Br. J. Pharmac. (1971), **41**, 629–639.

Comparative study of antral gastrin activity in some mammals

B. O. AMURE AND A. OMOLE

Department of Physiology, University of Ibadan, Ibadan, Nigeria

Summary

1. The amounts of crude gastrin extract and the gastrin activities of extracts from antral mucosa of several mammalian species have been determined.

2. The yield of crude gastrin powder per gramme wet weight of antral mucosa was greater in goats and rabbits than in cat, dog, man or pig.

3. Statistical differences do not appear to exist between the potencies of gastrin from the various species. The potencies of the powders were within the ranges 1·2–1·8 μg porcine gastrin II/mg with the exception of the extract from frozen dog antra (0·8 μg/mg).

4. During prolonged freezing of animal antra and consequent thawing before extraction, significant losses in gastrin activity occurred in the dog.

5. The amounts of gastrin activity per gramme wet weight of antral mucosa from herbivores (goat, rabbit and cattle) were greater than those from non-herbivores (cat, dog, man and pig).

Introduction

The isolation of the hormone gastrin, the elucidation of its structure and the more comprehensive characterization of its activities have now placed the endocrine function of some cells of the gastric antrum beyond doubt. The work in the Pavlovian School reported by Babkin (1928) and investigations of other workers have shown that meat extracts and some other constituents of food, stimulate the release of the antral hormone, thus the gastrin content of the antral mucosa may reflect the dietary habit of the animal. The present paper is concerned with a comparative study of the gastrin activity extracted from the antral mucosa of various mammals including carnivores (dog and cat), herbivores (cattle, rabbit and goat) and omnivores (hog and man).

Methods

Sources of material

Antra from weighed adult cattle and hogs were collected at an abattoir within minutes of slaughter and transported on ice to the laboratory. Adult dogs, goats, rabbits and cats, bought in the local market were weighed, killed in the laboratory and the antra were removed and placed on ice before extraction began within an hour of slaughter. Some dog stomachs were, however, stored frozen at −20° C for 6–16 days before extraction. Since the local rabbits, dogs and cats used are usually exposed to a variety of edible substances, it could not be assumed that the animals

were strictly bred on their acknowledged species diets, so some rabbits and dogs were bred on regulated diets in the laboratory, after which the gastrin concentrations in antra were compared with those of members of the same species whose diets were not controlled.

For this purpose, ten 7 day old puppies were obtained locally and maintained on a carnivorous diet of milk, boiled meat and tinned dog food (Spratt's Patent, London) for 6 months before slaughter; similarly, sixteen freshly weaned rabbits (4 weeks old) were recovered from the laboratory stock and fed on a vegetable diet (*Aspilia africana* and *Centrocema* leaves) for 6 months before use. Most of the human stomachs were obtained within 12 h post-mortem in cases of accidental death of healthy patients and from patients who died from non-gastrointestinal diseases ; a few human antra were, however, collected soon after gastrectomy but work with these fresh specimens has not been included in this report since the stomachs were from patients with ulcers and therefore, not normal.

The antrum is sometimes difficult to demarcate from the rest of the stomach and in the present experiments, was defined as the area extending from the pylorus to the body of the stomach. Recognition of the aboral limits of the body was based upon the anatomical demarcation of the pyloric region from the body by the incisura angularis on the lesser curvature.

Extraction of gastrin

The mucosa and some adhering submosa were cut away from the muscular layers of the antrum and after maceration in a food blender were treated by the method of Blair, Harper, Lake, Reed & Scratcherd (1961) for the preparation of a crude extract containing gastrin. In man, cattle, hog, goat and dog the antral mucosa from each animal was sizeable and therefore was either extracted singly or in batches of three or more; the antra of individual rabbits and cats were much smaller and had to be extracted in batches of four or more at a time. The macerated tissue was taken up in four volumes of water blended in a Waring Blender for 1·5 min and then simmered at boiling point for 10 minutes. It was cooled under the tap until the temperature fell to 28° C, filtered through glass wool and the filtrate was centrifuged for 10 min at 2,500 r.p.m. The supernatant was removed and treated with twenty volumes of acetone; a white precipitate was formed which was removed by filtration, washed with ether and allowed to dry. This product is referred to as 'crude gastrin powder' (C.G.P.) and the material was stored in this state.

