human gastrin I experiments. These volumes usually provided sufficient plasma to allow duplicate assays on the samples except in the case of the 12–13 min sample after pentagastrin. The 1½ min samples were always assayed in duplicate or triplicate. When more than one estimation was made on a plasma sample, the mean result is recorded.

Experiments were carried out on nine cats and the results are shown in Table 1. The altering concentration of pentagastrin in circulating plasma was followed on nine occasions after single injections of pentagastrin in five animals. The doses of pentagastrin varied from 14 to 400 μg/kg body wt. The altering concentration of synthetic human gastrin I in the circulation was followed on five occasions after single injections of synthetic human gastrin I in five animals. The doses of synthetic human gastrin I varied from 14 to 57 μg/kg body wt. In two animals the effects following more than one pentagastrin injection in the same animal were studied and in one animal the changes following separate injections of both synthetic human gastrin I and pentagastrin were studied. When more than one injection was given these were separated by intervals of between 1 and 3 hr and no subsequent injection was given before the completion of the acid secretory response to the previous injection. The plasma concentrations on assay varied between the equivalence of approximately 4 and 1000 ng S.H.G.I./ml.

In order to be able to compare the half-lives of both pentagastrin and synthetic human gastrin I despite the injection of different doses into different animals, the gastrin concentrations in the 1½ min plasma samples are taken as 100 % and all subsequent plasma concentrations in the same experiment are expressed as a percentage of the concentration in the 1½

TABLE 1. An analysis of the changing concentrations of gastrin activity in the plasma with time, following the instantaneous, I.V. injection of synthetic human gastrin I or pentagastrin. Time refers to the interval elapsing after the injection of the gastrin peptide

Synthetic human gastrin I

Time (min)	Log_{10} values of plasma gastrin concn. expressed as a % of the concn. at 1½ min				
3	1·7930	—	1·7796	1·6902	1·7193
5½	—	1·5930	—	—	—
6	1·4440	—	1·4654	1·3243	1·3579
9	1·0860	—	1·1206	1·0828	1·0086
9½	—	0·9570	—	—	—
12	0·8870	—	0·8513	0·7482	0·7000
15	—	—	0·5441	—	0·3617
Correlation coefficient	−0·9940	−0·9921	−0·9986	−0·9909	−0·9975
P	< 0·001	< 0·1 > 0·05	< 0·01	< 0·01	< 0·001

Pentagastrin

Time (min)	Log_{10} values of plasma gastrin concn. expressed as a % of the concn. at 1½ min								
2	1·7404	1·8722	—	—	—	—	—	—	—
3	—	—	—	1·3874	1·6493	1·4969	1·5079	1·5575	1·4914
4	1·3160	1·3222	1·2355	—	—	—	—	—	—
6	0·8195	—	—	1·0000	1·0128	0·9031	0·8261	1·0864	—
6½	—	—	—	—	—	—	—	—	0·7076
8	—	—	0·4914	—	—	—	—	—	—
9	—	—	—	—	0·7709	0·6812	0·2788	—	—
9½	—	—	—	—	—	—	—	—	0·1761
12½	—	—	0·1139	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	1·8062
Correlation coefficient	−0·9943	−0·9999	−0·9675	−0·9494	−0·9781	−0·9636	−0·9940	−0·9852	−0·9736
P	< 0·01	< 0·01	< 0·05	> 0·1	< 0·05	< 0·05	< 0·01	> 0·1	< 0·01

min sample. The $\log_{10}$ values of these percentages are illustrated in Table 1 and Fig. 5. Statistically significant negative correlations were found between the changing plasma gastrin concentration and time after the injection of pentagastrin or synthetic human gastrin I in eleven of the fourteen experiments. In the remaining three experiments the values for r were $-0{\cdot}9921$, $-0{\cdot}9852$ and $-0{\cdot}9494$ but the Tables for the levels of

