- Fellows, R. E. & Rogol, A. D. (1969). *J. biol. Chem.* **244**, 1567.
- Fellows, R. E., Rogol, A. D. & Mudge, A. (1971). *Excerpta med. int. Cong. Ser. no. 236: Abstr. 2nd int. Symp. Growth Hormone*, p. 3.
- Li, C. H., Dixon, J. S., Lo, T.-B., Schmidt, K. D. & Pankov, Y. A. (1970). *Archs Biochem. Biophys.* **141**, 705.
- Li, C. H., Liu, W.-K. & Dixon, J. S. (1966). *J. Am. chem. Soc.* **88**, 2050.
- Liao, T.-H. & Pierce, J. G. (1971). *J. biol. Chem.* **246**, 850.
- Mills, J. B., Howard, S. C., Scapa, S. & Wilhelmi, A. E. (1970). *J. biol. Chem.* **245**, 3407.
- Niall, H. D. (1971). *Nature, Lond.*, **230**, 90.
- Niall, H. D., Hogan, M. L., Sauer, R., Rosenblum, I. Y. & Greenwood, F. C. (1971). *Proc. natn. Acad. Sci. U.S.A.* **68**, 866.
- Pierce, J. G., Liao, T.-H., Carlsen, R. B. & Reimo, T. (1971). *J. biol. Chem.* **246**, 866.
- Santomé, J. A., Dellacha, J. M. & Paladini, A. C. (1968). In *Growth Hormone*, p. 29. Ed. by Pecile, A. & Müller, E. E. Amsterdam: Excerpta Medica Foundation.
- Santomé, J. A., Dellacha, J. M., Paladini, A. C., Wolfenstein, C. E. M., Peña, C., Poskus, E., Daurat, S., Sese, Z. M., Sanguesa, V. F. & Biscoglio, M. (1969). In *Atlas of Protein Sequence and Structure*, vol. 4, p. D-159. Ed. by Dayhoff, M. O. Silver Spring: National Biomedical Research Foundation.
- Seavey, B. K. & Lewis, U. J. (1971). *Biochem. biophys. Res. Commun.* **42**, 905.
- Sherwood, L. M. (1967). *Proc. natn. Acad. Sci. U.S.A.* **58**, 2307.
- Wallis, M. (1966). *Biochim. biophys. Acta*, **115**, 423.
- Wallis, M. (1969). *FEBS Lett.* **3**, 118.
- Wallis, M. (1971). In *Atlas of Protein Sequence and Structure*, vol. 5. Ed. by Dayhoff, M. O. Silver Spring: National Biomedical Research Foundation (in the Press).

Structure-Activity Relationship of Synthetic Corticotrophin Peptides

By W. RITTEL. (Research Laboratories, Pharmaceuticals Division, CIBA-Geigy Ltd., 4000 Basle, Switzerland)

Three aspects of work undertaken to study the structure-activity relationship of corticotrophin analogues will be discussed: (1) a search for corticotrophin peptides with prolonged action; (2) an attempt at rendering corticotrophin analogues orally active by introducing lipophilic substituents; (3) a comparison of the influence of structural modifications on the lipolytic and corticotrophic activity of corticotrophin analogues.

All the peptides studied were prepared by fragment-coupling synthesis by the methods developed previously (Schwyzer, Rittel, Kappeler & Iselin, 1961; Kappeler & Schwyzer, 1961).

Since it was observed that the introduction of D-serine in position 1 of corticotrophin fragments

markedly increases their corticotrophic activity (Boissonnas, Guttman & Pless, 1966; Kappeler *et al.* 1967), a number of analogues bearing further modifications besides the D-serine-1 were synthesized. Of these, [1-D-serine,17-,18-dilysine]- β -corticotrophin-(1-18)-octadecapeptide amide (Riniker & Rittel, 1970) was found to possess a very potent and prolonged corticotrophic activity, not only in animals (Desaulles, Riniker & Rittel, 1969) but also in man (Walser & Müller, 1969). Additional evidence (Felber, Aubert & Deguillaume, 1969) indicates that this short-chain analogue may also be administered by the intranasal route.

