Preparation of Internally Labelled Rat Pituitary Somatotropin (Growth Hormone)

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Rat somatotropin (growth hormone) was labelled biosynthetically by incubating anterior pituitary lobes with radioactive amino acids for 24 h in a simple buffered salts medium containing glucose. The labelled hormone was isolated by preparative polyacrylamide-gel electrophoresis or by chromatography on Sephadex G-100 and then DEAE-cellulose. The labelled material was pure by several criteria and cross-reacted immunologically with unlabelled rat somatotropin. When a mixture of ^{14}C -labelled amino acids was used for labelling the protein, label could be introduced into these same amino acids of somatotropin, though relative specific radioactivities varied considerably. Somatotropin labelled by the procedures described in the present paper was suitable for structural studies and could be used for a variety of other biochemical experiments.

When the rat anterior pituitary gland is incubated *in vitro* in a simple buffered salts solution containing glucose and a labelled amino acid, label is incorporated into pituitary proteins, including somatotropin (growth hormone) and prolactin. This system has been used extensively to study the biosynthesis of such pituitary hormones and its regulation (e.g. MacLeod & Abad, 1968; Betteridge & Wallis, 1973). The amount of label incorporated is sufficient to allow the possibility of preparing internally labelled proteins for use in various biochemical systems. Catt & Moffat (1967) used such an approach to prepare labelled prolactin.

We have explored the possibility of preparing labelled rat somatotropin by this method, with the intention of producing sufficient material for structural studies. Amino acid-sequence studies have been carried out on somatotropins from various mammalian species, but so far these have been mainly large domestic animals (and man). Structural studies on pituitary hormones from smaller animals are limited by shortage of material. If somatotropin that was labelled in several or all amino acids could be prepared, it should be possible to use this for sequence studies, detecting peptides, amino acids and their derivatives by virtue of their radioactivity and thereby greatly increasing the sensitivity of detection. The studies described here were designed to explore the possibility of producing labelled somatotropin suitable for such structural studies, though such material may be useful for other purposes also.

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Materials and Methods

Materials

Radioactive amino acids were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. They were L-[^{35}S]methionine (sp. radioactivity 280 Ci/mmol), U- ^{14}C -labelled protein hydrolysate (52 mCi/mg-atom of carbon), L-[U- ^{14}C]asparagine (100 mCi/mmol), L-[U- ^{14}C]glutamine (42 mCi/mmol), L-[U- ^{14}C]histidine (25 mCi/mmol), L-[methyl- ^{14}C]methionine (25 mCi/mmol), L-[methylene- ^{14}C]tryptophan (56 mCi/mmol) and DL-[3- ^{14}C]cysteine hydrochloride (25 mCi/mmol). The U- ^{14}C -labelled protein hydrolysate contained all the amino acids found in proteins except for asparagine, glutamine, tryptophan and cysteine; the content of methionine and histidine was low. In some experiments it was therefore supplemented with these six amino acids, added either individually or as a supplementary mixture obtained from The Radiochemical Centre (sp. radioactivity 10 mCi/mmol for each of these six amino acids).

Rat somatotropin (NIH-RP-GH1) and anti-serum to rat somatotropin, prepared in monkeys, (NIAMDD-Anti-rat GH-serum-3) were gifts from the Rat Pituitary Hormone Program of the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, MD, U.S.A. Bovine somatotropin was prepared by the method of Wallis & Dixon (1966).

Tissue-culture medium 199 was from Flow Laboratories (Irvine, Scotland, U.K.). Sephadex G-25 and G-100 were from Pharmacia Fine Chemicals (Uppsala, Sweden). DEAE-cellulose (DE-32) was from Whatman Biochemicals (Maidstone, Kent,

U.K.) and was washed before use as recommended by the manufacturers. Ampholine was from LKB Instruments (South Croydon, Surrey, U.K.). Trasylol was from Bayer (U.K.) (Haywards Heath, West Sussex, U.K.).

Animals

Pituitary glands were obtained from male rats (150–200g) of the albino Sprague–Dawley strain. These animals were obtained from the laboratory colony and were fed *ad lib*.