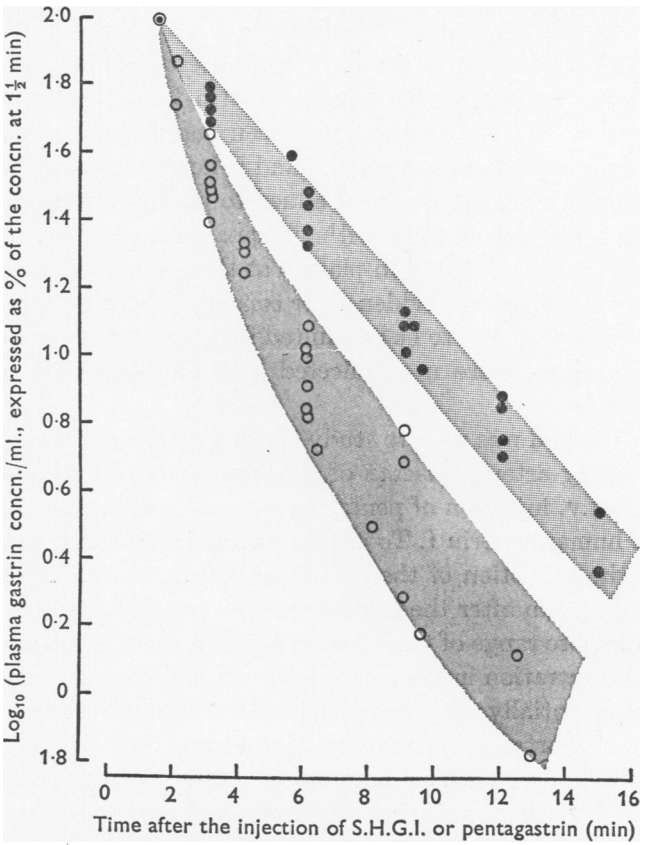

Fig. 5. Illustrating the changes with time in plasma gastrin concentration relative to the concentration in the plasma 1½ min after the injection of synthetic human gastrin I (●) and pentagastrin (○). The shaded areas include the range of values obtained after the injection of each of the gastrins.

significance of r had to be entered with only 1 degree of freedom. The mean value for the slopes of the regressions relating changing plasma gastrin concentration and time in the synthetic human gastrin I experiments is $-0{\cdot}115$ S.E. $\pm\, 0{\cdot}004$ and the mean value in the pentagastrin experiments is $-0{\cdot}206$ S.E. $\pm\, 0{\cdot}012$. There is a significant difference between these mean values as determined by Cochran's modification of the t test ($t = 6{\cdot}87$;

t at $P = 0{\cdot}05$ is 2·36). There is no significant correlation between the dose/kg body wt. and the slopes of the regressions in response to these doses when either synthetic human gastrin I or pentagastrin is injected ($P > 0{\cdot}1$). Calculated from these data, the half-life for pentagastrin in the circulating plasma is 1·50 min (S.E. $\pm$ 0·08) and for synthetic human gastrin I it is 2·65 min (S.E. $\pm$ 0·09).

DISCUSSION

The method of bio-assay of gastrin which is described can be carried out with as little as 30 ng synthetic human gastrin I at a minimum concentration of 2·5 ng/ml. The method has been successfully applied to the assay of gastrin activity in cat plasma and it has been shown that pentagastrin or synthetic human gastrin I added to plasma can be stored on ice or at 4° C for periods up to 27 hr without apparent loss of gastrin activity. When the gastrins were stored in plasma at these temperatures for longer periods, however, there was evidence for considerable loss of activity. This loss of activity is probably to be accounted for by bacterial contamination: the plasma samples were not collected and prepared with completely aseptic precautions.

