column. The asymmetrical or multiple peak elution patterns obtained with BGH plus ion solutions, therefore, appear to be the result of some hormone-ion interactions.

All the lyophilized powders derived from the BGH-ion complexes were assayed for total nitrogen and amount of the particular cation added and retained by the hormone. Ion contents were determined by atomic absorption. Specific binding of the ions by BGH was then defined by the relation % ion in sample/% N in sample. The obtained data were assembled in Figure 2. Significant amounts of Mg^{++}, Ca^{++}, and Na^{+} were complexed by the hormone. Mg^{++} was bound in largest amounts but in an unspecific manner, inasmuch as an increase in Mg^{++} concentration caused a proportional increase in specific binding. Specific binding of Ca^{++} reached a plateau extending from 0.01 to $0.02\,M$ Ca^{++}, thus indicating a possible change in the conformation of the protein chain when exposed to different concentrations of Ca^{++}. Na^{+} was also bound significantly by the hormone. A clear optimum for specific binding appeared evident at a concentration of $0.02\,M$. This indicates that a 0.02 molarity in respect to Na^{+} causes the protein chain to assume a particular molecular conformation which is optimal for Na^{+} binding. The most remarkable results were obtained with K^{+}. Some K^{+} was bound when present in concentrations below $0.02\,M$. However, at this molarity and above, virtually none was bound by the hormone. Thus a dramatic discriminatory effect of BGH against complexing of this cation became apparent.

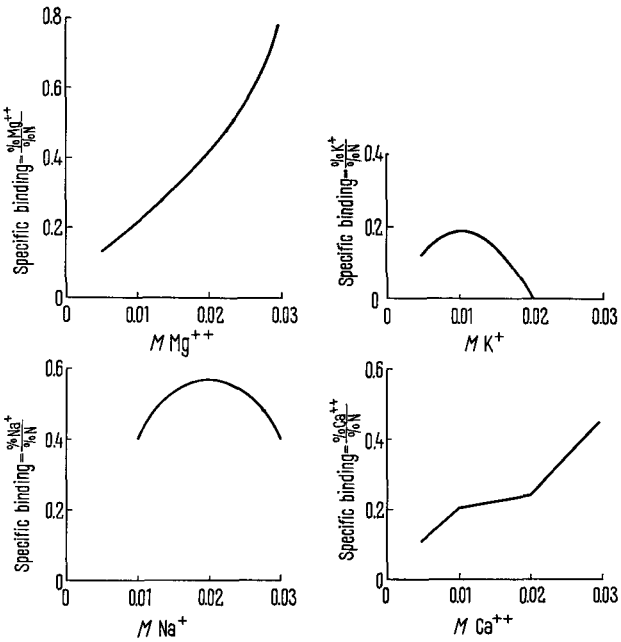

Fig. 2. Specific binding of Mg^{++} (upper left), Ca^{++} (lower right), Na^{+} (lower left) and K^{+} (upper right) by BGH.

Ashes of these samples were also subjected to semiquantitative emission spectrographic analyses. These indicated that the main bulk of the inorganic residues consisted of Na^{+}, Ca^{++}, Mg^{++}, Al^{+++}, and SiO_3^{--}, regardless of the particular ion added. K^{+} was only detected when added in small amounts, thus corroborating the results obtained with the atomic absorption method. These results indicated that the ions present in the starting material were only replaced to a limited extent by the particular ion added to the solution.

Zusammenfassung. Hochgereinigtes Rinderwachstumshormon besitzt ein ziemlich gutes Bindungsvermögen für Mg^{++}-, Ca^{++}- und Na^{+}-Ionen. Andererseits wird K^{+} je nach Konzentration überhaupt nicht, oder nur in Spuren gebunden.

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Fractionation of Highly Purified Bovine Growth Hormone on Sephadex G-25 Gel

In an earlier paper, evidence was presented which suggested some inhomogeneity among highly purified bovine growth hormone (BGH) preparations[1]. These inhomogeneities were observed during electrophoretic studies on polyacrylamide gel columns and showed the resolution of BGH into five immunologically active bands.

This preliminary report describes the resolution of BGH homogeneous by such criteria as starch gel electrophoresis and *N*-terminal amino acid analysis into two subfractions by Sephadex gel exclusion chromatography.

The preparation of BGH, polyacrylamide gel electrophoresis, and Ouchterlony immunoassay techniques were as described previously[1].

30 mg of pure BGH were dissolved in 3 ml $0.05\,M$ NH_3, applied to a Sephadex G-25 column (3.14 cm^2 by 40 mm) and eluted with deionized H_2O. Typical elution results are given in the Figure. These data show that a protein peak emerged with the void volume of the column. However, a substantial amount of material was retained slightly by the gel and emerged as a second peak. Both of these fractions were recovered and concentrated by lyophilization. The dry weight ratio between fractions 1 and 2 was 3:1 (recovery 80%). Reruns with either fraction resulted in the reformation of both peaks. Sephadex G-25 gel has an exclusion barrier around 5000 and should generally retain uncharged molecules with a molecular weight less than this value. Earlier investigators gave a value of 45,000 for the molecular weight of BGH[2,3]. If this value were

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correct and molecular size were the only factor, the hormone should not be retained by the Sephadex G-25 gel.

