A New Preparation of Gastrin : Preliminary Characterization

This Laboratory is examining the chemistry of 'gastrin', about which little has been reported. We have recently prepared a gastrin-like secretory stimulant from porcine antral mucosa by a new and somewhat novel method. This is a preliminary report on its chemical properties, and similar data on 'gastrin' as prepared by a more conventional procedure. The quotation marks signify only that it is not known whether gastrin is a single compound, or a group of similar structures with a common unique moiety, or a group of biologically, more or less, specific substances. The facts that gastrin has been reported as dialysable[1], non-dialysable[2], with an isoelectric point near *p*H 5·0[3], or *p*H 8·0[4], to name only a few of its properties, leave the question very much open. Recently, Gregory *et al.*[5] described a gastrin-like substance isolated from a pancreatic tumour.

Different from any method we have found in the literature of this substance, we have extracted gastrin from hog antrum with aqueous alkali, *p*H 11–12, at 40° C. Subsequent steps included isoelectric precipitations at *p*H 5·0, and precipitations in sodium chloride solution. At no time was the preparation more acid than *p*H 4·0, or warmer than 40° C. Dialysis of the crude material against running water and several changes of distilled water followed by lyophilization or isoelectric precipitation gave yields of 0·5–2·0 gm. per 20 antral mucosæ.

At this stage we have had consistently good stimulation. Solutions of 75–100 mgm. (as little as 50 mgm. has produced good response) in 50 ml. of sterile saline were injected during ~30 min., using a motor-driven syringe. A sharp rise in secretory flow and of $[H^+]$ resulted, followed by a decline after the injection. For the half-hour of greatest activity, eleven experiments gave us an average rate of secretion of 12·4 ml./hr., and a rate of hydrogen ion production equivalent to 18·0 ml. of 0·01 *N* hydrochloric acid/hr. 80 per cent, or more, of these amounts represented increase over the base line. Our best eight experiments with material prepared by a modification of the Uvnäs method[3] gave comparable figures of 5 ml. (volume) and 10·6 ml. (0·01 *N* hydrochloric acid equivalent.

In our previous work on dialysed gastrin, as prepared by the Uvnäs method, we had often noted vomiting, or an inactive preparation, or acquired tolerance. In the dialysed material from the new method we have not observed such symptoms, and we have had no inactive preparations. Of considerable additional interest are the excellent results obtained with subcutaneous injections. A good flow persisted for 2½–3 hr. after the injection, contrasting with the rapid decline after histamine injection. One such active sample had been kept dry and in the dark at −20° C. for 8 months before the assay.

In the material prepared by acid extraction and trichloracetic acid precipitation[3] we had found a small but consistent content of phosphorus (average 0·05 per cent). We have found 10–15 times as much phosphorus in the new preparation (dialysed), that is, 0·5–0·8 per cent. Treatment with 0·25 *N* alkali at 27° C., isoelectric precipitation and re-dialysis did not lower the phosphorus content appreciably, making the presence of a typical phosphoprotein unlikely[6].

In the ash of the dialysed gastrin, as the only unique constituent in measurable quantity, we have consistently found tin. On standing in dilute stannous chloride and prolonged re-dialysis, the gastrin retained considerable metal ion (ash ~1 per cent, of which ~20 per cent was tin).

We have subjected the dialysed gastrin to diethylaminoethylcellulose ion-exchange treatment, Tiselius electrophoresis (one main component, through the kindness of Dr. P. D. Goldsworthy), and curtain electrophoresis with veronal buffer (*p*H 8·6, ionic strength 0·02) on Whatman 3-*MM* paper at 300–500 volts. The most interesting results have come from the last method. Electrophoresis of a 1 gm. sample of dialysed material led to recovery of 65 per cent (redialysed and lyophilized material). One main protein fraction was observed (~40 per cent) which migrated (minus to plus) a moderate distance, and gave strong colour with tetrabromophenolphthalein ethyl ester (potassium salt)[7]. Displaced further toward the positive pole, there was a smaller fraction (average 2–3 per cent after dialysis), which gave no protein colour test, and another protein-like material (~1 per cent). The phosphorus content of the main protein fraction was low (0·05 per cent or less); that of the non-protein fraction was high (4–7 per cent). The main fraction had an ultra-violet spectrum typical for proteins. The ultra-violet spectrum of the non-protein fraction had a sharp intense peak at 260 mμ. Much of the remaining phosphorus, which had not been accounted for, appeared to be on the curtain in the path of the main protein fraction. The latter appears to be bound to the high-phosphorus fraction somewhat tenaciously.

The protein after electrophoresis had considerably less secretory stimulant activity than the material before electrophoresis and appeared to be less stable. The dialysed gastrin and the main protein fraction on electrophoresis have each given a 10 per cent yield, or more, of crystalline material, as long fibrous needles, by an ammonium sulphate procedure. Full details of the extraction procedure, the chemical properties and our experience in tracing the activity will be presented shortly.

This investigation was supported in part by Grant *A*–2341, from the National Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service.

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