The assay method was used to study the changing gastrin concentration in the circulating arterial plasma of cats for periods of 13 min after the instantaneous I.V. injection of pentagastrin and 15 min after the injection of synthetic human gastrin I. To ensure prior mixing of the gastrin in the circulation, the collection of the first plasma sample was in all instances delayed until 1½ min after the injection of the peptides. It is evident that despite a wide dose range of the injected gastrins there is a rapid reduction of gastrin concentration in the arterial plasma and that the concentration decreases exponentially with time (Table 1). The data relating $\log_{10}$ concentration in the arterial plasma with time can be fitted by a single term but could well be derived from populations better characterized by curvilinear functions that cannot with confidence be fitted to the present data. It is expected that dilution, destruction and elimination are all responsible for the reduction in the plasma concentrations of pentagastrin and synthetic human gastrin I. Because the data can be fitted by a single term it is likely that all of these factors persist during the period of study or if any are withdrawn they are too small a component of the total function or are withdrawn too slowly to be detected by the experimental method.

There is no reason to attribute the falling plasma gastrin concentrations to the destruction of the peptides within the circulating plasma. The stability of pentagastrin and synthetic human gastrin I in cat plasma was studied *in vitro*. They were added to freshly collected cat plasma to give concentrations well within the range observed during the course of the

half-life experiments. There was no evidence of destruction of the biological activity of either peptide incubated in freshly collected cat plasma at 37° C for periods up to 30 min. There is at present no indication of the stability in plasma of any other gastrointestinal hormones in a similar state of purity. Greengard, Stein & Ivy (1941) reported considerable destruction of the biological activity of crude secretin preparations incubated at 37° C in dog's whole blood, plasma or serum, although washed red cells were considered to be without effect. Greengard, Grossman, Woolley & Ivy (1944) found crude pancreozymin preparations showed some reduction in their biological activity after 6 hr incubation with dog serum.

It is unlikely that diffusion of peptides of the size of pentagastrin (mol. wt. 768) and synthetic human gastrin I (mol. wt. 2201) is completed within the first 1½ min after their intravenous injection. It has been shown that the 1 min dilution volume of D_2O in the cat is only 50% of the thiocyanate space and that it takes 3 hr for D_2O to equilibrate in cell water (Edelman, 1952). If diffusion into the extravascular fluids is a significant factor in the rapidly reducing concentrations of the gastrin peptides in the circulation after 1½ min, it is to be expected that the half-life of pentagastrin will be considerably shorter than that of synthetic human gastrin I. If in addition, their diffusion coefficients are mainly related to their molecular size, it is to be expected that the ratio of the observed half-lives of the two gastrins will be the same as the ratio of the square roots of their molecular weights. The ratio of the observed mean half-lives of pentagastrin and synthetic human gastrin I in the circulation is 0·57 (1·50:2·65) and the ratio of the square roots of their molecular weights is 0·58. The similarity of these ratios supports but does not prove the validity of the hypothesis that diffusion into the extravascular fluids is one important factor responsible for the observed, rapid reduction in the concentration of these gastrin peptides in the circulating plasma.

A number of other small molecular weight peptides have been shown to disappear rapidly from the circulation after intravenous injection. Isotopically labelled arginine-vasopressin (mol. wt. 1102) was shown to follow a single exponential function with time in the circulation of dogs and to have a half-life between 3½ and 7 min (Silver, Schwartz, Fong, Debons & Dahl, 1961). Similar results have been obtained with the unlabelled hormone (Share, 1962) and it is concluded that in the dog as in the rat (Dicker & Nunn, 1957), it is distributed in a fluid volume greater than the blood volume. Ginsburg & Heller (1953*a*) found this extravascular compartment in the rat was restricted to the renal and splanchnic regions. Oxytocin (mol. wt. 1025) also appears to penetrate into extracellular and intracellular fluid in the rat (Ginsburg & Smith, 1959). Glucagon (mol. wt. 3485) has been shown to have a half-life between 5 and 10 min in the cir-

culation of rabbits (Berson, Yalow & Volk, 1957) and in man (Unger, Eisentraut, McCall & Madison, 1961). Pure adrenocorticotrophic hormone has a half-life of about 5½ min in the circulation of intact male rats (Greenspan, Choh Hao Li & Evans, 1950). The half-life of 5 min for arginine-vasopressin in the circulation of the dog appears long compared with the half-life of 2·6 min in the circulation of the cat for the larger molecule of synthetic human gastrin I. There is, however, considerable species variation in half-life values of peptides in the circulation; vasopressin has a half-life of less than one minute in the circulation of intact male rats (Ginsburg & Heller, 1953*b*) and of about 3 min in rabbits (Chaudhury & Walker, 1959).

