PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 148, 1081–1086 (1975)

The Tissue Distribution of Tritiated Gastrin and Pentagastrin in the Rat (38692)

G. LANCIAULT, J. GISLON, J. P. ACCARY, AND S. BONFILS
(Introduced by F. P. Brooks)

Unité de Recherches de Gastroentérologie, INSERM U. 10 Hôpital Bichat, 170, Bd. Ney, F. 75877 Paris Cedex 18

The fate and tissue distribution of gastrin and pentagastrin has been studied in various species of animals including man. Newton and Jaffe (1) found that intravenously-administered radioiodinated gastrin in dogs disappeared very quickly from the blood and that the renal cortex, the gastric antrum and the gastric fundus significantly sequestered the labelled gastrin to a higher degree than did the other tissues sampled. No significant changes were seen in the liver. Stagg *et al.* (2) using ^{14}C pentagastrin in dogs, showed that the polypeptide also disappeared quickly from the blood but in contrast found that 80% of the radioactivity reappeared in the bile. Of this, 40% was unchanged pentagastrin.

Since gastrin and pentagastrin share a common C-terminal tetrapeptide which is essential for the biological activity of both molecules, it is of interest that the other amino acids found in gastrin alter the manner by which the hormone is handled in the body.

Recently Girma *et al.* (3) and Marche *et al.* (4) described methods for the labelling of gastrin and pentagastrin respectively with tritium having a sufficiently high specific radioactivity to be used in the study of the distribution of the hormone.

With the use of tritiated labelled gastrin and pentagastrin, studies were undertaken to determine:

1. the half-life ($t_{1/2}$) of gastrin and pentagastrin in the blood of the rat.
2. the distribution of gastrin and pentagastrin in the various tissues of the rat.
3. the distribution of the labelled materials in rat serum, using column chromotography, after passage in the animal.

Methods. Male Wistar albino rats with a weight range between 250 and 300 g were used throughout the study. After an overnight fast of 18 hr (water was given *ad lib.*), the animals were anesthetized with pentobarbital sodium, 50 mg/kg ip. Each animal received iv either gastrin, Human Synthetic Gastrin I: 1–17 (Imperial Chemical Industries, Macclesfield, England) or the C-terminal peptide analogue (BOC-Gly-Trp-Met-Asp-Phe NH_2), pentagastrin. The gastrin was tritiated according to the method of Girma *et al.* (3).[1] The method consists of a two-step tritiation in which a derivative iodinated on the tyrosine portion of the molecule, is used as an intermediate. Pentagastrin was labelled according to the method of Marche *et al.* (4) in which an o-nitrophenyl tryptophanyl derivative is made and submitted to catalytic hydrogenolysis in the presence of tritium gas. The tritiated forms of gastrin and pentagastrin retained 100% of their biological activity and had a specific radioactivity of 45 and 7 Ci/mmole respectively. A single dose of either 155 ng gastrin or 472 ng pentagastrin (equivalent to approximately 3.15 μCi) was given to each rat iv.

Disappearance of radioactivity from blood. Anesthetized and heparinized rats with a plastic catheter placed into the carotid artery were injected with either gastrin or pentagastrin. Arterial blood samples, 10 μl, were taken every 15 sec for the first min, every min for the first 10 min, every 5 min for the first hr and finally every 15 min for the second hr. Each sample was replaced with an equivalent amount of saline.

The whole blood sample was mixed with 1 ml of solubilizer and 15 ml of scintillator and counted. A quench curve was constructed to eliminate the influence of the color changes in the samples. The disappearance of radioactivity from the blood was

[1] We wish to thank Drs. J. P. Girma and J. L. Morgat of the Service de Biochimie, Centre d'études nucléaires de Saclay, for kindly supplying the tritiated materials.

plotted versus time on a semilog scale. The half-life ($t_{1/2}$) of gastrin and pentagastrin was determined by the use of the equation $y = Ae^{-kt}$.

