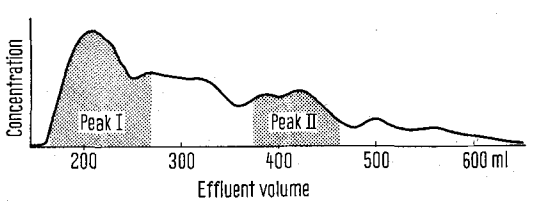
Evidence of Gastrin-Like Activity in the Rat's Antrum

Isolation and subsequent chemical analysis of gastrin obtained from hog, man, dog, cow and sheep has revealed close similarities in the structure of this polypeptide in the various species[1–6]. Gastrin resides mainly in the antrum, but some gastrin-like activity has been found also in the hog cardiac region[7–9] and in the first part of the duodenum in some other species[10–12]. At this institute the rat has become the most favoured species in studies of the nervous and chemical control of gastric secretion. It thus appeared desirable to establish whether gastrin activity can be extracted from the antrum or duodenum in rats.

In the first attempt we prepared crude acetone precipitated powders from the mucosa of the corpus, antrum and duodenum, employing the simple method of BLAIR et al.[13]. A preparation from the antrum, when injected s.c. into a conscious rat provided with a Heidenhain pouch, evoked a brisk flow of acid secretion. The preparations from corpus and duodenum were inactive.

The active antral preparation could unfortunately not be further purified by the well-known large-scale technique of GREGORY and TRACY[1], which proved unsuitable when the amount of starting material is small. As a suitable alternative, a water extract of the tissue was fractionated by a simple gel filtration procedure. In each pool the stomachs of 30–50 starved laboratory rats were used. Antral mucosa was scraped off with a blunt scalpel and for immediate use collected in an ice-chilled beaker or stored in a deep freezer for later processing. Mucosa, 2–3 g, frozen or fresh, was homogenized in 3 vol. of distilled water. The suspension was heated in a boiling water bath for 5 min, then cooled and sedimented by low-speed centrifugation (about 2000 *g*) for 5 min. The pellet thus obtained was resuspended in 1.5 vol. of distilled water and centrifuged again. The combined opalescent supernatants were centrifuged at 80,000 *g* for 30 min. A clear extract was obtained and tested for gastrin activity and histamine concentration. The concentration of this amine was less than 1.7 μg/ml, as assayed on an isolated guinea-pig ileum, an amount which is too low to interfere with the assays of gastrin activity. Samples of 6–7 ml of the extract were chromatographed on a Sephadex G-25 column (86 × 2.5 cm) and eluted with 0.03*M* NH_4HCO_3, pH 8.0. The transmission at 254 nm of the eluate was continuously recorded, and a typical run is shown in the Figure. Fractions of about 13 ml were collected, and gastrin activity was tested as follows: Fraction samples of 3–5 ml were evaporated to dryness under reduced pressure and the residue dissolved in 1 ml of Tyrode's solution. This volume was injected s.c. into a conscious Heidenhain pouch rat, and the secretory response of acid was recorded for at least 30 min or until the pre-injection level was restored. This type of pouch is particularly suitable because of its low and stable interdigestive secretion and consistency of secretory responses. No formal assay of gastrin activity was performed. Nevertheless, the procedure permitted a semiquantitative comparison between different samples. A threshold dose corresponded to about 0.1 μg hog gastrin II. Some tests required higher sensitivity which was achieved by a background infusion of a threshold dose of methacholine while the sample was injected i.v. This mode of administration of the extract appeared satisfactory as no adverse side-effects were noted.



Chromatography of rat antral extract on Sephadex G-25; 0.03*M* NH_4HCO_3, pH 8.0. Shaded areas indicate gastrin activity.

The Sephadex chromatography of the antral extract resulted in the separation into 2 peaks of gastrin activity (Figure), the major one (peak I) appearing in the macromolecular fractions eluted immediately after the void volume. The second peak (peak II), containing an estimated tenth of the total activity, was eluted at a larger volume and appeared in fractions containing low molecular weight substances. This separation technique gave highly reproducible results and in later experiments the positions of the activity peaks could be predicted from the shape of the elution curve, thus making it possible to escape some of the tedious and time consuming activity tests.

When pooled and concentrated fractions of peak I were rechromatographed, a further separation into 2 peaks of gastrin activity again occurred and were eluted at similar volumes as were the 2 peaks of the original extract. This suggests that the gastrin activity of our extract may be bound to some macromolecules forming a complex which easily dissociates when equilibrium is disordered. A logical step for continued work would be a search for methods which increase the yield of activity of peak II enough to make it accessible to further purification and characterization[14].

Zusammenfassung. In der Pylorusschleimhaut der Ratte wurde ein Gastrin ähnlicher Stoff nachgewiesen.

B. GRAHN and S.-E. SVENSSON

Institute of Physiology, University of Lund, Lund (Sweden), 27 November 1969.

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[14] We thank Prof. R. A. GREGORY, Liverpool, for a generous gift of hog gastrin II.