Incubation of pituitary glands

Rats were killed by cervical dislocation and the pituitary glands were removed. Anterior and posterior lobes were separated, and the former were placed in medium at room temperature (about 20°C). Media for incubation were tissue-culture medium 199 (Morgan *et al.*, 1950), Gey & Gey's (1936) medium or Krebs–Ringer bicarbonate medium (composition as given by Betteridge & Wallis, 1973). These media were equilibrated with CO₂/air (1:19) before use, and during incubations, and contained glucose (11.2mM), Phenol Red (20 µg/ml), penicillin (35 µg/ml) and streptomycin (100 µg/ml), and in some cases bovine serum albumin (1mg/ml). Pituitary glands were divided into halves and rinsed in medium before incubation. They were incubated at 37°C under CO₂/air (1:19).

After incubation, pituitary glands and media were separated. Most of the subsequent work was carried out on the glands, which contained the bulk of the labelled somatotropin, but in some cases the media were also analysed. Glands were homogenized in NH₄HCO₃/(NH₄)₂CO₃ buffer (0.2M-NH₄HCO₃ adjusted to pH8.5 with aq. NH₃).

Analytical polyacrylamide-gel electrophoresis

For analytical-scale fractionation of pituitary extracts and column eluates, analytical gels of the type described by Davis (1964) were used. These were formed by polymerization of 8.0% (w/v) acrylamide and 0.2% (w/v) methylenebisacrylamide in glass tubes of internal diameter 6mm. A discontinuous buffer system was used (Davis, 1964), but in most cases sample and spacer gels were omitted. The sample was mixed with 5 µl of a saturated solution of Bromophenol Blue in glycerol before layering on the gel. Electrophoresis was carried out at a current of 3mA per gel. After electrophoresis, gels were stained with 0.005% (w/v) Amido Black in 7.5% (v/v) acetic acid overnight, and then washed with several changes of 7.5% acetic acid.

For determination of the distribution of radioactive protein, the stained gels were cut into 5 or 2mm

slices, and these were solubilized by heating with H₂O₂ (0.2ml) at 50–60°C, overnight, in closed vials. The solubilized gel fragments were then mixed with 2–3ml of scintillation fluid (0.5% 2,5-diphenyl-oxazole/30% Triton X-100/70% toluene, w/v/v) and counted for radioactivity in a Beckman LS233 scintillation counter. Counting efficiency for ¹⁴C and ³⁵S was 90–95%. Some loss of ¹⁴C label occurred during this solubilization procedure (usually 20–25%). This was assessed by treating a known quantity of ¹⁴C-labelled amino acid mixture by the same procedure, and corrections were made to experimental results where appropriate.

When the amount of hormone to be run on a gel was small (less than 5 µg) and the sample was to be detected by virtue of its radioactivity, unlabelled rat somatotropin was added as carrier. This both provided a visible marker after staining, and minimized losses of the labelled hormone caused by adsorption.

Isoelectric focusing in polyacrylamide gels

Analytical-scale isoelectric focusing was carried out in polyacrylamide gels (Wrigley, 1968) prepared in the same manner as for polyacrylamide-gel electrophoresis, except that the buffer was replaced by 1% (w/v) ampholine (final concn.; pH range 3–10). The electrode solutions were 0.4% (v/v) ethylenediamine (cathode, top) and 0.2% (v/v) phosphoric acid (anode, bottom) and focusing was carried out for 4–6h at 200V. Gels were washed in several changes of 10% (w/v) trichloroacetic acid to remove Ampholine and were then stained in the same way as after polyacrylamide-gel electrophoresis. Slicing and solubilization of gels for determination of radioactivity and use of carrier rat somatotropin was as for polyacrylamide-gel electrophoresis.

Preparative polyacrylamide-gel electrophoresis

A commercial apparatus was used for preparative polyacrylamide-gel electrophoresis (Shandon Instruments, Camberley, Surrey, U.K.) and was operated at 4°C. The gels were prepared in the same way as for analytical polyacrylamide-gel electrophoresis, except that all buffers contained 7M-urea. Final acrylamide concentration was 7.5% (w/v); gels were 5cm in length and 2cm in diameter. The electrolyte buffers were 10 times more concentrated than those used for analytical gels. The eluting buffer (0.43M-Tris/acetate, pH8.3) was pumped at a flow rate of 12ml/h; fractions (1ml) were collected. Starting material was made 7M with respect to urea and was layered on the spacer gel. Electrophoresis was performed at 20mA (220V) for 1h and then at 35mA (400V). Total time for the run was about 5h. Radioactivity in the fractions from an electrophoresis run was measured

by scintillation counting of samples, with the scintillant used for analytical polyacrylamide-gel electrophoresis.