Distribution of radioactivity in the organs. In a second group of animals, tissue samples were taken 10, 30, 45, 60, 90 and 120 min after injection of either gastrin or pentagastrin. Samples were obtained from skeletal muscle, esophagus, proximal duodenum, gastric rumen, fundus and antrum, liver, kidney, pancreas and colon. The samples (approximately 100 mg) were digested in 1 ml of solubilizer and diluted with 15 ml scintillator and counted. Quench curves were obtained to correct for the differences between the various tissues.

The radioactivity found in the tissue expressed either as a percentage of the total injected per g of tissue or of the concentration of gastrin (ng/g wet wt of tissue) was plotted against time.

Chromatographic separation of serum. In a third group of rats, serum samples, 500 μl, obtained 5, 30 and 120 min after iv injection of tritiated gastrin or pentagastrin were placed onto 15-cm-long chromatographic columns of G-25 Sephadex and eluted with 0.02 *M* ammonium bicarbonate (5). One ml aliquots were recovered and placed into 10 ml of scintillator fluid and counted. The elution curves were plotted against sample number and compared with a gastrin or pentagastrin standard curve.

Results. Disappearance of gastrin and pentagastrin from blood. Figure 1a represents a semilog plot of the radioactivity remaining in the blood as a function of time after a single iv injection of tritiated gastrin. The exponential curve, when separated into three phases, shows inflection points at approximately 2 and 7 min. During the first 2 min, the slope probably represents a nonspecific mixing of the gastric hormone in the blood. The calculated $t_{1/2}$ for this mixing is about 30 sec. Between 2 and 55 min the slopes represent the true metabolic degradation of gastrin. The fast phase (slope 2) has a $t_{1/2}$ of 1.8 min while the slower phase (slope 3) has a $t_{1/2}$ of >30 min. Beyond 75 min the radioactivity tended to increase.

A similar plot for pentagastrin (Fig. 1b) produced comparable results. When the curve was partitioned into three component slopes, the $t_{1/2}$ for slopes 1, 2 and 3 were 30 sec, 3.0 min and >30 min respectively. In contrast to gastrin, the radioactivity in the blood did not increase after 75 min post-injection.

Distribution of radioactivity in the organs.

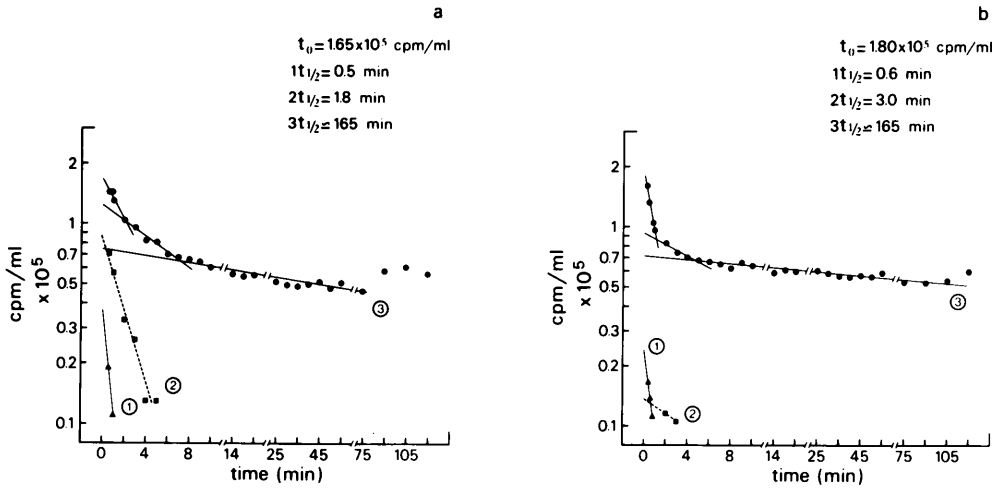

FIG. 1. A semi-log plot of the disappearance of a single iv injected dose of tritiated: (a) human synthetic gastrin I: 1-17 and (b) BOC-Gly-Trp-Met-Asp-PheNH$_2$, pentagastrin, from the blood of anesthetized rats. The curves are broken down into three components representing the various phases of distribution of the radiolabelled materials. Each point on the gastrin and pentagastrin curves represents the mean value obtained from 12 and 10 rats, respectively.

