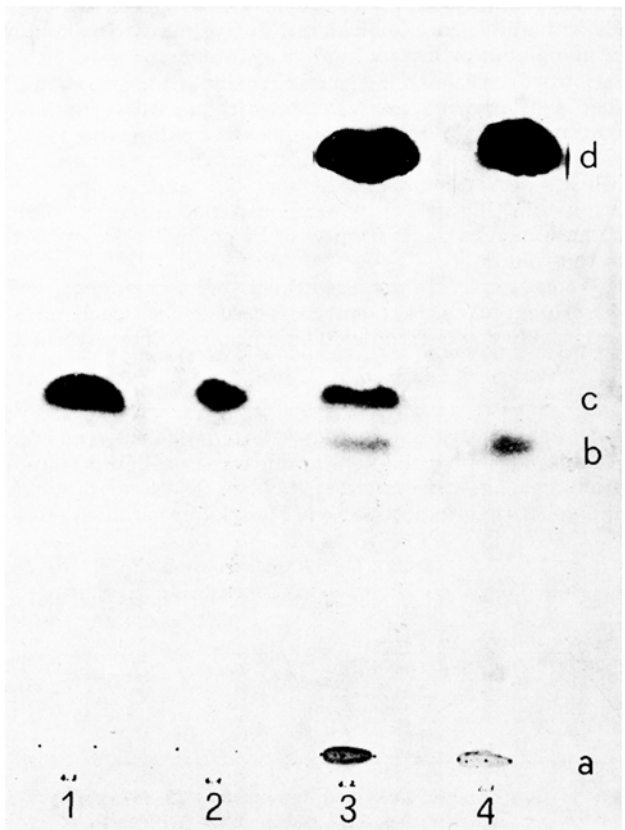
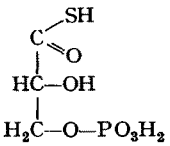
Produkten und Enzympräparat getestet. Anlagerungen von Sulfid an verschiedene Verbindungen sind aufgetreten. Sie beeinflussen aber die nachfolgenden Aussagen nicht. In der Figur sind die Resultate dargestellt. In Anwesenheit von $H^{35}S^-$ wird eine Verbindung markiert, welche sich als Phosphorsäureester der Thioglycerinsäure herausstellte:



Autoradiographie der Reaktionsprodukte. 1, 2, 3, 4 = verschiedene Ansätze (vgl. Text). a) Start; b) Phosphothioglyzerinsäure; c) Phosphoglyzerinsäure; d) anorganische Oxidationsprodukte des Schwefels.



Die beiden Isotope werden getrennt in die zwei C_3-Verbindungen eingebaut. Die Erklärung dazu liefert der Reaktionsmechanismus der Carboxylase[7]. Falls bei der Spaltung der äusserst unbeständigen C_6-Verbindung $H_2{}^{35}S$ die Funktion von H_2O übernimmt, entsteht neben einem ^{14}C-markierten Molekül Phosphoglyzerinsäure ein ^{35}S-markiertes Molekül Phosphothioglyzerinsäure. Die Carboxylase ist somit für die Synthese nicht direkt erforderlich, sondern lediglich für die Bereitstellung der instabilen C_6-Verbindung[8].

Summary. The presence of sulphide in the reaction mixture of the Ru-DP carboxylation leads to the synthesis of phospho-thioglyceric acid.

R. BRÄNDLE und J. MARTI

Pflanzenphysiologisches Institut der Universität, Altenbergrain 21, CH-3013 Bern (Schweiz),
6. Dezember 1970.

[1] K. H. ERISMANN und R. BRÄNDLE, Flora, Jena *A 159*, 379 (1968).
[2] R. BRÄNDLE, R. STRASSER und K. H. ERISMANN, Verh. schweiz. naturf. Ges. *68*, 122 (1968).
[3] R. BRÄNDLE und J. SCHNYDER, Experientia *26*, 1395 (1970).
[4] C. M. JOHNSON, Analyt. Chem. *24*, 736 (1952).
[5] P. SCHÜRMANN, J. Chromat. *39*, 507 (1969).
[6] Der GRD, Wimmis, Schweiz, danken wir für die Aufnahme und Interpretation der IR-Spektren.
[7] B. R. RABIN und P. W. TROWN, Nature, Lond. *202*, 1290 (1964).
[8] Die Arbeit wurde durch den Schweiz. Nationalfonds unterstützt.