Column chromatography

Purification of small quantities of labelled rat somatotropin was carried out by gel filtration and subsequent ion-exchange chromatography. Chromatography was performed at 4°C and eluants contained 0.03% toluene as a bacteriostat. Gel filtration was on columns (approx. 45 cm × 0.28 cm²) of Sephadex G-100, equilibrated with 0.02 M-Tris/HCl buffer, pH 8.5. Ion-exchange chromatography was carried out on small columns (2–5 cm × 0.28 cm²) of DEAE-cellulose (Whatman DE-32) equilibrated with 0.02 M-Tris/HCl buffer, pH 8.5. Samples were applied in this buffer and were then eluted by a linear 0–0.1 M-NaCl gradient prepared by mixing 40 ml of starting buffer and 40 ml of 0.1 M-NaCl in the Tris buffer. In most cases the column was then also eluted with steps of 0.2 M- and 2 M-NaCl in the starting buffer (about 25 ml in each case). Fractions were collected in polystyrene tubes to minimize adsorption of labelled protein. Radioactivity in these fractions was determined by scintillation counting, as described under 'Analytical polyacrylamide-gel electrophoresis'.

Amino acid analysis

For amino acid analysis, samples of labelled rat somatotropin were hydrolysed with 6 M-HCl in a sealed evacuated tube at 110°C for 24 h. Before hydrolysis, samples were mixed with unlabelled bovine somatotropin (1–2 mg) and desalted by chromatography on Sephadex G-25. The distribution of radioactivity in the amino acids of the hydrolysate was determined by using an amino acid analyser (Locarte Co., London W12 9RT, U.K.) linked to a flow scintillation counter (Tracerlab, Weybridge, Surrey, U.K.). Counting efficiency for ¹⁴C in this system was 30–35%. In some cases amino acids were fractionated by paper electrophoresis and chromatography.

The relative specific radioactivities of the amino acids in the labelled hormone were determined by dividing the d.p.m. associated with each amino acid in an analysis by the number of residues of that amino acid in rat somatotropin (as given by Ellis *et al.*, 1968).

Interaction of labelled rat somatotropin with antiserum to somatotropin

The antiserum was obtained freeze-dried, and was reconstituted (to 10% serum) in diluent buffer containing 1% (w/v) bovine serum albumin, 0.1% (w/v)

sodium azide, 0.15 M-NaCl, 0.01 M-NaH₂PO₄ and 0.02 M-EDTA, adjusted to pH 7.6 with NaOH. A portion (0.1 ml) of this antiserum was incubated with labelled rat somatotropin, with or without 300 µg of unlabelled rat somatotropin, for 16 h at 4°C. Trasylol (a proteinase inhibitor; 10 µl of a 2.8 mg/ml solution) was included in each incubation. The mixtures were then submitted to gel filtration on a column (approx. 45 cm × 0.28 cm²) of Sephadex G-100, under the conditions given above.

Sequence analysis with rat somatotropin labelled with [³⁵S]methionine

A Beckman sequencer (model 890; Beckman Instruments, Glenrothes, Fife, Scotland) was used, with the dimethylbenzylamine programme of Hermodson *et al.* (1972). Reagents for the sequencer were obtained from Beckman Instruments, or from Pierce Chemical Co. (Chester, U.K.).

• *Radioimmunoassay of rat somatotropin*

Radioimmunoassay of rat somatotropin was carried out with a kit obtained from the National Institute of Arthritis, Metabolism and Digestive Diseases (Bethesda, MD, U.S.A.). The procedure was that described in the instructions accompanying the reagents, except that precipitation of antibody-hormone complex was carried out by using poly(ethylene glycol), as described by Desbuquois & Aurbach (1971).