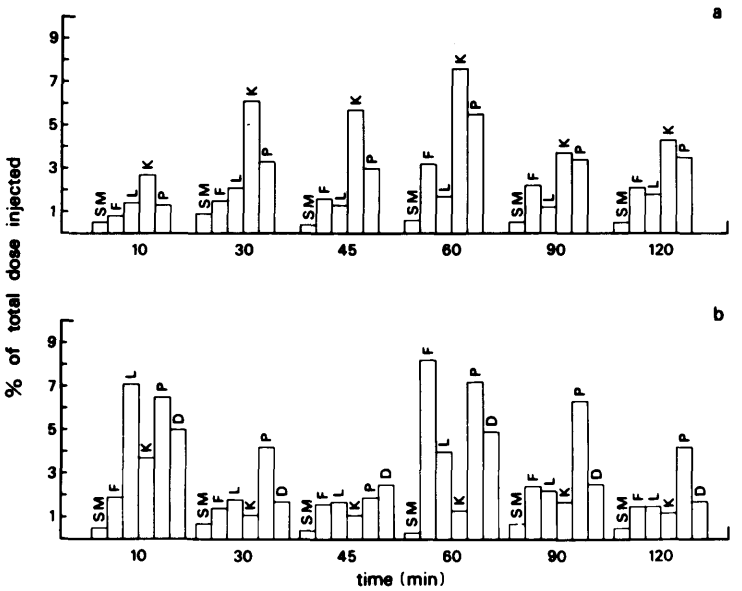

FIG. 2. Tissue distribution represented as a % of the total dose injected of tritiated: (a) human synthetic gastrin I: 1-17 and (b) BOC-Gly-Trp-Met-Asp-PheNH$_2$, pentagastrin, in the anesthetized rat over a period of 2 hr. Each value represents the mean from five rats. SM, skeletal muscle; F, gastric fundus; L, liver; K, kidney; P, pancreas; D, proximal duodenum.

Tissue gastrin and pentagastrin levels based on the calculated weight of the active material found in the tissue (ng of compound/g wet wt of tissue) or on a percentage of the total dose of labelled material recovered per g of tissue is represented in Figs. 2 and 3 respectively.

With tritiated gastrin, no real differences in tissue distribution of radioactivity were found between skeletal muscle (standard of reference), esophagus, gastric rumen and antrum, proximal duodenum and the colon. However, significant increases were seen in the gastric fundus, liver, kidney and pancreas (Figs. 2a and 3a). The levels of tissue radioactivity changed with time after injection. Within 10 min of injection, the kidneys showed the highest levels of radioactivity as compared with skeletal muscle. The kidneys continued to concentrate radioactivity and peaked at 60 min at which time the radioactivity represented 7.5% of the total dose injected. A similar pattern was also noted for the gastric fundus and pancreas. The highest activity for these two tissues was also found to be 60 min after injection. The liver showed significant increases after 10 min but then oscillated between greater or less activity during the remaining time of observation.

In contrast, with pentagastrin (Figs. 2b and 3b) radioactivity was found predominantly in the liver, pancreas and proximal duodenum. Ten min after injection, the liver contained approximately 7% of the total dose injected. Some radioactivity was found in the kidney but was not comparable to that seen with gastrin. The gastric fundus showed a single peak of approximately 8% of the total dose injected at 60 min. Highest activities in the tissues occurred 10 min after injection in contrast with 60 min for gastrin. However, a second peak was seen at 60 min with pentagastrin. The pancreas, 60 min after injection, appeared to have the greatest affinity for the tritiated pentagastrin in contrast to the kidney for gastrin at the same time period.

