posed of a natural silk cocoon, which was obtained by thorough extraction of queen cells with diethyl ether.
One of these cocoons was impregnated with an extract of drone pupae or fractions of it, the other (control) with the same amount of solvent.
The test cage was kept in a dark room with a temperature of 15 °C. After 30 min the running activity of the bees decreased and a cluster built up. This process was finished 1 or 2 h after the start of the test.
Then it was noted which cocoon was covered by the bees and integrated into the now well-ordered bee cluster. Tests which resulted in the covering of both or non of the cocoons were rejected.
3000 drone pupae were extracted with 300 ml diethyl ether at room temperature. The crude extract was directly injected on a HPLC column (Perkin-Elmer Series 2, Lichrosorb Si 60, 16×250, UV-detector 247 nm, $CHCl_3$, 17 ml/min) and collected fractions were subjected to the biotest. The biological active fraction was purified by HPLC and analyzed by MS under electron impact and field desortion (FD) ionization conditions (Finnigan MAT 311 A, equipped with a combined FD/FI-EI ion source), by ^{1}H-NMR (Bruker WM 300, 300 mHz, $CDCl_3$) and IR-spectroscopy (Perkin-Elmer 355, $CHCl_3$).
Results. The FD-mass-spectrum of the biological active substance (HPLC retention time 5 min) shows a single peak for the molecular ion at m/z 858; high resolution MS establishes the elemental composition $C_{55}H_{102}O_6$ of the molecule. The fragment ions in EJ-MS m/z 603, 577, 339, 313, 265, 264 and 239 indicate a triglyceride with 1 palmitate and 2 oleate residues with the palmitate acid likely present in position 3 of the glycerol[1].
The presence of the glycerol unit is definitely identified by the signals (in ppm from TMS) at 5.33 (1 H), 4.25 (2H), while in the IR-spectrum the carbonyl absorption of the ester functions appears at 1725 cm^{-1}. Based on these results the glyceryl-1,2-dioleate-3-palmitate was synthesized.
The reaction of 25 mg 1,2-dioleine in 40 ml CH_2Cl_2 and 5 mg triethylamine with 11.5 mg palmitoylchloride was catalyzed by 1 mg dimethylamino pyridine[2]. The obtained triglyceride was purified by TLC (silica gel, Merck, $CHCl_3$, R_F: 0.6) and characterized by spectroscopic methods.
The test cocoon was impregnated with 10–20 µl ether extract containing the equivalent of 5–12 drone pupae and the control cocoons with the same amount of ether. The test showed that 72 times the test cells were more attractive compared to 12 times for the controls.
The HPLC fraction (retention time 5 min) gave a proportion of 65 (fraction) to 11 (control). The synthetic glyceryl-1,2-dioleate-3-palmitate (7 µg) was also offered on the test cocoon, resulting in 32 (test) to 12 (control). Due to the results of chemical analyses we suspected that olive oil contained the active substance also.
So the test cocoons were impregnated with 0.4 µl olive oil. The results were 34 (oil) to 6 (controls). All ratios reported here, are at least significant on a 1% level (χ^2).
Experiments on other substances and a more detailed discussion will be published elsewhere.

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Preparative electrophoresis for large scale preparation of highly purified bovine growth hormone[1]

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Summary. A method allowing large scale preparation of bovine growth hormone from pituitary glands in relatively few steps is described. In comparison to conventional purification techniques previously reported, the use of preparative polyacrylamide gel electrophoresis reduces the number of steps and the amount of time needed for the isolation of the hormone. In addition, the yield is several times greater, the hormone is purer and it has greater bioactivity (1.83 IU/mg).