The efficiency of the extraction procedure was assessed in order to avoid differential degrees of antral gastrin recovery from species to species. For this purpose, minced meat material recovered from filtration through glass wool of the mixture of water and boiled antral mucosa from specimen stomachs of each species during the first extraction process, was re-extracted beginning with the mincing stage and running the mucosa through the entire procedure up to precipitation with twenty volumes of acetone. The residual boiled mucosa from this re-extraction exercise was subjected to a third extraction, and the products were assayed against standard extracts to estimate the amounts of gastrin activity contained in the mucosa after each extraction and to obtain information on the number of extractions necessary to exhaust the gastrin activity contained in the antra of the respective species.

The thickness and toughness of the antral mucosa differed from species to species and in these extractions mucosae from the hog, cattle and goat which were thicker

were as a rule minced six or more times to attain a soft uniform texture; softer specimens recovered from man, rabbits, dogs and cats required less mincing (three or more times). On the day of assay, an aqueous extract of the powder was obtained by homogenizing 20 mg of powder in 10 ml of 0·9% NaCl solution and removing the insoluble remnant by centrifugation; this preparation (or dilutions of it) was injected into the test animals.

Assay of gastrin

This was based on the perfused rat stomach preparation described by Ghosh & Schild (1958). Male rats of the Wistar strain weighing 130–300 g were used. During the 24 h preceding the start of the assay, the rats were allowed free access to water but the normal pellet diet was replaced by cane sugar lumps to facilitate clearing of food debris from the stomach.

The animals were anaesthetized with urethane (0·6 ml/100 gm b.w. of a 25% w/v solution intramuscularly) and the stomach was prepared for perfusion by cannulation of the duodenum and intubation of the oesophagus. NaOH (0·00025–0·001 N) was perfused through the stomach at $1 \pm 0{\cdot}1$ ml/min and the pH of the effluent detected by a glass electrode in a flow assembly was recorded continuously. The pH values of the gastric effluent fluid from the unstimulated stomachs were usually within the range 6·5–7·5 in the assay rats during perfusion with 0·00025 N-NaOH at 1 ml/ minute. Occasionally it was necessary to use higher concentrations of NaOH (0·001 N). For instance, in some unstimulated stomachs the basal secretory activity was high (effluent pH 4·0 or less) and in these circumstances the addition of further acid on stimulation of the stomach would effect little or no change in the pH of the perfusing fluid. In two rats, the perfusion fluid (0·00025 N) was calibrated to determine the range of acidity over which it would act as a linear buffer. This was done by passing the perfusion fluid into the unstimulated rat stomach at a rate of $1 \pm 0{\cdot}1$ ml/min and the pH of the gastric effluent was continuously recorded until it became reasonably stable. Then the effluent fluid over each period of 8 min was collected and titrated in each case to given different acid pH values with 0·01 N–HCl.

The principle behind this procedure is that the amount of 0·01 N–HCl required for titrating to a given pH could be taken to indicate the rate of HCl secretion by the stomach required to effect the same pH change in the gastric effluent. For the two rats, it was found that within the range pH 8·5–4·0 for one and pH 7·0–3·6 for the other, pH was linearly related to the moles of added HCl (Fig. 1). In both animals, identical doses of porcine gastrin (800 μg of aqueous extract of the crude powder) elicited similar rates of acid secretion which were indistinguishable from the effect of 25 μg pentapeptide (I.C.I. 50,123) in spite of the difference in the basal levels of acid secretion in the rats. All assays were designed as a $(2+2)$ dose assay comparison of the gastrin activity of the extracts from the antral mucosae of the various species with a laboratory standard extract prepared from hog stomach. The $2+2$ assay procedure consisted of high and low doses of the standard and test drugs in a randomized order to obviate possible cumulative effects. The doses of antral extracts used were within the range 100–800 μg and the standard porcine extract supplied by Brown, which contained known amounts of gastrin II, was injected in doses of 5–40 μg.

