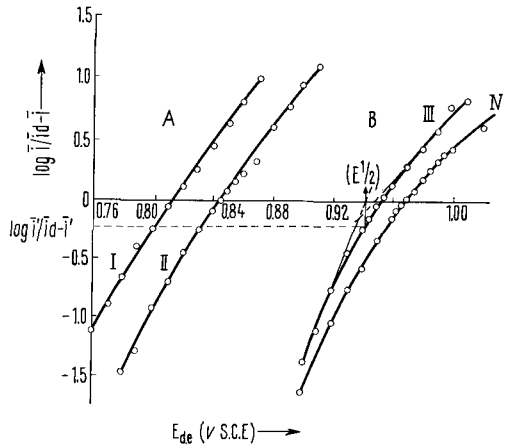
reversibly with the transfer of 2 electrons ($n = 2$). In view of the irreversible discharge of Cd (II) it was considered worthwhile to investigate the kinetics of electrode reaction.



Plots of $E_{d.e.}$ *v.* $\log \bar{i}/(\bar{i}d - \bar{i})$. Cd^{2+} in (I) 0.05 *M* TGA, (II) 0.06 *M* TGA, (III) 0.4 *M* TGA and (IV) 0.5 *M* TGA.

S. No.	TGA Concentration (*M*/l)	α	$K°$ cm/sec (22 °C)
1	0.05	0.540	2.770×10^{-3}
2	0.06	0.50	2.288×10^{-3}
3	0.40	0.505	2.343×10^{-3}
4	0.50	0.484	2.774×10^{-3}

α, mean = 0.506. $K°$, mean = 2.544×10^{-3}.

Determination of $K°$ and α. From the logarithmic analysis of the *c–v* curves of Cd^{++} in different concentrations of thioglycolate the kinetic parameters $K°$ and α are calculated according to the relations proposed by KORYTA[2].

The Figure represents the plots of $E_{d.e.}$ *v* $\log \bar{i}/(\bar{i}d - \bar{i})$. From the slope of the asymptote drawn at the negative part (marked A) of the log plots the value of n was evaluated, which in each case was found to be 2, and also the values of reversible half wave potential ($E_{1/2}$) given by the point of intersection of asymptote at voltage axis. The values of transfer coefficient (α) were calculated from the slope of the asymptote at the positive portion (marked B) of the log plots. After knowing the values of α and i' (obtained from ($E_{1/2}$) as shown in the Figure) (curve II) the values of $K°$ were determined. The Table illustrates the concentrations of TGA employed and the values of α and $K°$ obtained.

It is apparent from the Table that the values of α and $K°$ are almost constant. The rate of discharge of Cd (II) at D.M.E. is independent of thioglycolate concentration.

Zusammenfassung. Das polarographische Verhalten von Cd^{2+} wird beschrieben.

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[2] J. KORYTA, Electrochim. Acta *6*, 67 (1964).

[3] Acknowledgment. I wish to express my gratitude to my teacher, Prof. R. S. SAXENA, who interested me in polarography, put the best working facilities at my disposal and stimulated this work by many valuable discussions.

Isolation of Canine Gastrin

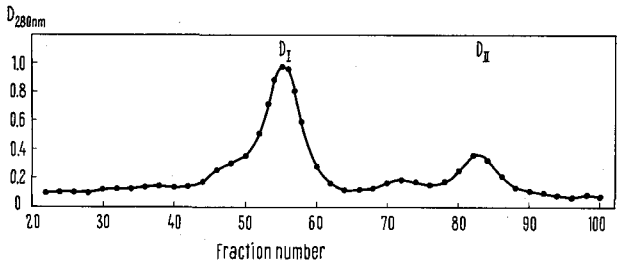
The hormone gastrin has now been isolated from the gastric antral mucosa of hog, man, sheep and cow[1]; the present account describes its isolation from antral mucosa of the dog. The chief difficulty, as anticipated, was the collection of sufficient antral mucosa to provide the minimal amounts of the purified peptides required for a definitive study of their constitution. On the basis of previous experience it was expected that about 10 µg gastrin would be obtained from one antrum, so that at least 100 antrums would be required for a single preparation; 2 such batches were processed. As opportunity offered, the antral mucosa was dissected from the stomachs of freshly killed animals and the strips boiled in a small volume of water for 5 min to inactivate enzymes; the mixture was then stored in the deep-freeze. When 100 such preparations had been obtained, the isolation was carried out following the general procedure already described for hog and human antral mucosa[2,3]. As in the latter case, the boiled antral strips were homogenised and re-extracted with water to obtain the maximal yield. The final stage of isolation, as before, was by gradient elution chromatography on a small column (1×5 cm) of aminoethylcellulose, using a concentration gradient of 0.04 to 0.4 *M* ammonium bicarbonate buffer. The Figure shows the result. The fractions corresponding to the peaks DI and DII were pooled and dried in vacuo until free from ammonium bicarbonate; they were then rerun in similar conditions to those described above to remove traces of contaminants.