Results

Incorporation of labelled amino acids into somatotropin

When anterior pituitary lobes were incubated *in vitro* with labelled amino acids, label was incorporated into pituitary proteins, including somatotropin (Fig. 1). Incorporation into this hormone was approximately linear with time for 24 h in simple incubation media (Krebs-Ringer bicarbonate buffer with added glucose; Gey & Gey's medium), but subsequently slowed considerably. In tissue-culture medium 199, incorporation into somatotropin continued for much longer periods (up to 72 h), but the total amount of radioactivity incorporated was decreased, presumably because the amino acids in the medium diluted out the added label.

Various attempts were made to increase the overall incorporation of labelled amino acids into somatotropin, by using alternative incubation media, or supplementing the incubations with various factors. Marked improvements compared with the results obtained with simple media have not yet been achieved.

Fig. 1. Analytical polyacrylamide-gel electrophoresis of an extract of rat anterior pituitary gland incubated with ^{14}C -labelled amino acids

One rat anterior pituitary gland was incubated for 24 h in 0.4 ml of Krebs-Ringer bicarbonate medium containing 11.2 mM-glucose and 50 μCi of ^{14}C -labelled amino acid mixture. The gland was homogenized in 0.4 ml of 0.2 M- $\text{NH}_4\text{HCO}_3/(\text{NH}_4)_2\text{CO}_3$ buffer, pH 8.5, and samples (2 μl) of the homogenate (a) and the incubation medium (b) were submitted to polyacrylamide-gel electrophoresis, with 40 μg of unlabelled rat somatotropin as carrier. Gels were stained, sliced and solubilized for determination of radioactivity. Above each histogram (distribution of radioactivity) is a diagram of the stained gel for the homogenate and the medium respectively (showing carrier somatotropin). The anode is to the right.

Purification of labelled rat somatotropin

Various procedures have been explored for the purification of the labelled rat somatotropin produced by biosynthesis *in vitro*.

Preparative polyacrylamide-gel electrophoresis has been used successfully for the isolation of the labelled hormone, and some results obtained with this technique have been presented elsewhere (Davies & Wallis, 1971; Wallis & Davies, 1976). Losses encountered with this method were considerable, but substantial amounts of labelled somatotropin were

Fig. 2. Gel filtration of a labelled pituitary extract on Sephadex G-100

A pituitary homogenate, labelled as described in the legend to Fig. 1 (13.3×10^6 d.p.m.), was applied to a column ($46.7 \text{ cm} \times 0.28 \text{ cm}^2$) of Sephadex G-100 equilibrated with 0.02 M-Tris/HCl buffer, pH 8.5. Fractions (0.33 ml) were collected and 5 μl of each fraction was subjected to scintillation counting.

prepared by using a single electrophoretic step. This material was suitable for structural studies (Wallis & Davies, 1976). Some studies on labelled somatotropin made by this method are considered in the next section.

A column-chromatographic method was also investigated as an alternative way of purifying rat somatotropin that might be simpler, more readily scaled down and subject to smaller losses of material. Gel filtration of the labelled pituitary extract was followed by ion-exchange chromatography. Gel filtration of a rat pituitary extract (labelled with a ^{14}C -labelled amino acid mixture) on Sephadex G-100 is illustrated in Fig. 2. Analytical polyacrylamide-gel electrophoresis showed that the labelled somatotropin ran primarily in peak B. Recovery of radioactivity from this column was 94% of that applied. Peak B from this column was rechromatographed on DEAE-cellulose (Fig. 3). Polyacrylamide-gel electrophoresis demonstrated that the main peak eluted by the NaCl gradient (peak I) contained somatotropin (Fig. 4). Material eluted by 0.2 M-NaCl from the DEAE-cellulose column (peak II in Fig. 3) included some material with an electrophoretic mobility similar to that of rat prolactin. The total recovery of radioactivity from this DEAE-cellulose column was 73%. The recovery of labelled somatotropin in fraction I represents 39% of that in the original anterior pituitary (as determined by polyacrylamide-gel electrophoresis). This column-chromatographic procedure has now been applied successfully to the purification of labelled rat somatotropin with several different labelled amino acids and most of the studies described in the next section were carried out with material made by this method.