The responses of the rat to the varying doses of antral extracts used in assays as well as to some doses of histamine (100–800 μg histamine dihydrochloride) and pentapeptide I.C.I. 50,123 (6·25–25 μg) were preliminarily sought by assaying graded doses of a secretagogue on a rat. For this purpose, both the standard and test gastrins were required to have equal logarithmic spacing of doses injected, implying that when the standard solution was given in doses of S and 2S units, the test solution was administered in doses of U and 2U units. The assay of each single extract against the standard laboratory gastrin was performed in up to twenty different rats and for the calculation of relative potencies of extracts as described by Irwin (1937) and Bliss & Marks (1939) it was accepted as a prerequisite that in the same animal preparation, the 2+2 dose assay log dose—response lines for the standard and test gastrin extracts should be parallel. In this work, Student's *t*-test was used to assess the significance of differences between means, and values of $P \leqslant 0{\cdot}05$ were regarded as statistically significant.

Assay of histamine

Extracts were tested for the presence of histamine with the isolated guinea-pig ileum using a modification of the technique of Adam, Hardwick & Spencer (1954).

Results

The extraction procedure adopted in this work was capable of uniformly recovering in a single extraction 95% and more of the gastrin activity present in the antral mucosa of each of the species used, provided the differential requirements of mucosal mincing as previously indicated were satisfied. In most species no further activity could be recovered after the first extraction.

The yield of crude gastrin powder from the antral mucosa of each of the species largely reflects the quantity of the starting material (Table 1, columns 1–4). How-

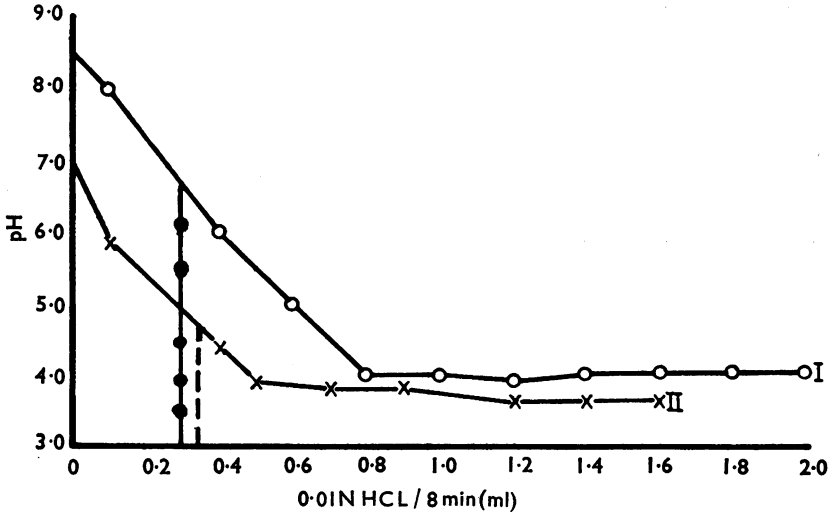

FIG. 1. Titration curve for N/$_{4000}$ NaOH (perfusion fluid) after passage through unstimulated rat stomach in two experiments on two rats. (●–●–●), Response to 800 μg porcine gastrin or 25 μg pentagastrin (I.C.I. 50,123); (– – –), response to 800 μg crude porcine gastrin.

TABLE 1

Animal	No. of animals used	Average weight (g) of antral mucosa per animal Mean±S.D.	Total weight of crude gastrin powder (g)	Crude gastrin powder (mg) per gramme wet weight antral mucosa Mean±S.D.	Gastrin activity: porcine gastrin II equivalent		
					μg/kg body weight	μg/g antral mucosa wet weight Mean±S.D.	μg/mg *C.G.P. Mean±S.D.
Goat	18	37·9± 4·9	26·2	38·5±6·4 (3)	101·4	54·1± 5·8 (3)	1·4±0·14 (3)
Rabbit	30	4·2± 0·6	2·67	21·3±2·8 (4)	48·6	32·8± 2·6 (4)	1·5±0·10 (3)
Rabbit (diet)	16	2·3± 0·2	0·90	24·0±3·6 (4)	43·7	34·1± 2·0 (4)	1·4±0·10 (4)
Cattle	24	160·1±15·0	61·68	16·1±2·5 (6)	13·4	28·5±15·7 (6)	1·8±0·5 (6)
Cat	20	4·2± 1·7	1·29	15·3±3·5 (6)	31·9	23·4± 9·6 (6)	1·5±0·7 (6)
Dog (frozen antra)	32	16·6± 3·8	8·3	15·6±4·3 (5)	23·6	11·4± 0·3 (5)	0·8±0·06 (4)
Dog (fresh antra)	20	20·7± 4·0	7·1	17·1±3·8 (4)	42·6	22·9± 1·5 (4)	1·3±0·15 (4)
Dog (diet)	10	7·4± 1·2	1·32	17·8±3·5 (3)	44·2	23·8± 3·3 (3)	1·4±0·20 (3)
Man	24	22·3± 3·0	7·38	13·8±2·6 (9)	16·9	19·1± 3·4 (9)	1·4±0·2 (8)
Pig	22	72·3± 6·6	19·7	12·4±3·3 (10)	11·2	15·2± 5·4 (10)	1·2±0·4 (9)