Throughout the isolation procedure, the behaviour of the activity was followed by testing small aliquots of appropriate fractions for their power to stimulate gastric acid secretion in conscious dogs provided with denervated pouches of the gastric fundus. The final products showed the expected high potency in this respect; but owing to the very small amount of material available, no studies were made of the actions on other gastro-intestinal struc-

[1] R. A. GREGORY, Proc. R. Soc. B *170*, 81 (1968).
[2] R. A. GREGORY and H. J. TRACY, Gut *5*, 103 (1964).
[3] R. A. GREGORY, HILDA J. TRACY and M. I. GROSSMAN, Nature *209*, 583 (1966).

tures known to be displayed by the gastrin peptides of other species[1]. Detailed studies with synthetic material[4] will be reported.

From the first batch of antrums, a total of approximately 1 mg gastrin was obtained (DI + DII); in the second preparation, the yield was smaller, being approximately 800 µg total gastrin. As is evident from the Figure, the proportion of the unsulphated (DI) to the sulphated (DII) form is about 2:1. This is similar to the proportion found in man, but different from that found in the hog, sheep and cow, in which the sulphated form of the hormone predominates.



Separation by gradient elution chromatography of the dog gastrins (DI and DII) on aminoethylcellulose in ammonium bicarbonate buffer.

Zusammenfassung. Die Isolierung von zwei Gastrinen aus der Magenschleimhaut von Hunden wird beschrieben.

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The Physiological Laboratory, University of Liverpool (England), and Veterans Administration Center, Los Angeles (Illinois 90073, USA), 3 January 1969.

[4] K. L. Agarwal, G. W. Kenner and R. C. Sheppard, Experientia *25*, 346 (1969).

Structure and Synthesis of Canine Gastrin

Amino-acid analysis of canine gastrins I and II[1] showed that both DI and DII had the composition (Ala_2, Asp, Glu_5, Gly_2, Met_2, Phe, Pro, Trp_2, Tyr) and that the tyrosine in canine gastrin II was O-sulphated, as in human, porcine, bovine, and ovine gastrins II[2]. Amongst those gastrins, structural variations are confined to positions 5 and 10 in the heptadecapeptide chain, and the foregoing composition could be tentatively accommodated in an analogous [Met^5, Ala^{10}]-structure, which would make canine gastrin a hybrid of the porcine and bovine-ovine hormones. Papain degradation of canine gastrin I[3] afforded L-Glu-Gly-Pro-Trp-Met, confirming one of the tentative assignments, but the central portion of the molecule was cleaved so extensively that no clear results emerged and doubt was cast on the analogy with the bovine-ovine hormones. We therefore adopted the broader working hypothesis that canine gastrin I has one of the 5 structures summarized below; each is derived from porcine gastrin I by a single substitution of alanine for glutamic acid.

L-Glu-Gly-Pro-Trp-Met-(Ala, Glu_4)-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2
1 2 3 4 5 6–10 11 12 13 14 15 16 17

In order to conserve the small supply of canine gastrin, our next step was to synthesize all 5 heptadecapeptide amides for comparison of their enzymic 'fingerprints' with those of the natural hormone. The compounds were made by a general method originally devised for synthesis of porcine gastrin I[4] and also employed with minor improvements for synthesis of human gastrin I[5]; it has been superseded for preparation of pure gastrins in quantity, but it was particularly convenient for the present purpose of permuting the central sequence. The 5 octapeptide derivatives, comprising the sequence 6–13, with the general structure shown below were prepared by active

$$\text{BOC-(Ala, } \underset{\displaystyle \text{OBu}^t}{\text{Glu}_4}\text{)-Ala-Tyr-Gly-OMe}$$

ester condensations and then coupled, after saponification of the methyl ester, with the C-terminal tetrapeptide. Finally, after removal of protective groups by trifluoroacetic acid, the N-terminal pentapeptide was added by means of L-Glu-Gly-Pro-Trp-Met-N_3 (1–5 azide). The 5 heptadecapeptide amides were purified by chromatography on G-25 Sephadex and appeared to be homogeneous (thin-layer chromatography, electrophoresis). Samples (0.1 mg) were digested with papain at pH 7.0, subtilisin at pH 8.0, and thermolysin at pH 8.0, and the concentrated digests were examined by electrophoresis on Whatman No. 1 paper (pH 6.5, 6 Kv) alongside digests of canine gastrin I. Subtilisin differentiated the 5 synthetic compounds, while the papain digests of the [Ala^8] and [Ala^9]-isomers and the thermolysin digests of the [Ala^7] and [Ala^8]-isomers were not clearly distinguishable. The total conclusion was that the [Ala^8]-isomer was most similar in behaviour to canine gastrin I, but the identification was marred by the presence of an extra Ehrlich-positive spot on each of the patterns from syn-

[1] R. A. Gregory, H. J. Tracy, M. I. Grossman, D. de Valois and R. Lichter, Experientia *25*, 345 (1969).
[2] G. W. Kenner and R. C. Sheppard, Proc. R. Soc. B *170*, 89 (1968).
[3] P. H. Bentley, Ph. D. Thesis, Liverpool (1967).
[4] J. C. Anderson, G. W. Kenner, J. K. MacLeod and R. C. Sheppard, Tetrahedron Suppl. *8*, Part I, 39 (1966).
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