Fig. 3. Chromatography on DEAE-cellulose of the somatotropin-containing peak from a Sephadex G-100 column. Peak B from the column shown in Fig. 2 (1.25×10^6 d.p.m.) was applied to a column ($3.9 \text{ cm} \times 0.28 \text{ cm}^2$) of DEAE-cellulose equilibrated with 0.02 M -Tris/HCl buffer, pH 8.5. Fractions (1.26 ml) were collected and $20 \mu\text{l}$ of each fraction was subjected to scintillation counting. A linear gradient (0 – 0.1 M -NaCl in starting buffer) was applied between fraction nos. 9 and 70 (G), and steps of 0.2 M -NaCl (S1) and 2 M -NaCl (S2) in starting buffer were applied at fraction nos. 71 and 91 respectively.

Characterization of labelled rat somatotropin

The homogeneity of the material believed to be labelled somatotropin (peak I, Fig. 3) was studied by analytical polyacrylamide-gel electrophoresis (Fig. 4) and isoelectric focusing (Fig. 5). In each case the material from the peak tube (tube 31) and the leading edge (tube 28) showed a single peak corresponding to the main component of the rat somatotropin marker. The trailing edge of the peak (tube 37) showed some labelled material running faster than this marker on electrophoresis, and with a slightly lower isoelectric point on isoelectric focusing. This may represent deamidated somatotropin; it can be excluded readily by pooling the material in peak I without the tail of the peak. Peak II from DEAE-cellulose contained no material in the position of the rat somatotropin marker on polyacrylamide-gel electrophoresis.

Rechromatography of labelled rat somatotropin (peak I from DEAE-cellulose) on Sephadex G-100, under the conditions shown in Fig. 2 and with 0.2 mg of bovine somatotropin as carrier, showed a single peak of radioactivity running in the position of peak B in Fig. 2, which is that expected for a somatotropin. Rechromatography of the labelled hormone on DEAE-cellulose, under the conditions shown in Fig. 3 and with 1.0 mg of unlabelled rat somatotropin as carrier, gave a single peak of radioactivity running in the position of peak I in Fig. 3.

Fig. 4. Analytical polyacrylamide-gel electrophoresis of fractions obtained during purification of rat somatotropin on DEAE-cellulose.

Samples ($100 \mu\text{l}$) of various fractions obtained during purification of rat somatotropin (Fig. 3) were submitted to analytical polyacrylamide-gel electrophoresis, with $20 \mu\text{g}$ of unlabelled rat somatotropin as carrier. Gels were sliced (2 mm) and solubilized for determination of radioactivity. The arrow shows the position of the main band of carrier somatotropin, seen on the stained gels (pattern as in Fig. 1). (a) Leading edge of peak I (tube 28; 2100 d.p.m.), (b) middle of peak I (tube 31; 6900 d.p.m.), (c) trailing edge of peak I (tube 37; 1900 d.p.m.) and (d) peak II (tube 72; 5600 d.p.m.). The anode is on the right.

Antiserum to rat somatotropin was used to demonstrate the immunological similarity of the labelled material to rat somatotropin. The labelled hormone

Fig. 5. Isoelectric focusing of labelled rat somatotropin. Labelled rat somatotropin (peak I, Fig. 3; 6900 d.p.m.) was submitted to isoelectric focusing, with 20 μ g of rat somatotropin as carrier. Gels were sliced and solubilized for determination of radioactivity. (a) Diagram of stained gel showing carrier somatotropin; (b) distribution of radioactivity. The anode is on the right.

was incubated for 16h with the antiserum in the presence or absence of 300 μ g of unlabelled rat somatotropin, and the mixtures were then chromatographed on Sephadex G-100. Results are shown in Fig. 6. In the absence of unlabelled somatotropin, radioactivity was eluted from the column in a fast-running peak (X) at the void volume of the column, whereas in the presence of an excess of unlabelled hormone, nearly all of the radioactivity ran in the position expected for somatotropin (peak Y). We conclude that the antiserum binds the labelled hormone, and that an excess of unlabelled hormone can displace the labelled material.