The number of extracts obtained from all groups of pooled antra for each species is shown in parentheses in columns 5, 7 and 8. *C.G.P., crude gastrin powder.

ever, when results are expressed as milligrammes powder per gramme wet weight mucosa, clear differences appear (Table 1, column 5), the highest value (goat) being more than three times the lowest value (pig) and also significantly greater ($P<0.001$) than in any other of the species tested. The second highest yield of crude powder per gramme mucosa was obtained in rabbit and although this was not significantly greater than the value for cattle ($P=0.3$), dog ($P=0.3$) and cat ($P=0.1$), the difference between the rabbit and pig values is significant ($P=0.01$).

After the intravenous injection of gastrin the pH of the rat gastric effluent fluid fell within 2–3·5 min (2·78 ± 0·10 min; mean ± S.D. in 100 observations), reached a minimum within 15 min (10·1 ± 0·5 min; mean ± S.D. in 100 observations) and returned to the preinjection level, in most cases completely, within 30 min of response (21·8 ± 1·1 min; mean ± S.D. in 150 observations). The second dose was, as a matter of procedure, injected as soon as the response to an earlier dose was over and the recovery pH of the effluent was maintained for a minute or two (Fig. 2). The responses of a rat to repeated injection of identical doses of gastrin extract were constant with no obvious sign of tachyphylaxis provided a steady stomach sensitivity had been attained. This was achieved by giving repeated equal doses of the test extract until constant responses of magnitude preferably 2·5–3·0 pH units were obtained for two consecutive doses (Fig. 3). In several instances when a single dose at the start of an assay experiment procured this change, the sensitivity of the stomach was thereafter largely unaltered. Figure 3 shows that for standard porcine gastrin, pentapeptide (I.C.I. 50,123) and crude porcine gastrin, the first dose of a secretagogue elicited a response which is quantitatively smaller than responses to subsequent identical doses. The graded doses of the antral extracts (100–800 μg) and doses of histamine dihydrochloride (100–800 μg) and pentapeptide I.C.I. 50,123 (6·25–25 μg) gave graded secretory effects which, when plotted, were linearly related to the logarithms of the doses (Fig. 4), hence the doses of the extracts used for the assays were restricted within this dose range. The index of precision of the assay technique as calculated from linear log dose-response regressions for graded doses of gastrin extracts was 0·15 or less, thus making the procedure acceptable for quantitative work (Loraine & Bell, 1966). The assays were reproducible, since the gastrin activity detected in any one extract was the same although responses to identical doses of an extract varied considerably from rat to rat. The laboratory standard preparation which was the extract prepared from the first hog antrum obtained, contained the equivalent of 1·2 ± 0·15 μg of gastrin II/mg powder (mean ± S.D.) from assay experiments in our laboratory against a histamine-free, partially

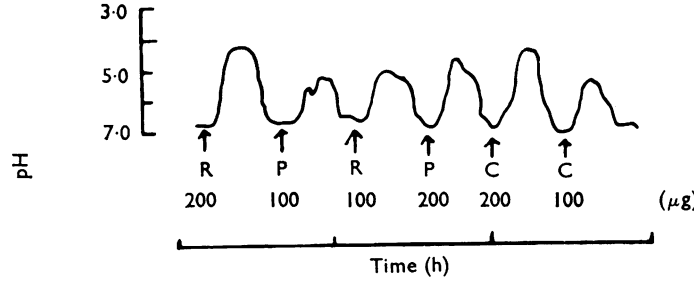

FIG. 2. Effects of intravenous doses of crude gastrin extracts in an anaesthetized rat. Doses (μg) are indicated in figures. R, Crude rabbit gastrin; P, crude porcine gastrin; C, crude cattle gastrin.