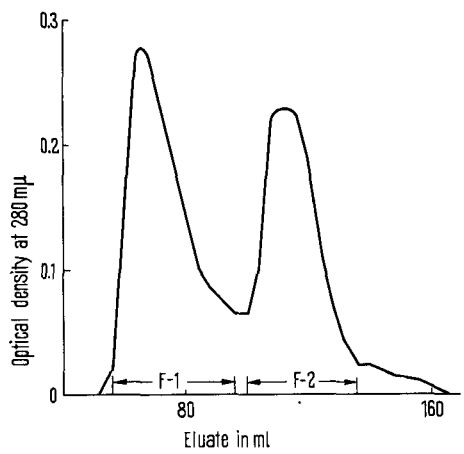
Polyacrylamide gel electropherograms prepared with material from both fractions (Figure) were essentially identical in respect to each other and the unfractionated hormone. In all cases a distinct pattern of five bands was formed as described earlier for the unfractionated hormone[1].

Immunoassays by the Ouchterlony technique showed that both fractions reacted positively with BGH-antiserum. A single precipitation band was obtained in each case. These bands and the one formed by standard growth hormone were confluent. However, the band formed by fraction 1 material (Figure) was quite weak and very indiscrete in shape.

Bioassays by the rat tibia assay[4] showed that both fractions possessed somatotropic activity as shown in Table I.

The determination of the relative amino acid composition[5] in 22 h hydrolysates yielded the results shown in Table II. These data were not corrected for hydrolysation losses. These results show no significant differences between either of the fractions and the unfractionated hormone in respect to amino acid composition. A spectrophotometric tryptophan determination[6] done on the unfractionated hormone indicated that our hormone preparation was devoid of tryptophan. This is in contrast to the findings of LI and CHUNG[2] who found a value of three residues of tryptophan based on a molecular weight of 45,000. However, their results were published nearly 10 years ago and consequently were obtained with materials prepared by less advanced protein purification techniques than are available today. BGH would therefore represent the second growth hormone described lacking tryptophan, since rat growth hormone also was found to contain none[7].

Sedimentation velocity studies in the Spinco Model E ultracentrifuge with the unfractionated hormone and the materials derived from the two peaks in the Figure showed evidence of at least two components within each preparation (Table III). Under the condition of analysis, a small amount of a slow-moving component was present in all the samples with a minimum molecular weight corresponding to approximately 6000. The predominant portion of each preparation had sedimentation coefficients corresponding to minimal molecular weights greater than



1% BGH solution in 0.05 *M* NH_3 run over Sephadex G-25. F-1 and F-2 = recovered fractions. Flow rate, 1 ml/min; eluted at room temperature; eluant, deionized H_2O.

Table I. Growth hormone assay of BGH fractions obtained by Sephadex gel exclusion chromatography (see the Figure)

Sample	No. of rats	Dose/rat (μg)	Tibia width (micra)
Control	7	none	159 ± 2.59[a]
Fraction 1	7	160	309 ± 3.26
Fraction 2	7	160	265 ± 11.0

[a] Standard error of average.

Table II. Amino acid composition of fractions 1 and 2 (see the Figure) expressed in residues per 31 residues of alanine

Amino acid	Fraction 1	Fraction 2	BGH unfractionated
Lysine	22.9	22.7	20.9
Histidine	7.2	8.5	7.7
Arginine	22.0	21.2	19.0
Aspartic acid	33.7	32.5	32.9
Threonine	24.6	23.3	23.9
Serine	25.0	24.7	24.6
Glutamic acid	46.0	43.4	45.0
Proline	15.8	14.9	14.7
Glycine	22.0	22.0	21.8
Alanine	31.0	31.0	31.0
Valine	14.5	14.5	14.8
Methionine	8.3	7.4	8.0
Isoleucine	12.0	10.9	11.1
Leucine	48.2	46.1	46.5
Tyrosine	12.9	11.9	11.8
Phenylalanine	25.8	24.0	24.5
Tryptophan			none

Table III. Sedimentation analysis of BGH fractions shown in the Figure

Sample	Sedimenting component (% of total)	$S_{20,w}$[b] (95% confidence limits)	Apparent minimum[a] MW (95% confidence limits)
Unfractionated	Slow (18%)	(1.17 ± 0.48)S	7,100 (3,200–11,900)
BGH	Fast (82%)	(2.74 ± 0.04)S	25,400 (24,900–26,000)
Fraction 1	Slow (8%)	(1.12 ± 0.40)S	6,600 (3,400–10,500)
	Fast (92%)	(2.99 ± 0.07)S	29,000 (28,000–30,000)
Fraction 2	Slow (< 10%)	~1S present but not well enough resolved	
	Fast 1 (> 80%)	(3.15 ± 0.15)S	31,400 (29,000–33,600)
	Fast 2 (< 10%)	> 3S present but not well enough resolved	

[a] Assumption of an unsolvated, spherical particle ($f/f_0 = 1$). [b] Values corresponding to 1% protein concentration. Samples were run as 1% solutions in 0.05 *M* NH_3 containing 0.1 *M* KCl.