The distribution of labelled amino acids in the radioactive somatotropin was investigated after hydrolysis, as described in the Materials and Methods section. Label was found in all types of amino acids of the hormone, provided that these had been included in the incubation medium. The relative specific radioactivities of the amino acids in the labelled

Fig. 6. Interaction of labelled rat somatotropin with anti-serum to rat somatotropin

Labelled rat somatotropin (peak I, Fig. 3) was incubated with antiserum to rat somatotropin as described in the Materials and Methods section, and the mixture was submitted to gel filtration on Sephadex G-100. (a) Anti-(rat somatotropin) serum (0.1 ml; 10%) plus a sample (7000 d.p.m.) of labelled somatotropin; (b) anti-(rat somatotropin) serum (0.1 ml; 10%) plus a sample (4200 d.p.m.) of labelled somatotropin plus 0.3 mg of unlabelled rat somatotropin. The column size was 45.6 cm $\times$ 0.28 cm²; fractions of volume 0.37 ml were collected and 200 μ l of each was taken for scintillation counting.

hormone varied considerably, those of phenylalanine, leucine, isoleucine and valine generally being high and those of glycine, alanine, proline and serine low. The relative specific radioactivities of several amino acids, especially aspartic acid and glutamic acid, were variable from one experiment to another. The reason for this is not clear and its interpretation is complicated by the fact that asparagine and glutamine were converted into the corresponding acids during hydrolysis. Batches of labelled hormone made by both the electrophoretic and chromatographic methods were used for these experiments and did not appear to differ significantly.

The identity and homogeneity of the labelled rat somatotropin was further demonstrated by amino acid-sequence studies. Fig. 7 shows the degradation in the sequencer of rat somatotropin labelled with [³⁵S]methionine and purified by chromatography on Sephadex G-100 and DEAE-cellulose. Label was released only at cycle 4 of the degradation, in accordance with the presence of methionine at residue 4, but nowhere else in the *N*-terminal sequence of the

Fig. 7. Sequenator analysis of rat somatotropin labelled with $[^{35}\text{S}]\text{methionine}$

Rat somatotropin was labelled by incubation of one rat anterior pituitary gland with $50\mu\text{Ci}$ of $[^{35}\text{S}]\text{methionine}$ in Krebs-Ringer bicarbonate medium with added glucose for 24h. The hormone was then purified from the pituitary homogenate by chromatography on Sephadex G-100 and DEAE-cellulose, and desalted on a column of Sephadex G-25 (medium) equilibrated with 1mM-acetic acid (with 1mg of bovine serum albumin as carrier). A sample (33nCi) of the labelled hormone was subjected to sequence analysis with 5mg of apomyoglobin. Samples from the sequenator were extracted as usual, and one half of each extract was submitted to scintillation counting [after mixing with scintillation mixture containing 0.35% 2,5-diphenyloxazole and 0.005% 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene in toluene]. The remainder of the extract was submitted to t.l.c. to confirm that the phenylthiohydantoin expected during the Edman degradation of myoglobin were obtained.

protein (Wallis & Davies, 1976). Further sequence studies on labelled hormone (prepared by preparative polyacrylamide electrophoresis) have confirmed its identity and homogeneity (Wallis & Davies, 1976, and unpublished work).

Specific radioactivity of labelled rat somatotropin

The specific radioactivity of the labelled rat somatotropin prepared in the experiment illustrated in Fig. 3 was determined by using radioimmunoassay to determine the somatotropin concentration in peak I. The specific radioactivity of this material

was $2.4\text{nCi}/\mu\text{g}$. This value is probably an underestimate, since the rat somatotropin used as a standard in the assay (NIAMDD-Rat GH-RP-1) is rather impure, and the content of somatotropin estimated in peak I is therefore an overestimate.

Discussion

It is evident that labelled rat somatotropin can be prepared by biosynthesis *in vitro* in quantities sufficient for use in biochemical experiments. Yields in the chromatographic steps were reasonably high, and losses caused by adsorption on glass, chromatographic media etc. were apparently not excessive. However, attempts to rechromatograph trace quantities of the labelled hormone in these systems in the absence of carrier material did give very poor recoveries, presumably due to such adsorption, and this may limit the extent to which the method can be scaled down.

The identification of the labelled material as somatotropin is based on comparison with a sample of unlabelled hormone in various separation systems, its specific association with antiserum to rat somatotropin and on sequence studies. Identification by conventional biological assay was not possible, since the amount of material present was insufficient. The labelled hormone was homogeneous by several criteria, and showed less sign of microheterogeneity than the unlabelled rat somatotropin preparation available (and indeed most somatotropin preparations available from other species).