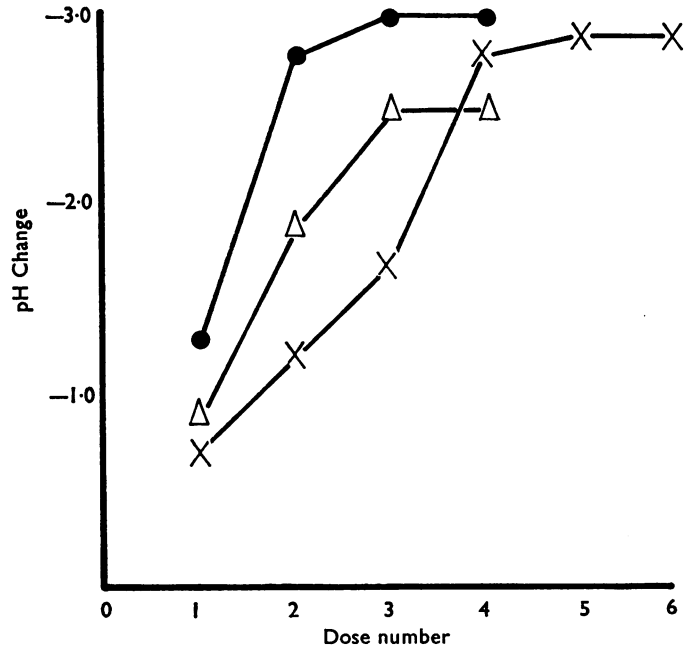

FIG. 3. Effect of repeated intravenous injections of secretagogues on acid secretion in the perfused stomach in three rats at the beginning of assay experiments. (●——●), Standardized gastrin (30 μg); (△——△), pentagastrin (25 μg); (×——×), crude porcine gastrin (800 μg).

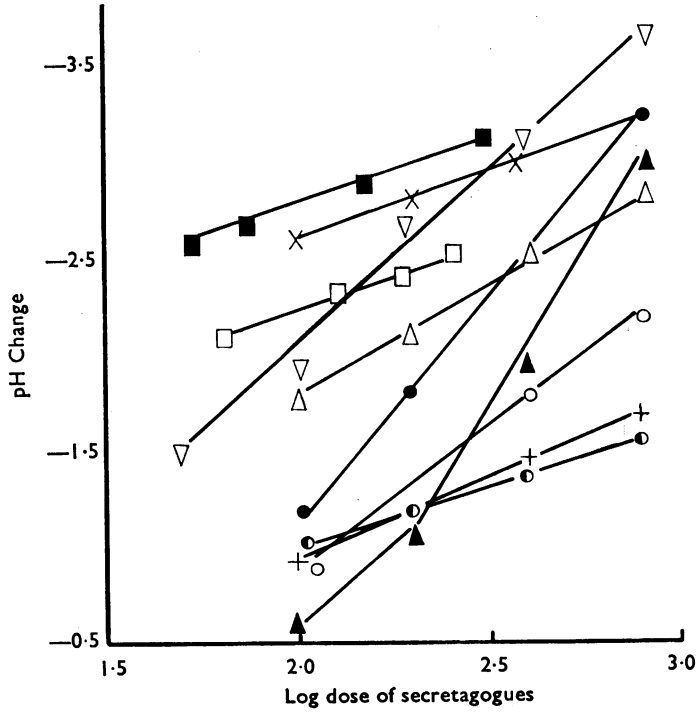

FIG. 4. Responses to graded doses of secretagogues in a rat. (■——■), Standardized porcine gastrin (units ×10 on dose scale); (×——×), histamine dihydrochloride; (□——□), pentagastrin I.C.I. 50,123 (×10 on dose scale); (▽——▽), crude cattle gastrin; (△——△), crude porcine gastrin; (●——●), crude human gastrin; (◐——◐), crude rabbit gastrin; (+——+), crude dog gastrin; (○——○), crude cat gastrin; (▲——▲), crude goat gastrin.

purified porcine gastrin extract prepared and standardized against pure gastrin II (gastrin II supplied by M.R.C., London) by Brown (1968).