Immunochemical Differentiation between Gastrin and Related Peptide Hormones through a Novel Conjugation of Peptides to Proteins

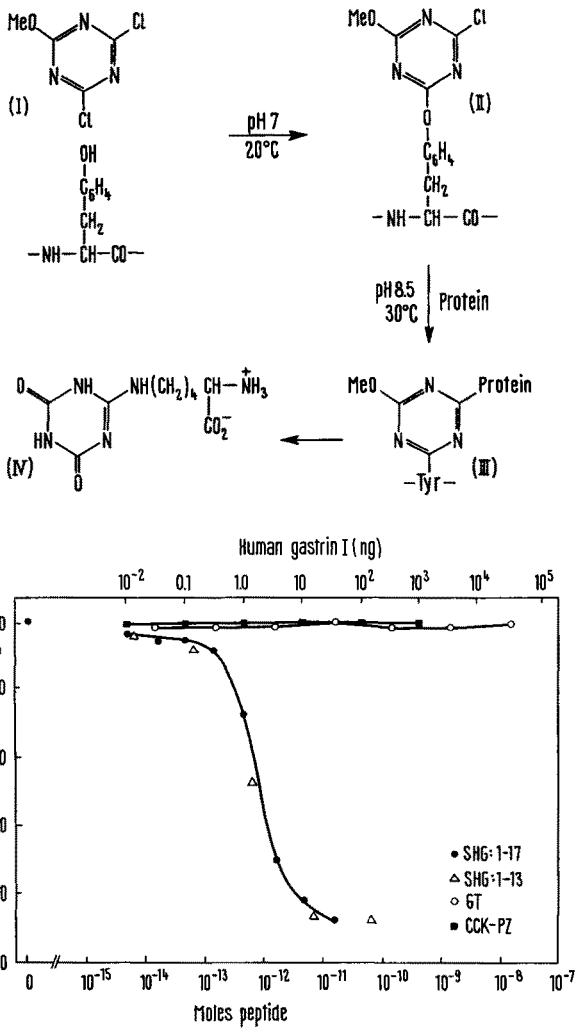
Gastrins from different mammalian species are all heptadecapeptide amides with amino-acid sequences varying in the central region[1–3]. At the outset of the chemical work it was discovered that gastrin-like hormonal activity is possessed by any peptide containing the same four C-terminal residues as the gastrins, viz. Trp-Met-Asp-Phe-NH_2[4]. The same sequence has since been discovered as a structural component of other naturally occurring peptides[5,6], notably cholecystokinin-pancreozymin (CCK-PZ)[7]. There is a clear need for a sensitive technique of differentiating between gastrin and CCK-PZ in biological tissues. Several immunochemical assays have been developed for gastrin (e.g. refs.[8–11]), but in their present form there is risk of cross reactivity with CCK-PZ. In this communication we describe a logical method of achieving the desired selectivity in immunochemical assay of gastrin.

The problem was approached with the aim of raising antibodies to that part of the gastrin molecule lacking the C-terminal tetrapeptide. The functional groups in this 1–13 sequence comprise several side-chain carboxyl groups of glutamic acid and one phenolic group of tyrosine at position 12. The latter seemed to us to offer the best link for peptide-protein conjugates which would be specifically antigenic.

Halogenated-1, 3, 5-triazines are excellent reagents for attaching small molecules to macromolecules[12–14], and they can be used successfully for coupling peptides, through their amino-groups, to proteins[1]. 2, 4-Dichloro-6-methoxy-1, 3, 5-triazine (I)[15] reacts with the phenolic hydroxyl group of tyrosine residues in neutral solution at surprising speed, yielding the mono-chloro derivative (II)[16]. The remaining chlorine retains sufficient reactivity for convenient displacement by, for example, the side-chain amino-groups of a protein. Thus the conjugates (III) are accessible. Acidic hydrolysis of the conjugates and amino-acid analysis in the usual way reveal the new amino-acid (IV), which is eluted in the analyzer close to leucine[17]. Conjugation of (II) with proteins can be run at pH 8.5 (pH stat), but on a small scale it is convenient to use potassium bicarbonate to buffer both this stage and the initial formation of (II) from (I).

Both synthetic human gastrin I[18] and the 1–13 peptide, Glu-Gly-Pro-Trp-Leu-Glu$_5$-Ala-Tyr-Gly-OH, were conjugated with bovine serum albumin (BSA) by this novel technique. The 1–13 peptide was prepared by the usual removal of the blocking groups from its penta-t-butyl monomethyl ester, prepared either by the method of MORLEY[19] or an adaptation of our own latest method for synthesis of human gastrin[20]. In a typical experiment, the triazine (I) (11.14 mg) and $KHCO_3$ (30.72 mg) were added to 1–13 peptide (35.76 mg), followed by acetone (0.8 ml) and water (0.8 ml). The mixture was kept at 20 °C (N_2) for 1.5 h and then washed well with ether. BSA (60.81 mg), $KHCO_3$ (38.96 mg) and water (0.4 ml) were then added, and the mixture was kept at 30 °C (N_2) for 22 h. The conjugate (73.00 mg) was isolated by gel chromatography (Sephadex G-25, 0.4% aqueous NH_4HCO_3), and amino-acid analysis indicated that 12 moles of 1–13 peptide had conjugated to each mol of BSA.

The peptide-BSA conjugates were dissolved in 0.15 *M* NaCl – 0.01 *M* phosphate, pH 7.4, and emulsified with an equal volume of 4:1 mineral oil – arlacel A (v/v); the mineral oil contained *Mycobacterium butyricum* (500 µg/ml). Randomly bred New Zealand white rabbits (3 in each group) were immunized with 1 mg of conjugate by 4 foot-pad injections at intervals of 2 months.