The specific radioactivity of somatotropin labelled with ^{14}C -labelled amino acids is of course very low (about $2.4\text{nCi}/\mu\text{g}$). It is possible that altered incubation conditions may increase this value somewhat, but since the specific radioactivity of the ^{14}C -labelled amino acids used cannot be significantly increased, that of the product is also limited. Material of higher specific radioactivity can be prepared if ^3H -labelled amino acids are used (or $[^{35}\text{S}]\text{methionine}$ and/or $[^{35}\text{S}]\text{cysteine}$), though cost eventually becomes a limiting factor. However, internally labelled somatotropin was prepared in this study primarily for structural studies, and for these the absolute amount of radioactivity incorporated is more important than the specific radioactivity of the product.

A more serious problem than low specific radioactivity for the present purposes is the marked variation in the specific radioactivities of different amino acids within the labelled protein. The specific radioactivities of the most heavily labelled amino acids (including phenylalanine, leucine, isoleucine and valine) were 10–30 times those of the least heavily labelled (such as glycine, alanine, serine and proline). If the protein is to be used for amino acid-sequence studies, the specific radioactivity of the least heavily labelled amino acids will become limiting, and this therefore presents a considerable problem. The

reasons for the differences in specific radioactivities for different amino acids have not been determined in detail, but they probably include differences in specific radioactivity of labelled amino acids added (in most cases a function of the number of carbon atoms in each amino acid), differences in amounts of labelled amino acids added, different pool sizes for different amino acids in the pituitary and different extents of metabolism of the different amino acids.

Several authors have proposed that amino acids utilized for protein synthesis in mammalian tissues do not pass through the general amino acid pool of the cell (Hider *et al.*, 1969; Samli *et al.*, 1971). However, it is clear that in the present study the relative specific radioactivities of the amino acids in labelled somatotropin are not correlated with the specific radioactivities or the total quantities of labelled amino acids in the incubation mixture. Metabolism of amino acids was certainly occurring during these incubations, since amino acid analysis showed that the distribution of label among free amino acids after incubation was markedly different from the initial distribution, both in the incubation medium and in the pituitary homogenate. The detailed nature of such amino acid metabolism has not been investigated yet; information about it might well suggest ways of increasing the specific radioactivity of those amino acids least heavily labelled.

Despite the problems incurred owing to the incorporation of some amino acids at low specific radioactivities, it seems likely that the labelled somatotropin prepared by the method described here will be useful for various types of biochemical study. We have shown (Wallis & Davies, 1976) that such material can be used for studies on the amino acid sequence of rat somatotropin, and these studies are continuing. Others have also used internally labelled proteins for sequence studies (Jacobs *et al.*, 1974; McKean *et al.*, 1974), and it is clear that many variations of the labelling and sequencing techniques can be used.

Internally labelled somatotropin should be useful for a variety of other biochemical purposes, including studies on the degradation of the hormone and on binding to tissues, cells and membrane receptors *in vivo* and *in vitro*. In most previous studies of this type protein hormones have been labelled with radioactive iodine (by iodination primarily of tyrosine residues); such an approach has disadvantages in that the iodine may be removed from the hormone, the iodinated hormone may behave differently from the natural one and any derivatives produced from the hormone that do not contain iodinated tyrosine will not be labelled and therefore cannot be traced. Clearly, material produced by biosynthesis does not have these disadvantages, and the fact that the position of the label can be varied according to the nature of the amino acid used may have advantages

in the location and identification of specific degradation products. On the other hand, the specific radioactivity of labelled somatotropin prepared biosynthetically is as yet much too low to replace iodinated hormone in many types of study.

Labelling of somatotropin by biosynthesis is a particularly favoured case because this hormone represents a high proportion of the total protein synthesized in the male rat pituitary gland (Fig. 1). Whether this method can be applied generally to the preparation of other labelled hormones, and indeed other animal proteins, will depend partly on whether other preparations can be found that make the required protein as a high proportion of the total protein synthesized. In many cases this should be possible. Thus, in the pituitary, use of female glands (in mammals) should enable application of the approach to the preparation of labelled prolactin (Catt & Moffat, 1967). For other pituitary hormones it may be necessary to carry out a partial separation of the appropriate hormone-secreting cells before biosynthetic labelling is attempted.

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