Bovine growth hormone[2] is a single polypeptide chain consisting of 191 residues, with a known sequence[3–5]. The availability of large amounts of homogeneous bGH might be useful for studies on regulation of bGH secretion or for clinical use. To date, few bGH isolation procedures have employed preparative electrophoresis[6,7].
In view of the shortcomings of classical methods of hormone fractionation[8], the present investigation, involving the use of a rapid preparative polyacrylamide gel electrophoresis (Prep-PAGE), was developed. In the apparatus used, a slab shaped gel is cast directly between 2 cooled glass surfaces. The major problem associated with the Prep-PAGE technique, inefficiency of heat dissipation, is overcome by making the gel with an enlarged surface area. The apparatus also adopts a symmetrical elution chamber in which the buffer enters equally from all points, which results in a ready elution of separated components. Protein better than 90% pure and free of detergents, urea or carrier ampholytes were recovered with 73% efficiency. The procedure was also directly applicable to preparation of a product for lyophilization.
Materials and methods. Reagents. Acrylamide and N,N′-methylene-bis-acrylamide of commercial grade were recrystallized from chloroform and acetone respectively. Coomassie Brilliant Blue R-250, silver nitrate and other chemicals were of analytical grade (all obtained from Serva Heidelberg, FRG). Carrier Ampholines of various pH range were purchased from LKB (Bromma, Sweden) and the low molecular weight calibration kit for SDS-PAGE (range 84,000–14,300 i.e. phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme) from Bio-Rad Laboratories (Richmond, CA).
Hormone preparation. The starting material was prepared as previously described using the alternative method of precipitating the crude extract by ammonium sulfate at

Table 1. Sequence of purification of bGH

Stage	Total protein (mg)	Total activity (units)	Specific activity	Purification rate	Recovery (%)
Homogenate	20,000±4000 (10)[a]	254±20 (8)	0.012±0.04 (8)	1	100
C-2-S	224±20 (10)	159±20 (7)	0.71±0.14 (7)	59.2	62.6
p-bGH	85±12 (10)	155±9 (7)	1.83±0.17 (7)	152.5	61.0

[a]Determined as freeze-dried weight corrected for residual water; values are given as means ±SE with number of preparations in parentheses.

Table 2. Amino acid composition[a] of bGH

Amino acid	p-bGH found[b]	p-bGH calculated
Lys	11.1	(11)
His	3.1	(3)
Arg	12.2	(12)
Asp	16.3	(16)
Thr[c]	12.3	(12)
Ser[c]	12.3	(12)
Glu	24.2	(24)
Pro	6.1	(6)
Gly	10.5	(10)
Ala	14.3	(14)
½Cys	4.0	(4)
Val	6.3	(6)
Met	3.9	(4)
Ile[d]	6.6	(7)
Leu[d]	24.5	(24)
Tyr	5.9	(6)
Phe	12.1	(12)
Trp	1.0	(1)
No. of residues		184

[a]Molar ratios; [b]mean of single analyses of 4 preparations; [c]corrected for destruction; [d]the 72-h values for isoleucine and leucine were used.

pH 7.9 (C-2-S)[9]. Bovine growth hormone standard reference was the preparation NIH-GH-b16 with a sp. act. of 0.91 IU/mg obtained from NIAMDD and stored at −70 °C until needed.

Preparative polyacrylamide gel electrophoresis. Prep-PAGE was performed following the pH 9.9 system of Duesberg and Ruechert[10] in 7% acrylamide gel slabs, (165×30×7 mm), overlaid with 3% stacking gel in a ELCAM 84 vertical gel electrophoresis system (CAMAG, Muttenz, Switzerland). A 30% stock solution was made up and kept in a dark bottle at 4 °C. In the stock solution, treated extensively with mixed ion exchanger for removal of charged species, the acrylamide-bis ratio was 30:1. Efficient cooling of the whole electrophoresis system was achieved by connecting the apparatus to a thermostatic circulator. Volatile elution buffers (NH_4HCO_3 0.1 M, pH 9.6 or ethanolamine-CH_3-COOH 0.1 M, pH 9.4) were used. Gel composition was similar to that of Duesberg and Ruechert except that urea was omitted to prevent its crystallization in the gel as a result of cooling (2-4 °C) during the run. Freeze-dried protein samples were dissoved in the stacking buffer at 10 mg/ml concentration. The elution chamber of the apparatus is fitted below the actual separation chamber. A polyethylene frit defines the bottom of the elution chamber. Elution buffer entering through this porous frit provides a flow of liquid counteracting the downward migration of eluted protein material. A constant current of 12 mA was applied until all proteins had eluted (about 16 h).