Chromatographic separation of serum. Elution of standard tritiated gastrin on a G-25 Sephadex column gave a separation curve (Fig. 4) consisting of a single major peak which came off the column in the void volume (4 ml). Standard tritiated pentagas-

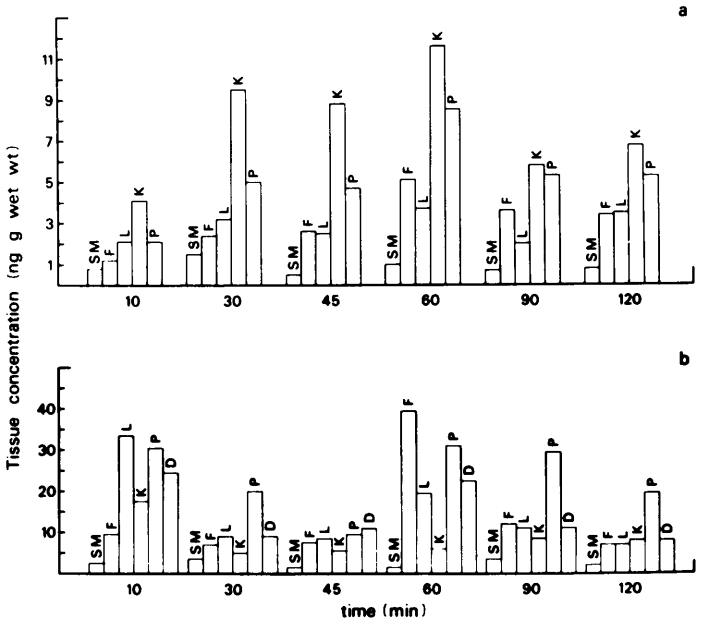

FIG. 3. Tissue distribution represented as the weight (ng/g wet wt of tissue) of tritiated: (a) human synthetic gastrin I: 1-17 and (b) BOC-Gly-Trp-Met-Asp-PheNH$_2$, pentagastrin, in the anesthetized rat over a period of 2 hr. Each value represents the mean from five rats. SM, skeletal muscle; F, gastric fundus; L, liver; K, kidney; P, pancreas; D, proximal duodenum.

trin also gave a single peak in a later volume consistent with its smaller molecular weight.

Rat serum obtained 5, 30 and 120 min after iv injection of either gastrin or pentagastrin and eluted in a similar fashion gave separation curves which were comparable to the standard curves in resolution but had a much lower radioactivity (Fig. 4).

Within 5 min of injection of gastrin, the chromatographic peaks were comparable with the standard curve. However, at 30 and 120 min, a second peak began to appear. After 120 min, although the total cpm of the curve was lower, the second peak was significantly increased.

Pentagastrin (Fig. 4), 5 min after injection gave an elution curve completely different from its standard. Two major peaks were obtained, one corresponding to the standard gastrin peak, and the second to the standard pentagastrin peak. At 30 and 120 min the peak corresponding to the gastrin standard increased such that at 120 min, it was significantly greater than the peak corresponding to standard pentagastrin. When the serum albumins of the rat were marked with bromphenol blue (BPB) the first peak corresponded with the BPB-marked albumins suggesting that with time pentagastrin or its radioactive breakdown products become attached to serum albumins.

Discussion. Tritiated labelled gastrin and pentagastrin injected iv into the anesthetized rat disappears very quickly from the blood. The half-life of 1.8 min for gastrin was found to be less than that previously obtained using different experimental conditions.

