Table 2. *Immunological activity of derivatives of insulin measured by immune-haemolysis inhibition and radioimmune assays*

The percentage activity in the immune-haemolysis inhibition assay was determined from the number of picomoles of inhibitor needed to produce 50% inhibition. The percentage activity in the radioimmune assay was determined from the number of picomoles needed to give a C_0/C_1 ratio 1.5. Insulin was considered to be 100% active in both systems.

Insulin derivative	Immunological activity (%)	
	Radioimmune assay	Immune-haemolysis inhibition assay
Insulin	100	100
Moniodo-insulin	58.4	3.9
Mononitro-insulin	37.7	6.7
Proinsulin	26.3	0.06*
Des-asparagine-insulin	3.5	0.17
Des-octapeptide-insulin	2.0	0.08

* Calculated at 20% inhibition of immune haemolysis.

tyrosine-A19 that are not detected by antibodies in the radioimmune assay.

The data *in toto* suggest that most antibodies that are functional in the immune-haemolysis assay combine with determinants that are dependent on the tertiary relationship between the C-terminal portions of the two chains. The slightly greater immunological activity in the radioimmune assay is interpreted to mean that antibodies functional in the radioimmune assay are directed to determinants less involved with the two C-termini. Alterations in the regions of the N-terminus of the B-chain have little influence on either immune assay (Brandenburg & Ooms, 1968; Arquilla *et al.* 1969). It is possible that relatively few antibodies in insulin antisera are directed to residues in the C-terminus of the B-chain. This region is involved in the dimer site and may not be exposed when insulin is used as an antigen.

Of interest in these studies is the fact that both mononitro- and moniodo-insulin retain considerable biological activity compared with other derivatives with the same degree of immunological alteration (Table 2). This suggests that the regions of tyrosine-A14 and possibly tyrosine-A19 are not involved directly in the areas of the insulin molecule that are essential for biological activity.

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Structural Relationships among Growth Hormones and Prolactins

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The pituitary growth hormones and prolactins comprise a group of protein hormones with similar structural features, homologous amino acid sequences and, in some species, overlapping biological properties. There is considerable species specificity within the group. Relationships between these hormones will be considered here in the light of information currently available about their primary structures.

An amino acid sequence for human growth hormone was first proposed by Li, Liu & Dixon (1966), but has since been modified (Niall, 1971; Niall, Hogan, Sauer, Rosenblum & Greenwood, 1971). The hormone possesses a single polypeptide chain (molecular weight about 20000), though there is some tendency for the chains to aggregate. Bovine and ovine growth hormones normally exist as dimers, with a molecular weight of about 45000. These can dissociate under various conditions (Andrews & Folley, 1963, and many subsequent reports). Studies on the primary structure, particularly at the N- and C-termini, have shown that bovine growth-hormone preparations contain two types of polypeptide chain, but that these are very similar and may differ only by the presence or absence of a single N-terminal alanine residue (Wallis, 1969).

The two chains of bovine growth hormone have been separated by isoelectric focusing (Ellis, Lorenson, Callahan & Grindeland, 1971); biological activity is retained after separation. These authors suggest that there may be several differences between the chains in addition to the *N*-terminal groups (one chain possesses *N*-terminal alanine, the other *N*-terminal phenylalanine). The difference in isoelectric point (about 0.2pH unit) could be due solely to differences between the pK_a values of the α -amino groups of the *N*-terminal alanine and phenylalanine residues (the α -amino group is likely to be the only one with a pK_a close to the isoelectric point of the protein, about pH 8). It is not clear how the two chains of bovine growth hormone arise; possible explanations involve enzymic degradation or gene duplication (Wallis, 1969).

The amino acid sequence of bovine growth hormone has been studied in several laboratories (Fellows & Rogol, 1969; Fellows, Rogol & Mudge, 1971; Santomé, Dellacha & Paladini, 1968; Santomé *et al.* 1969; Wallis, 1966, 1969, 1971). Using homology with human growth hormone to help order some peptides, we are able to propose a complete sequence for the hormone except for a short region near the middle of the molecule. Our results are in good agreement with those of Fellows *et al.* (1971), but there are substantial differences from the sequence proposed by Santomé *et al.* (1969). Comparison of the sequences of bovine and human growth hormones shows many differences (about 35% of residues are different) and indicates a rapid rate of evolutionary divergence.

Studies have been reported on the amino acid sequences of growth hormones from other species. Ovine growth hormone is very similar to the bovine hormone (Bellair, Morely & Burger, 1971; Davies & Wallis, 1971; Dellacha, Enero, Santomé & Paladini, 1970). Porcine growth hormone, however, is substantially different from both the bovine and human hormones (Mills, Howard, Scapa & Wilhelmi, 1970). Comparison of primary structures indicates that the rate of evolution during divergence of the mammalian growth hormones has been very variable, a feature related presumably to the observable differences in biological activity.

Less work has been reported on the amino acid sequences of prolactins, partly perhaps because the existence of an independent human prolactin has been in doubt until recently. The amino acid sequence of ovine prolactin was reported by Li, Dixon, Lo, Schmidt & Pankov (1970), and studies on the bovine hormone have also been reported (Seavey & Lewis, 1971; Wallis, 1971). The prolactins (like the growth hormones) from these two species are very similar; only two clear-cut differences have been identified. Whether prolactins

from other species will prove (like the growth hormones) to be more variable must await further work.

There is clearly homology between the sequences of growth hormones and prolactins. Approximately 20% of residues are identical. It is noteworthy that human growth hormone, which possesses lactogenic activity, is not more like bovine prolactin than are bovine or porcine growth hormones (which are not lactogenic).

The sequences of growth hormones and prolactins show homology with those of some other protein hormones. Extensive homology exists between human growth hormone and human placental lactogen (Sherwood, 1967; Niall *et al.* 1971); over 80% of the residues are identical. The structural resemblance between these two hormones is greater than that between human growth hormone and growth hormones from other mammals, suggesting that the placental lactogen may have arisen (by gene duplication) after the separation of the major eutherian orders. In this case the placental lactogens which have been found in the rat and other mammals must presumably have arisen independently of the human lactogen and may well be structurally very dissimilar. It should be noted that, although human placental lactogen has biological properties similar to those of prolactin (very low growth-promoting activity and high lactogenic activity), it is structurally much more closely related to the growth hormones than to the prolactins.

The amino acid sequences of the α -chain of thyrotropin and the C1-chain of luteinizing hormone (Liao & Pierce, 1971; Pierce, Liao, Carlsen & Reimo, 1971) also show some homology with the sequences of growth hormone and prolactin, certainly sufficient to suggest a common evolutionary origin. The homology of primary structure among the protein hormones of the pituitary and placenta is thus very widespread; it seems possible that there may also be similarities in tertiary structure and even mode of action.

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Structure-Activity Relationship of Synthetic Corticotrophin Peptides

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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).

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