Analytical electrophoresis. SDS-polyacrylamide gel electrophoresis. SDS-PAGE was performed using an electrophoretic apparatus (constructed in this laboratory) in which the entire surface of both faces of the gel sandwich is in contact with cold running fluid. Slab gels (170×90×0.8 mm) were prepared as described by Laemmli[11] in a 7–20% acrylamide gradient gel, overlaid with 4% stacking gel. Freeze-dried samples were solubilized in the sample buffer (0.1 M Tris-HCl, pH 6.8, containing 2% SDS, 5% 2-mercaptoethanol and 10% glycerol) at a final concentration of 2 mg/ml. The samples were then heated at 100 °C for 2 min. Electrophoresis was performed at a constant current of 8 or 10 mA for 6 h at 16 °C.

Polyacrylamide gel isoelectric focusing (PAGIF). The homogeneity of isolated hormone was tested by isoelectric focusing in 5% polyacrylamide gel with ampholines of 3–11 pH range at 4 °C using LKB 2117-010 Multiphor equipment. After 1 h prefocusing, PAGIF was carried out at a constant wattage of 5 W/gel (0.4×100×120 mm) using the Pharmacia model 2000/300 ECPS constant power supply. The cathodic and anodic electrolytes were fresh solutions of 1 M NaOH and 1 M H_3PO_4 respectively. The pH gradient was determined as previously described[12]. Samples were freshly prepared solutions of lyophilized extracts at a concentration of about 4 mg/ml in water 1% Ampholine pH 9–11. After electrophoresis the gels were dipped in the staining solution of procedure 'A' of Vesterberg[13]. Silver staining was performed following Coomassie Brilliant Blue R-250 staining according to Morrissey[14]. The scanning of the electrophoretic patterns at 595 nm were obtained with a Quick Scan R & D (Helena Laboratories, Beaumont, TX) densitometer with a scanning device provided with an automatic peak integrator.

Amino-acid analysis. Protein samples (1.2–1.5 mg) were hydrolyzed in glass-distilled 6 N HCl in vacuo at 110 °C for 24 h and 72 h. Cystine determination was carried out according to the method of Spencer and Wold[15] after oxidation to cysteic acid and the methionine was determined as methionine sulphone following performic acid oxidation according to the method of Hirs[16]. Tryptophan content was estimated after methane sulfonic acid hydrolysis as suggested by Simpson[17]. All analyses were performed on an automatic amino-acid analyser (Beckman 120 B) according to standard procedures recommended by the manufacturer. All values found represent composition in molar ratio, based on 24 and 72 h hydrolysis, corrected for losses.

Protein determination. Protein contents were determined by the method of Lowry et al.[18] using gamma-globulins as a standard.

Bioassay. Growth hormone potencies were determined by the tibia-test of Greenspan[19]. The experimental methodology was suggested by other authors[20]. Calculation of relative potencies and confidence limits were carried out as recommended by the statistical test of Bliss[21] with an Olivetti P 6060 computer.

Results and discussion. Yield and specific activity at each stage of the purification procedure are given in table 1. Total yield of purified bGH was about 2.24 g/kg wet wt of pituitaries (as C-2-S extract)[9].

The recovery was about 73% calculated by the protein content as determined by the Lowry method as applied to the layered sample and to the collected fractions in peak II

of figure 1. The estimated potency of p-bGH was 1.83 IU/mg (with upper and lower 95% confidence limits of 1.35 and 2.67 respectively). The dose response curves of NIH-GH-b16 and p-bGH were linear and parallel.

With respect to preparative electrophoresis, it was not convenient to use gels longer than 4–5 cm, as the longer electrophoresis time will bring about diffusion of the sample bands[22] and will also adversely affect the aggregation state of bGH.

In all the elution diagrams shoulders at each side of the main peak could be seen: the prep-PAGE system partially resolves this heterogeneity probably due to the presence of isohormones[23,24]. This conclusion is confirmed by the data from a Ferguson plot[25] which enables these components to be identified as isohormones on the basis of their different net charge in analytical disc-electrophoresis. A plot of log R_f vs the gel concentration was constructed for selected major bands of p-bGH obtained and for a variety of reference proteins. In the present study it was found that the apparent relative mobilities of bands of p-bGH did not vary with acrylamide concentration, the p-bGHs behaving rather as charge isomers.