Although highly active on parenteral administration, [1-D-serine,17-lysine,18-lysine]-corticotrophin-(1-18)-octadecapeptide amide and related analogues show only a very slight activity when given orally to rats (P. A. Desaulles & P. Barthe, unpublished work). With a view to increasing their absorption from the gut by introducing lipophilic substituents, several derivatives of [1-D-serine,17-,18-dilysine]- β -corticotrophin-(1-19)-nonadecapeptide modified at the C-terminal were prepared (Brugger, 1971). In these peptides the proline-19 residue is present either in the form of an alkyl ester, -Pro-OR, or an alkyl-substituted amide, -Pro-NHR or -Pro-NR₂, the alkyl groups R containing 5-35 carbon atoms. An increase in the lipophilic character of these derivatives was evident from their behaviour in distribution experiments as well as on chromatography. However, no significant increase in oral corticotrophic activity over that of the unsubstituted nonadecapeptide could be observed (D. Raps, P. Barthe & P. A. Desaulles, unpublished work).

Besides their adrenal effect, corticotrophin peptides are known to exert adipokinetic activity in adipose tissue. The influence of various structural alterations in corticotrophin analogues of chain length 1-16 to 1-39 on their lipolytic and corticotrophic activities was studied. The results obtained in experiments *in vivo* (rat) indicate that the two effects cannot be dissociated by any significant degree (W. Rittel, M. Brugger, B. Kamber, B. Riniker, P. Sieber, P. Barthe, P. A. Desaulles & R. Maier, unpublished work), as was suggested by previous findings (Lebovitz, 1965; Lebovitz & Engel, 1964).

Boissonnas, R. A., Guttman, S. & Pless, J. (1966). *Experientia*, **22**, 526.

Brugger, M. (1971). *Helv. chim. Acta* (in the Press).

Desaulles, P. A., Riniker, B. & Rittel, W. (1969). In *Protein and Polypeptide Hormones*, p. 489. Ed. by Margoulies, M. Amsterdam: Excerpta Medica Foundation.

Felber, J. P., Aubert, M. L. & Deguillaume, R. (1969). *Experientia*, **25**, 1196.

- Kappeler, H., Riniker, B., Rittel, W., Desaulles, P. A., Maier, R., Schär, B. & Staehelin, M. (1967). In *Peptides*, p. 214. Ed. by Beyermann, H. C., van de Linde, A. & Maassen van den Brink, W. Amsterdam: North-Holland Publishing Co.
- Kappeler, H. & Schwyzler, R. (1961). *Helv. chim. Acta*, **44**, 1136.
- Lebovitz, H. E. (1965). In *Endocrinology*, p. 1231. Ed. by Taylor, S. Amsterdam: Excerpta Medica Foundation.
- Lebovitz, H. E. & Engel, F. L. (1964). *Metabolism*, **13**, 1230.
- Riniker, B. & Rittel, W. (1970). *Helv. chim. Acta*, **53**, 513.
- Schwyzler, R., Rittel, W., Kappeler, H. & Iselin, B. (1961). *Chimia*, **15**, 312.
- Walser, A. & Müller, Th. (1969). In *Protein and Polypeptide Hormones*, p. 487. Ed. by Margoulies, M. Amsterdam: Excerpta Medica Foundation.