Newton and Jaffe (1) calculated a $t_{1/2}$ of 10 min, using a single injection technique of radioiodinated gastrin in dogs, while Accary *et al.* (6) obtained a half-life of 6 min in the rat. Reeder *et al.* (7) and McGuigan *et al.* (8), using a RIA technique, measured the disappearance of immunoreactive gastrin after a constant infusion of gastrin in dogs and found the half-life to be 10 and 4 min respectively.

Recently Walsh *et al.* (9) reported a $t_{1/2}$ value for gastrin which was comparable to the value obtained in our study. They found that the $t_{1/2}$ for immunoreactive human gastrin was about 3.2 min in the dog.

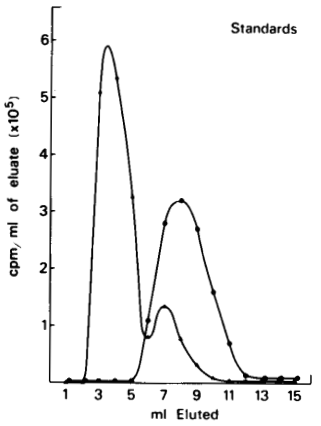

FIG. 4. Elution curves for standard tritiated human synthetic gastrin I: 1-17 (▲—▲), tritiated BOC-Gly-Trp-Met-Asp-PheNH$_2$, pentagastrin, (●—●) and for rat serum 5, 30 and 120 min after injection of the radioactive materials. The standards and serum samples were passed over 15 cm columns of Sephadex G-25, equilibrated and eluted with 0.02 *M* ammonium bicarbonate. One ml aliquots were recovered. Each point of the rat serum curves represents the mean value obtained from five rats.

The $t_{1/2}$ of pentagastrin has not been previously reported.

The difference in the half-life of gastrin in our study may be due to the fact that a tritiated gastrin given as a single dose may be metabolized more rapidly in the anesthetized rat as compared with the dog.

After gastrin had left the blood, it appeared that the kidney had the highest predilection for the labelled material. Elevated levels of radioactivity were also found in the liver, pancreas and gastric fundus. The localization of tritiated gastrin in the kidney is in agreement with the results of Newton and Jaffe (1) for radioiodinated gastrin in the dog. They found that within 15 min of injection, radioactivity was seen mainly in the kidneys. Although they did not measure the radioactivity in the gastric fundus, they found significant activity in the gastric antrum 60–180 min after injection. Some radioactivity was also seen in the liver and pancreas.

The high levels of radioactivity in the kidney and gastric fundus support the hypothesis that these are organs of catabolism for gastrin. Clendinnen *et al.* (10) have shown that the kidney is able to clear gastrin from the blood while Thompson *et al.* (11) reported that during periods of stimulated gastrin release approximately 30% of the gastrin is extracted by the gastric fundus of the dog. Further supporting evidence that the gastric fundus is able to modify gastrin was given by Bianca *et al.* (12). They found that radioiodinated gastrin administered to the dog with Heidenhain pouches could be recovered in the gastric juice of the pouch.

The tissue distribution of tritiated pentagastrin differed from that of gastrin. It appeared that the pancreas had the greatest affinity, although the liver and proximal duodenum sequestered significant quantities. Gastric fundic tissue showed a single but delayed augmentation in radioactivity. The cause of this phenomenon is not known. The peak biological response for the dose of pentagastrin administered is attained between 10 and 15 min postinjection, therefore this peak of radioactivity probably represents a nonspecific accumulation of either pentagastrin or its breakdown products.

Thompson *et al.* (13) and Stagg *et al.* (2), using ^{14}C pentagastrin, found that the liver was the primary metabolic organ for pentagastrin. Recently Wyllie *et al.* (14) demonstrated that pentagastrin is rapidly deaminated by rat, dog and human liver. Our results, showing that the pancreas had a high affinity for pentagastrin was surprising, for to date no evidence has been presented to substantiate this phenomenon. However, Walsh and Laster (15) reported that the pancreas and small intestine of the rat contains significant quantities of tissue amidase which could play a role in the catabolism of pentagastrin.