Prep-PAGE of the C-2-S extract results in a complete separation of GH from other proteins, more than 90% of protein content being associated with the major peak. Reelectrophoresis of the main lyophilized fraction separates about 5% of the total UV-absorbance into an earlier peak.

SDS-PAGE was used to test protein homogeneity and to determine the mol.wt of the native bGH. In addition, sample loads were intentionally increased to detect minor components and the silver stain, which has been reported to be about 100 times more sensitive than Coomassie Blue in detecting proteins on PAGE[26], was used. SDS-PAGE give evidence for several components in the NIH-GH-b16 standard preparation and C-2-S extract. Streaking and smearing of samples, not visible in Coomassie Blue stained gels, also occurred, suggesting reaggregation and disaggregation was taking place.

A major component near 21,000 mol.wt and some naturally occurring fragments of about 15,000 mol.wt (fig. 2), as determined by manufacturer's standard protein molecular weights, were found.

The p-bGH preparation, although much more homogeneous than the standard preparation, exhibits in SDS-PAGE slight heterogeneity due to the presence of 2 faint bands located ahead of the major one. The fractions of peak II were pooled separately as fraction D (No. 18–20); E (No. 12–17); F (No. 21–25). These samples behave similarly on SDS-PAGE, resolving into 1 major protein band at 21,000 mol.wt with 1 faint minor band behind the major one. Fraction D seems to be homogeneous with silver nitrate staining. This fraction was at least 97% pure as could be quantified by scanning densitometry.

The p-bGH preparation, when subjected to PAGIF (fig. 1), shows 2 bands with isoelectric points of 7.72 and 7.89 described as native bGH with phenylalanine and alanine as amino termini[6]. NIG-GH-b16 resolves into numberless bands ranging in isoelectric points from 6.30 to 7.90[9].

The results of the quantitative amino acid analysis of p-bGH are shown in table 2. The amino acid composition was constant in several analyses and there was agreement between our results and those described in the literature[3–5,23]. The amino acid residues in the present paper were estimated on the basis of a mol.wt of 21,000 as determined by the analytical studies mentioned above.

The following conclusions may be drawn from this study: 1. the final product (p-bGH) has a potency greater than that of the other preparation previously obtained[20,27]. 2. GH purified with preparative electrophoresis is homogeneous as judged by SDS-PAGE, in contrast to NIG-GH-b16 (standard) which is clearly heterogeneous. When both samples were subjected to PAGIF, the p-bGH preparation, although much more homogeneous than the standard preparation, exhibits slight heterogeneity.

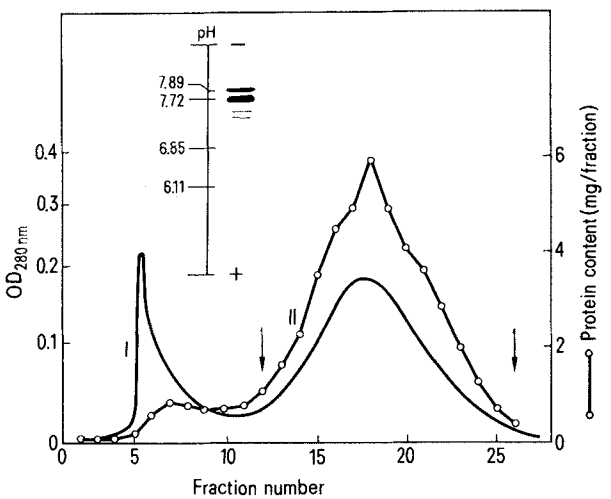

Figure 1. Elution profile obtained after application of 100 mg of alkaline pituitary extract (C-2-S) to 30 ml prep-PAGE. Fractions (6 ml each, flow rate 12 ml/h) between the arrows were pooled. In the insert PAGIF of pooled peak II, on the left the pH scale measured at 4 °C as described in the text.