When the species were ranked according to the gastrin activity obtained from each gramme of antral mucosa (Table 1, column 7) cattle fell into the same group as the other two herbivores, rabbit and goat. When ranking is based on the yield of dried powder (Table 1, column 5) the cattle value is lower than that for fresh dog antra. The upgrading of cattle in the former instance is dependent on the relatively high potency of the crude powder from that species, 1·8 μg gastrin II equivalent/mg powder. The range for all the species is 1·2–1·8 μg gastrin II equivalent/mg powder with the exception of the dog antra frozen for 6–16 days before extraction; this had a value of 0·8 μg gastrin II/mg crude powder, which is significantly lower than the values for both the freshly extracted dog antra and the diet-controlled dogs ($P=0·002$) (Table 1, column 8). There are no significant differences in either the yield of crude powder per gramme wet weight antral mucosa or the gastrin activity per gramme wet weight mucosa between the diet controlled dogs and the normal dogs ($P=0·9$; $P=0·7$, respectively) or between locally obtained rabbits and rabbits bred on a vegetable diet ($P=0·6$; $P=0·7$, respectively).

The goat had a significantly higher concentration of gastrin than the other species (Table 1, column 7); nevertheless, the potency of their crude gastrin powder did not differ significantly from that of the other animals and this outstanding gastrin concentration was attributable to the high yield of the crude gastrin per gramme antral mucosa provided by the species (Table 1, column 5). The extremes of mucosal gastrin concentration (pig and goat) (Table 1, column 7) differ by a factor of almost four and the general pattern emerges that the concentration is higher in the herbivores (goat, rabbit and cattle) than in non-herbivores (cat, man, pig and dog). On statistical consideration, however, the gastrin concentration in the goat is significantly higher than in all other species, and the value for the rabbit is significantly higher than for man and pig, both omnivores ($P<0·001$), and for dog and cat, both carnivores ($P=0·05$), while the cattle are not significantly different from the cat, man, dog or pig, presumably because of the tremendous variation in values from the individual groups of pooled antra extracted (μg gastrin II per gramme antral mucosa $28·5 \pm 15·7$; mean $\pm$ S.D.). The goat apart, in which the gastrin concentration was significantly higher than in the other herbivores, no statistical differences exist between the gastrin concentrations in individual species in other dietary groups; $P=0·1$ for the difference between pig and man, both omnivores, and $P=0·8$ for the difference between the cat and dog, both carnivores.

The gastrin II activity extracted per kilogramme body weight (Table 1, column 6) varied considerably from species to species, the highest value being obtained in the goat and the lowest in the pig. The values for the individual species within each dietary class are fairly close with the conspicuous exception of the herbivorous group where the values differ immensely, the gastrin II activity in goat being up to eight times that in the cattle but only double that in rabbit value. The diet controlled dogs and the dogs whose antra were extracted fresh gave similar values, which were higher than the value for the dogs whose antra were frozen for a period before extraction; the values for the diet controlled rabbits and normal rabbits were also similar.

The crude gastrin obtained from the various antra contained less than 0·05 μg histamine estimated as dihydrochloride per miligramme crude gastrin powder. The

stimulatory agent on the guinea-pig ileum was shown to be histamine and not gastrin itself, by the abolition of responses by the antihistaminic drug mepyramine maleate (Anthisan).

Discussion

The simple extraction method used in this work gave maximum yields and was chosen in preference to those which yield highly purified preparations of gastrin, because in the latter large and, possibly, variable losses could be incurred during the elaborate preparative procedures as suggested by Blair *et al.* (1961) and Gregory (1967). There is no reason to believe that the biological activity extracted from the crude powder was due to substances other than gastrin peptides. The perfused rat stomach is rather insensitive to histamine and in this work unequivocal gastric acid secretory responses were usually obtained after injection of a saline extract obtained from 100 μg of dried powder ; this amount of powder would have contained less than 0·005 μg histamine dihydrochloride which is only one-thousandth of the smallest dose of histamine (6 μg) that produces a definite effect in this preparation.