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25,000. These latter coefficients differ from each other but are within the range of sedimentation coefficients reported by LI and PEDERSON[8] for BGH when measured in buffer solutions of different pH values. The fraction 2 material contained a trace of a fast-moving component with an S_{20}, w > 3.15.

BGH dissolved in low ionic strength aqueous solutions of ammonia was reproducibly resolved into two fractions of identical amino acid composition by exclusion chromatography on Sephadex G-25 gel. These results were unexpected since the molecular weight reported for this hormone should exceed the exclusion barrier of the gel for uncharged molecules by a factor of at least 9. Inhomogeneity within our BGH preparation was also indicated by electrophoretic techniques and ultracentrifugational studies. Such micro-inhomogeneities among highly purified proteinaceous hormones are not uncommon[9,10], but were never accounted for in a satisfactory manner. Two interpretations as applicable to our observations with BGH are considered in the following: a causative factor might be the existence of several conformational forms of the hormone differing in their mode of protein chain folding. These structures might represent different hormonal salt forms. Retardation of the hormone on the gel columns might also occur by means of the weak ion exchange properties of Sephadex G-25. Two major forms of BGH molecules would then be present with each having different affinities for the charges on Sephadex gel but with similar molecular weights. On the other hand, we might also assume that aggregational phenomena are responsible for these observations. A molecular weight of 45,000 for BGH could conceivably represent the value of a stable polymer of the hormone. The size of the monomeric basic unit would then be considerably smaller. The experimental conditions chosen for Sephadex G-25 gel exclusion chromatography might favor the temporary formation of the monomeric and possibly some intermediary forms of the hormone while running through the column bed. These forms would be very labile and subject to immediate reaggregation to higher molecular weight complexes after elution and lyophilization. The same argument might explain the discrepancies in weight distribution observed between the sedimentation and chromatographic studies[11].

Zusammenfassung. Hochgereinigtes Rinderwachstumshormon wurde durch Sephadex G-25 Gel-Chromatographie in zwei Komponenten zerlegt. Beide Fraktionen wie auch das unfraktionierte Präparat erwiesen sich in bezug auf Aminosäurenzusammensetzung, Elektrophorese und biologische Aktivität identisch.

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Culture of Macrophages under Homogeneous Static Magnetic Field

The number of macrophages kept in vitro decays during the first hours and later starts to increase. We have used macrophages obtained from the guinea-pig peritoneal cavity, culturing them in guinea-pig serum with Hanks solution.

In a preliminary group of experiments, series of tubes were kept in an incubator and other series were placed in the gap of an electromagnet. Every hour for 4 h we sampled one tube from each series and proceeded to count the number of macrophages in a Neubauer chamber. In each case the tubes which had been in the magnetic field gave a greater number of macrophages than the tubes which had been in the incubator. The magnetic field intensity was either 4200 oersteds or 5650 oersteds, and the temperature was either constant (around 27 °C, 30 °C, 37 °C etc.) or increasing (i.e. from 20–32 °C). Although a set of 6 practically homogeneous experiments from this group showed that differences from 27–33% in the number of macrophages were only statistically significant at a probability level ranging from 10–30%, the fact that the differences in this preliminary group were always in the same direction suggested that it would be desirable to carry out more experiments.

Another group of experiments was carried out subjecting the cultures to field intensities of 2000, 4000, 6000, and 8000 oersteds. The number of macrophages was compared to that given by tubes maintained in the pole gap, but without magnetic field. The temperature was always around 25 °C. Positive differences of 36, 46, 55, and 56%; 74%; 23, 30, 31, and 84%; 21 and 47%; and 17, 20, and 50% were significant at probability levels 1, 2, 5, 10, and 20% for the respective groups. A plotting of the number of macrophages against field intensity showed that the differences are greatest around 4000 oersteds.

We draw the conclusion that there is a magnetic effect in cultures of macrophages manifested by a larger number of viable cells, and that the effect is strongest at approximately 4000 oersteds. These results suggest that the magnetic field alters the metabolism of the macrophages and that the culture dynamics is changed.

Résumé. En cultivant des macrophages dans des champs magnétiques statiques et homogènes, on constate que le nombre de macrophages est le plus grand sous l'effet de champs de 2000 à 8000 oersteds, avec un maximum à 4000 environ.

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