It is not known whether the peptide hormones are normally secreted into the circulation as free peptides or bound to a carrier protein. Nor is it known whether if secreted free or bound they are transferred to a carrier in the blood. There is some evidence that in the rat, vasopressin forms a larger molecular weight complex in the plasma when either naturally released (Thorn & Silver, 1957) or presented as the free octapeptide (Ginsburg & Heller, 1953*b*; Thorn, 1959). In the dog, however, it appears that endogenous vasopressin is present in the plasma in an ultrafilterable form (Bocanegra & Lauson, 1961). Even if these peptides are bound to a carrier in the plasma the binding may not be irreversible or be incompatible with rapid diffusion. Thyroxine which is avidly bound to plasma protein has a volume distribution of about 10 l. in man (Ingbar & Freinkel, 1960).

Diffusion alone cannot, however, be the only quantitatively important factor responsible for the falling gastrin concentration in the circulating plasma following the injection of pentagastrin or synthetic human gastrin I. If diffusion alone is assumed to be responsible, estimates can be made of the volumes in which the peptides are diluted at any point in time. Such estimates of the volume dilutions of the gastrins 1½ min after their I.V. injection are shown in Table 2. As would be expected, there is a tendency for the dilution volumes to increase as the dose increases. The 1½ min dilution volumes are all greater than the calculated blood volumes of the cats (Conley, 1941; Hamlin & Gregersen, 1939) and with the largest doses these volumes are considerably in excess of the thiocyanate spaces of the animals (Lands, Cutting & Larson, 1940). Concentration equilibrium was not reached even in those experiments in which the arterial plasma concentration of gastrin was followed for the longest periods. If concentration equilibrium is reached in the body fluids following single, rapid, I.V. injections of pentagastrin or synthetic human gastrin I, the peptide concentration at this stage must be a very small percentage of the concentration at 1½ min (Fig. 5). Because of this and the fact that the thiocyanate space in the cat is approximately 45% of total body water (Eggleton,

1951; Skelton, 1927) it follows that in the present studies the injected gastrin peptides must have been subjected not only to diffusion but also to considerable destruction or elimination or both. It is of interest that *in vitro* studies with homogenates of rat liver and small intestine show that these tissues contain amidase activity which will inactivate the N-acylated

TABLE 2. Volume of distribution of gastrin peptides in the body fluid of the cat, 1½ min after their injection (calculated on the assumption that no significant destruction or elimination of the peptides has taken place). The estimated volumes of dilution are also expressed as a % of the estimated thiocyanate space of the same animals (calculated on the assumption that the thiocyanate space is 288 ml./kg, Lands, Cutting & Larson, 1940)

Wt. of cat (kg)	Dose (μg/kg)	Plasma concn. at 1½ min (ng/ml.)	Volume of distribution at 1½ min (ml.)	Estimated thio-cyanate space (ml.)	Estimated vol. distribution at 1½ min as a % of estimated thiocyanate space
		Pentagastrin			
2·9	170	280	1790	835	214
2·9	170	274	1820	835	218
2·5	400	1000	1000	720	139
3·0	167	440	1140	864	132
3·0	83	322	780	864	90
3·0	42	150	830	864	96
3·0	17	137	370	864	43
3·6	14	95	530	1037	51
1·6	125	619	320	461	70
		Synthetic human gastrin I			
2·8	36	199	500	806	62
3·6	14	102	490	1037	47
2·1	57	260	460	605	76
2·5	56	290	480	720	67
3·85	37	382	380	1108	34

C-terminal tetrapeptide amide of gastrin (Laster & Walsh, 1968). If the rates of destruction and/or elimination of pentagastrin and synthetic human gastrin I are similar, this could account for the similarity in the ratios of the observed half-lives of the two gastrins and of the square roots of their molecular weights.

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