The short latency of responses to gastrin and the fairly accelerated nature of these responses as obtained in these experiments agree with the findings of Amure & Ginsburg (1964) in rats and Makhlouf, McManus & Card (1964) in man. This, coupled with the observation of Ghosh & Schild (1958) that there is a long onset of response of gastric acid secretory cells of rats to histamine and some choline esters renders it unlikely that any of these substances were responsible for our results. As already observed by Ghosh (1956) and Lai (1964) and now reported in this work, the first response of a rat stomach is usually irregular and this is reasonably attributable to a low state of responsiveness of the acid secretory cells. The subsequent increases in the responses of the stomach to identical doses of secretagogue up to the first three or four doses substantiate the findings of Rosenoer & Schild (1962) who reported that they found it necessary to give, at the start of an assay, three or more identical doses of carbachol in succession to stabilize the sensitivity of the acid secretory cells. Once a steady state of sensitivity was attained, no further alteration in the responsiveness of the secretory cells to secretagogues occurred. Constant responses to identical doses of gastrin extracts given in rapid succession—that is, a second dose given as soon as the response to the previous one ceased—have been reported by some workers (Vasseur & Parrot 1964 ; Amure & Ginsburg 1964). However, Barrett, Raventos & Siddall, 1966) reported the development of tachyphylaxis to gastrin when doses were administered at intervals of less than 90 min while Lai (1964) observed increases in the sensitivity of the rat stomach after four-five identical doses of gastrin.

The results of the biological assay of the extracts are expressed as equivalent weight of porcine gastrin II. The active principles in the extracts of four of the species, man, dog, cattle (Kenner & Sheppard 1968) and cat (Agarwal, Kenner & Sheppard 1969) are peptides closely related to, but not identical with, porcine gastrin II and therefore the absolute potencies in the rat very probably differ from that of the standard preparation. Thus, the results of the gastrin activity reported in this investigation do not give the actual amounts of the gastrin appropriate to each of the species.

Detection of the highest gastrin concentration in the three herbivorous species, goat, rabbit and cattle, was rather surprising since the non-herbivorous species

which require a much higher degree of acidity and peptic activity in the stomach for digestion would normally be expected to produce and, perhaps, store more of the hormonal stimulant of gastric secretion. The herbivores depend largely on bacteria and plant enzymes in the stomach for the digestion of cellulose which predominates in their vegetable diets. It is known, however, that the herbivores, especially those with ruminant stomachs, secrete considerable amounts of gastric juice daily (Kuznetsova 1950 ; Masson & Phillipson 1952)—even more than in the non-herbivores—and since the cephalic phase of gastric secretion is either absent or unimportant in the herbivores (Popov 1932 ; Espe & Canon 1937), the voluminous secretion of juice in these species would necessarily require a large production of gastrin. In herbivores too, the concentration of hydrochloric acid in the gastric juice appears to be less than is found in the non-herbivores (Grosser 1905 ; Masson & Phillipson 1952 ; Duke 1955), suggesting that the acid inhibition of gastrin production described by Dragstedt (1957), Posey, Smith, Turner & Aldridge (1965) and recently reiterated by Grossman (1968) would be less in the herbivores than in the non-herbivores. If, therefore, the herbivores could be viewed as producing more gastrin, it is not improbable that more gastrin would be stored in their gastric antra, particularly if the rate of release of the hormone relative to synthesis is lower in the herbivores than in the non-herbivores. A higher concentration of gastrin in the antra of the herbivorous species would necessarily imply either a higher count of gastrin-containing cells per unit area of antral mucosa in these species compared to the others or a greater storage per gastrin-containing cell ; both of these alternatives, however, remain speculative until the gastrin cell can be identified without doubt.

The observation that rabbits and dogs fed in the laboratory for a period on vegetables and meat, respectively, have the same concentration of gastrin in their antral mucosa as animals used for the comparative study would support the comparison made in this report, in spite of the fact that some animals were obtained from the local markets and that some antral mucosa were collected from abattoirs. The ranking of the values of the antral gastrin II activity per kilogramme of animal body weight while, approximating to a dietary pattern, appears to indicate that the concentration of gastrin in the animal antrum does not simply reflect the body weight of the animal. This latter aspect resembles a similar report published earlier by Marks & Young (1940) for insulin extracted from the pancreas in a variety of animal species.

REFERENCES

ADAM, H. M., HARDWICK, D. C. & SPENCER, K. E. V. (1954). Assay of histamine on the isolated guinea-pig intestine by the method of superfusion. *Br. J. Pharmac. Chemother.*, **9**, 360–365.

AGARWAL, K. L., KENNER, G. W. & SHEPPARD, R. C. (1969). Feline gastrin. An example of peptide sequence analysis by mass spectrometry. *J. Am. chem. Soc.*, **91**, 3096–3097.