Calibration diagram indicating the percent of total [^{125}I]-human gastrin I precipitated using antisera from immunization with 1–13 peptide-BSA conjugate with inclusion of human gastrin I (●), 1–13 peptide (△), Trp-Met-Asp-Phe-NH_2 (○), and CCK-PZ (■) respectively.

Blood was obtained by cardiac puncture before the first injection and 2 weeks after the final injection of the series. Antibody activity was determined by assessment of antibody binding of [^{125}I]-human gastrin I[21].

As shown in the Figure, identical inhibition of binding by human gastrin and 1–13 peptide was displayed when the antibodies had been raised to 1–13 conjugate. Moreover there was no detectable cross reactivity with either CCK-PZ or Trp-Met-Asp-Phe-NH_2. On the other hand, antibodies raised to human gastrin I-BSA conjugate (prepared in the same manner) showed cross reactivity with CCK-PZ. The biological work is described in detail and its implications are discussed elsewhere[22].

Zusammenfassung. Nachweis, dass mit Hilfe von 2,4-Dichlor-6-methoxy-1,3,5-triazin Tyrosinreste von Peptiden und Lysinreste von Proteinen miteinander verknüpft werden können. Auf dieser Basis wurde ein immunologischer Test für die Sequenz 1-13 von menschlichem Gastrin entwickelt.

K. L. AGARWAL, S. GRUDZINSKI,
G. W. KENNER, N. H. ROGERS,
R. C. SHEPPARD and J. E. MCGUIGAN

Robert Robinson Laboratories,
University of Liverpool 7 (England), and
Division of Gastroenterology, Department of Medicine,
University of Florida, College of Medicine,
Gainesville (Florida 32601, USA), 9 November 1970.

[1] G. W. KENNER and R. C. SHEPPARD, Proc. R. Soc. B *170*, 89 (1968).
[2] K. L. AGARWAL, G. W. KENNER and R. C. SHEPPARD, Experientia *25*, 346 (1969).
[3] K. L. AGARWAL, G. W. KENNER and R. C. SHEPPARD, J. Am. chem. Soc. *91*, 3096 (1969).
[4] H. J. TRACY and R. A. GREGORY, Nature, Lond. *204*, 935 (1964).
[5] A. ANASTASI, V. ERSPAMER and R. ENDEAN, Experientia *23*, 699 (1967).
[6] L. BERNARDI, G. BOSISIO, R. DE CASTIGLIONE and O. GOFFREDO, Experientia *25*, 7 (1969).
[7] V. MUTT and J. E. JORPES, Biochem. Biophys. Res. Comm. *26*, 392 (1967).
[8] J. E. MCGUIGAN, Gastroenterology *53*, 697 (1967).
[9] J. E. MCGUIGAN, Gastroenterology *54*, 1005 (1968).
[10] A. C. CHARTERS, W. D. ODELL, W. D. DAVIDSON and J. C. THOMPSON, Surgery *66*, 104 (1969).
[11] R. S. YALOW and S. A. BERSON, Gastroenterology *58*, 1 (1970).
[12] K. VENKATARAMAN, *Chemistry of Synthetic Dyes* (Academic Press, New York 1952), p. 583.
[13] G. KAY and E. M. CROOK, Nature, Lond. *216*, 514 (1967).
[14] G. KAY and M. D. LILLY, Biochim. Biophys. Acta *198*, 276 (1970).
[15] J. R. DUDLEY, J. T. THURSTON, F. C. SCHAEFER, D. HOLM-HANSEN, C. J. HULL and P. ADAMS, J. Am. chem. Soc. *73*, 2989 (1951).
[16] The crystalline derivative, mp 126–127°, from Z-Ala-Tyr-OMe was fully characterized by elemental analysis, NMR- and mass-spectrum. Kinetic studies showed that (I) attacks the phenolic anion, as expected.
[17] (IV) is considerably decomposed under normal conditions of protein hydrolysis, yielding lysine, and therefore it appears in much less than stoichiometric proportion. Consequently the possibility that some of the triazine rings are linked to residues other than lysine, e.g. tyrosine, in the protein cannot be excluded.
[18] J. BEACHAM, P. H. BENTLEY, G. W. KENNER, J. K. MACLEOD, J. J. MENDIVE and R. C. SHEPPARD, J. chem. Soc. (C) *1967*, 2520.
[19] J. S. MORLEY, J. chem. Soc. (C) 2410 (1967); we thank Dr. MORLEY for a gift of this material.
[20] K. L. AGARWAL, G. W. KENNER and R. C. SHEPPARD, J. chem. Soc. (C) 2213 (1969).
[21] J. E. MCGUIGAN and W. L. TRUDEAU, Gastroenterology *58*, 139 (1970).
[22] J. E. MCGUIGAN, Am. J. med. Sci., in press (1971).