Although radiolabelled gastrin and pentagastrin is rapidly cleared from the blood, the chromatographic results presented support the hypothesis that the amount of material that remains circulating in the blood does not stay in its original form. This could be due to the fact that once metabolized, the radioactive breakdown products are re-released into the circulation. The results also suggest that a considerable quantity of pentagastrin or its breakdown products become bound to serum albumin.

Whether or not these modified forms are

biologically active cannot be determined since the concentrations are well below the sensitivity of a bioassay.

Summary. Tritiated synthetic gastrin I: 1-17 and a C-terminal peptide analogue (BOC-Gly-Trp-Met-Asp-PheNH$_2$) with a specific radioactivity of 45 and 7 Ci/mmole respectively, was found to disappear very rapidly from the blood when given iv to anesthetized rats. The calculated half-life for gastrin and the peptide analogue was 1.8 and 3.0 min respectively. The tissues which showed the greatest affinity for radioactivity with gastrin was the kidney, gastric fundus, liver and pancreas in the order of concentration. With pentagastrin, the pancreas, liver and proximal duodenum concentrated radioactivity to the highest degree.

The results also indicate that, once injected into the rat, the labelled products which remain in the blood undergo modification. Although the results presented do not give any indication how these modifications take place, it is possible that the organs which sequester the products modify them and then re-released them into the blood stream. Whether or not these modified forms are active biologically is open to conjecture.

This research was supported in part by a grant from the Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

Supported in part by a grant from the D.G.R.S.T. No. 73-7-1633.

1. Newton, W. T., and Jaffe, B. M., Surgery **69,** 34 (1971).
2. Stagg, B. H., Temperley, J. M., and Wyllie, J. H., Gut **12,** 825 (1971).
3. Girma, J. P., Morgat, J. L., Fromageot, P., Dubrasquet, M., Accary, J. P., Vatier, J., and Bonfils, S., *Biochimie* **56,** 763 (1974).
4. Marche P., Girma, J. P., Morgat, J. L., Fromageot, P., Chelis, C., Dubrasquet, M., and Bonfils, S., Europ. J. Biochem. **50,** 375 (1975).
5. Yalow, R. S., and Berson, S. A., Gastroenterology **58,** 1 (1970).
6. Accary, J. P., Sizonenko, P. C., Dubrasquet, M., and Bonfils, S., C.R. Soc. Bio. (Paris) **163,** 2538 (1969).
7. Reeder, D. D., Jackson, B. M., Brandt, E. N., and Thompson, J. C., Amer. J. Physiol. **222,** 1571 (1972).
8. McGuigan, J. E., Isaza, J., and Landor, J. H., Gastroenterology **61,** 659 (1971).
9. Walsh, J. H., Debas, H. T., and Grossman, M. I., J. Clin. Invest. **54,** 477–485 (1974).
10. Clendinnen, B. G., Davidson, W. D., Reeder, D. D., Jackson, B. M., and Thompson, J. C., Surg. Gynecol. Obstet. **132,** 1039 (1971).
11. Thompson, J. C., Reeder, D. D., Evans, J. C., Becker, H. D., and Brandt, E. N., Gastroenterology **64,** 810 (1973).
12. Bianca, S. S., Yip, C., and Jordan, P. H., Surgery 74, 412 (1973).
13. Thompson, J. C., Reeder, D. D., Davidson, W. D., Charters, A. C., Bruckner, W. L., Lemmi, C. A. E., and Miller, J. H., Ann. Surg. **170,** 493 (1969).
14. Wyllie, J. H., Stagg, B. H., and Temperley, J. M., Br. J. Surgery **61,** 22 (1974).
15. Walsh, J. H., and Laster, L., Biochem. Med. **8,** 432 (1973).

Received October 7, 1974. P.S.E.B.M. 1975, Vol. 148.