Hormonal Polypeptides of the Upper Intestine

By V. MUTT and E. JORPES. (*Kemiska Institutionen, Karolinska Institutet, S 104 01 Stockholm, Sweden*)

When Bayliss and Starling discovered secretin in 1902 they did not call it a hormone. This word did not exist at the time, but was invented 2 years later as a consequence of the discovery of secretin (Bayliss, 1920). In the decades that followed its discovery numerous attempts were made to obtain secretin in chemically pure form. The early work has been the subject of several comprehensive reviews (Greengard, 1948; Grossman, 1950). Although non-toxic preparations that could be used in medicine were obtained, and also some of its chemical characteristics could be established, the isolation of secretin had to await the elaboration of more efficient techniques for the fractionation of polypeptide mixtures than were available to the early workers. When such techniques had become available further progress was possible and highly active preparations of secretin were obtained in several laboratories (Legge, Morieson, Rogers & Marginson 1957; Newton, Love, Heatly & Abraham, 1959; Fishman, 1959).

Pure (porcine) secretin was first obtained in our laboratory in 1961 (Jorpes & Mutt, 1961), and in the following year its (corrected) amino acid composition and partial structure were published (Jorpes, Mutt, Magnusson & Steele, 1962). The biological activity of the pure hormone is such that 1 μ mol of secretin will induce the pancreas to secrete approx. 2.5 mol of bicarbonate, in 0.1 M solution.

The determination of its amino acid sequence posed no problems except that it had to be carried out on small quantities of polypeptide, since to obtain 1 μ mol of secretin it was necessary to process the intestines of 3000 hogs. The amino acid sequence is: His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-

Arg-Leu-Arg-Asp-Ser-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH₂ (Mutt, Magnusson, Jorpes & Dahl, 1965; Mutt, Jorpes & Magnusson, 1970). The sequence has been confirmed by synthesis (Bodanszky, Ondetti, Levine & Williams, 1967).

Secretin is strikingly similar to glucagon (Bromer, Sinn & Behrens, 1957). In 14 out of 27 positions the amino acids are identical in the two hormones, and in several other positions the differences are of a conservative nature. The difference between secretin and glucagon is no greater than may be found between polypeptides of identical function from distantly related species (Smith, 1967). Yet secretin and glucagon have widely different biological activities. In the method worked out for its isolation the destruction of secretin by the proteolytic enzymes of the intestine is prevented by heating the intestine for a few minutes in boiling water. This destroys the enzymes. From the coagulated proteinaceous mass secretin and other polypeptides are extracted with 0.5 M-acetic acid, adsorbed from the extract on alginic acid, eluted with 0.2 M-hydrochloric acid and precipitated from the eluate by saturating it with sodium chloride. The precipitate is a concentrate of the thermostable polypeptides of the intestinal wall, although not of all types.

The method of preparation obviously excludes very acidic peptides and also peptides of low molecular weight. From among the numerous peptides of this concentrate we next, after secretin, isolated a substance that exhibits the physiological properties ascribed to the hormone cholecystokinin (Ivy & Oldberg, 1928) and to the hormone pancreozymin (Harper & Raper, 1943), i.e. it both causes contraction of the gall bladder and leads to the secretion of enzymes from the pancreas (Jorpes, Mutt & Toczko, 1964; Jorpes & Mutt, 1966).

This substance is a polypeptide composed of 33 amino acid residues, the sequence of which has been determined (Mutt & Jorpes, 1968, and unpublished work). It is: Lys-Ala-Pro-Ser-Gly-Arg-Val-Ser-Met-Ile-Lys-Asn-Leu-Gln-Ser-Leu-Asp-Pro-Ser-His-Arg-Ile-Ser-Asp-Arg-Asp-Tyr(SO₃)-Met-Gly-Trp-Met-Asp-Phe-NH₂.

The C-terminal pentapeptide sequence is identical with that of the gastrins (Gregory, Hardy, Jones, Kenner & Sheppard, 1964) and of caerulein (Anastasi, Erspamer & Endean, 1967). As in caerulein and in gastrin II there is also in cholecystokinin-pancreozymin a residue of tyrosine O-sulphate. In gastrin II this residue is linked directly to the C-terminal pentapeptide; in cholecystokinin-pancreozymin, as in caerulein, it is displaced from this by an interposed amino acid residue, threonine in caerulein, methionine in cholecystokinin-pancreozymin. Whereas no fragment with more than trace