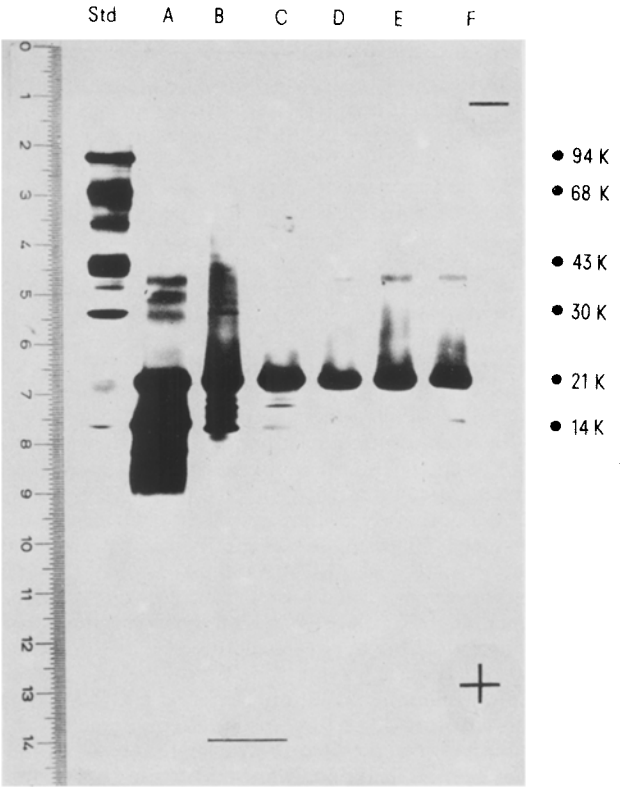

Figure 2. SDS-PAGE (0.1% SDS, 7–20% T) of different bGH preparations. Std, protein standards; A, NIH-GH-b16; B, C-2-S extract; C, p-bGH extract; D, pooled fractions 18–20 of figure 1; E, pooled fractions 12–17 of figure 1; F, pooled fractions 21–25 of figure 1. Stain $AgNO_3$ after Coomassie Blue R-250.

The bGH (up to 70 mg) can be purified from crude extract by a single electrophoresis run in 15 h so that time consuming steps and losses were eliminated. The methodology described permits a high recovery of material which is then readily available for further characterization.

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2 Abbreviations used: bGH, bovine growth hormone; C-2-S, intermediate extract of bGH obtained by 2 M ammonium sulfate precipitation at pH 7.9; p-bGH, fractions of bGH obtained with ELCAM 84 apparatus; NIAMDD, National Institute of Arthritis, Metabolism and Digestive Disease; NIH-GH-b16, reference preparation from NIAMDD; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PAGIF, polyacrylamide gel isoelectric focusing; Prep-PAGE, preparative polyacrylamide gel electrophoresis.

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Announcements

USA

5th international congress of Laser medicine and surgery

Detroit, MI, October 7–9, 1983

The congress will be held at the Sinai Hospital in Detroit. Information by the Registration Supervisor, c/o Charles B. Slack, Inc., 6900 Grove Road, Thorofare, NJ 08086/ USA.

England

1st international conference on Biointeractions 84

London, January 4–6, 1984

The conference 'Biointeractions 84' on materials/interactions will be held at the City University, London. Information may be obtained from Mary Korndorffer, Conference Organizer, Butterworth Scientific Ltd, Journals Division, P.O. Box 63, Westbury House, Bury Street, Guildford, Surrey GU2 5BH, England.

Austria

2nd international conference on human tumor markers

Vienna, February 20–22, 1984

Sponsored by International Society for Preventive Oncology (IPSO), the Austrian Cancer Society-Austrian Cancer League, and the Association of Clinical Scientists USA. – An update on biological and clinical applications of tumor markers for risk assessment, cancer detection and patient surveillance is presented. Sessions are structured as workshops; podium and poster presentations, panel discussions and scientific and technical exhibits are scheduled. Abstracts of presentations on relevant topics are invited by September 15, 1983. Further information by Herbert E. Nieburgs, Cell Pathology Laboratory, Mount Sinai Medical Center, 1 Gustave L. Levy Place, New York, N.Y. 10029, USA.

Italy

1st world conference on inflammation

Venice, April 16–18, 1984

The 1st world conference on inflammation, antirheumatics, analgesics, immunomodulators, sponsored by the International Association for Advancement of Biomedical