AMURE, B. O. & GINSBURG, M. (1964). Inhibitors of histamine catabolism and the action of gastrin in the rat. *Br. J. Pharmac. Chemother.*, **23**, 476–485.

BABKIN, B. P. (1928). *Die Äussere Sekretion der Verdaungs drüsen.* 2nd ed. Berlin: J. Springer. Cited in *Secretory Mechanisms of the Gastrointestinal Tract* (1962), ed. Gregory, R. A. London: Edward Arnold.

BARRETT, A. M., RAVENTOS, J. & SIDDALL, R. A. (1966). Influence of some anaesthetics on pharmacologically stimulated gastric secretion in the rat. *Br. J. Pharmac. Chemother.*, **28**, 51–63.

BLAIR, E. L., HARPER, A. A., LAKE, H. J., REED, J. D. & SCRATCHERD, T. (1961). A simple method of preparing gastrin. *J. Physiol., Lond.*, **156**, 11–12*P*.

BLISS, C. I. & MARKS, H. P. (1939). Cited in *Methods in Hormone Research*, Vol. **2**, *Bioassay*, ed. Dorfman, R. I. (1962). New York and London: Academic Press.

BROWN, P. (1968). The storage and binding of gastrin. Ph.D. thesis, University of London.

DRAGSTEDT, L. R. (1957). The physiology of the gastric antrum. *Archs. Surg. Lond.*, **75**, 552–557.

DUKE, H. H. (1955). *The Physiology of Domestic Animals.* 7th ed., pp. 330–331. London: Baillière, Tindall and Cox, Duke, H. H.

ESPE, D. L. & CANNON, C. Y. (1937). Gastric secretion in ruminants. *Am. J. Physiol.*, **119**, 720–723.

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

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

GREGORY, R. A. (1967). Isolation and chemistry of gastrin. In: *Handbook of Physiology*, Section 6, *The Alimentary Tract*, ed. Code, C. Washington D.C.: American Physiological Society.

GROSSMAN, M. I. (1968). Immunoassay of gastrin. *New Engl. J. Med.*, **278**, 1348.

GROSSER, P. (1905). Untersuchungen über den Magensaft der Wiederkauer. *Zbl. Physiol.*, **19**, 265–266.

IRWIN, J. O. (1937). Cited in *Methods in Hormone Research* (1962), Vol. **11**, *Bioassay*, ed. Dorfman, R. I. New York and London: Academic Press.

KENNER, G. W. & SHEPPARD, R. C. (1968). Chemical studies of some mammalian gastrins. *Proc. Roy. Soc. B.*, **170**, 89–96.

KUZNETSOVA, V. M. (1950). Cited in Pavlov, I. P. *Theories and Farm Animal Physiology*, ed. Serebryekov, P. N. Moscow: Selkhozgiz.

LAI, K. S. (1964). Studies on gastrin. *Gut*, **5**, 327–341.

LORAINE, J. A. & BELL, E. T. (1966). *Hormone Assays and their Clinical Application*, p. 13. Edinburgh and London: E. and S. Livingstone Ltd.

MAKHLOUF, G. M., MCMANUS, J. P. A. & CARD, W. I. (1964). Dose response curves for the effect of gastrin II on acid gastric secretion in man. *Gut*, **5**, 379–384.

MARKS, H. P. & YOUNG, F. G. (1940). Species variation in the insulin content of pan creas. *Nature, Lond.*, **146**, 31–32.

MASSON, M. J. & PHILLIPSON, A. T. (1952). The composition of the digesta leaving the abomasum of sheep. *J. Physiol., Lond.*, **116**, 98–111.

POSEY, E. L., SMITH, P., TURNER, C. & ALDRIDGE, J. (1965). Effects of anticholinergics, antacids and antrectomy on gastrin production and relation of antral motility to gastrin release. *Am. J. Dig. Dis.*, **10**, 399–409.

POPOV, A. N. (1932). *On the Physiology of Sheep*, edit. by Popov, A. N. Moscow: Izdanke Enlio.

ROSENOER, V. M. & SCHILD, H. O. (1962). The assay of urogastrone. *J. Physiol., Lond.*, **162**, 155–162.

VASSEUR, B. & PARROT, J. L. (1964). Dosage biologique de la gastrine sur le rat. *J. Physiol., Paris*, **56**, 460.

(*Received April* 10, 1970)