that our experimental results are in correlation with the shifting of equilibrium between synthesis and breakdown of adenylyl 2,3-DPGA, discovered by HASHIMOTO et al[10].

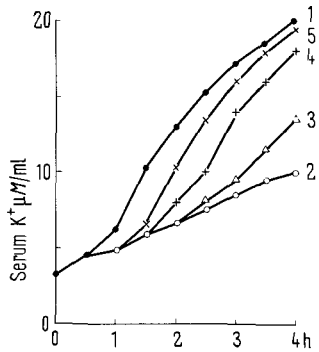

Fig. 3. Effect of $NaHSO_3$ on the K^+ transport of human blood in the presence of $10^{-3}M$ IAA + $10^{-2}M$ adenosine at 37 °C. For $NaHSO_3$ concentrations, see Figure 2.

Zusammenfassung. Der 2,3-Diphosphoglyzerat-Stoffwechsel menschlicher roter Blutkörperchen bedingt die Geschwindigkeit des infolge von ATP-Mangels auftretenden K^+-Austritts. Der rasche K^+-Austritt der Erythrocyten kann dann auftreten, wenn die Zellen kein 2,3-Diphosphoglyzerat mehr enthalten.

G. GÁRDOS[11]

Department of Cell Metabolism, Research Institute of the National Blood Centre, Budapest (Hungary), December 6, 1965.

[10] T. HASHIMOTO, Y. ISHII, M. TATIBANA, and H. YOSHIKAWA, J. Biochem., Tokyo *50*, 471 (1961).

[11] The excellent technical assistance of Miss E. MÉSZÁROS is gratefully acknowledged.

Demonstration of a Negative Binding Effect of Bovine Growth Hormone Toward Potassium

Studies with enzymatically modified bovine growth hormone (BGH) preparations revealed that some of these fragments retained large amounts of inorganic residue despite dialysis or Sephadex gel exclusion chromatography. The same phenomenon, but to a lesser extent, was found to apply to undegraded BGH[1]. The bulk of this inorganic material consisted of Na^+, Mg^{++}, Ca^{++}, Al^{+++}, and SiO_3^{--}. These results suggested that some of the ions were quite intimately bound by the hormone. Several mono- and divalent cations were, therefore, added singly at various concentrations to BGH solutions on the premise that different external concentrations of these ions might induce specific conformational changes of the protein chain, which in turn might allow for optimal ion binding.

Reaction mixtures were prepared as 3 ml aliquots containing 1% BGH. Cations added singly to the hormone solutions were Mg^{++}, Ca^{++}, Na^+, or K^+, all as their chloride salts. The ions were tested over concentrations ranging from 0.005–$0.03M$. The BGH-Mg^{++} and the BGH-Ca^{++} solutions were prepared in $0.05M$ acetic acid to avoid precipitation of the hormone since addition of these divalent ions to basic solutions caused an immediate precipitation of the protein. Na^+ and K^+ were added to the hormone in $0.05M$ NH_3 solutions. These test solutions were passed over Sephadex G-25 gel columns (3.14 cm^2 by 40 cm) to effect separation of the hormone plus ion complexes from the unbound ions. Elution was done with deionized H_2O. Studies by GELOTTE[2] have shown that small inorganic cations, in particular Na^+ and K^+, were retained to extents up to twice the total volume of Sephadex G-25 columns due to the presence of negative charges in the gel. Surprisingly, the hormone emerged from the columns in the form of 2–3 usually poorly resolved peaks as exemplified in Figure 1 for the BGH-Mg^{++} test solution. Only in the case of the BGH-K^+ solution was a fair resolution into two peaks obtained. All the protein-containing tubes were pooled to give one fraction. These solutions were reduced to dryness by lyophilization.

Control solutions containing 1% hormone in 0.1 and $0.05M$ NH_3 emerged as one asymmetrical peak in the first case and as 2 symmetrical peaks in the latter case. Hormone solutions in $0.05M$ acetic acid yielded one symmetrical peak which emerged with the void volume of the

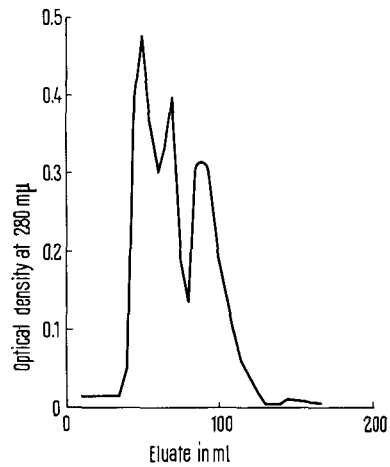

Fig. 1. BGH + $0.02M$ $MgCl_2$ solution passed over Sephadex G-25. The solution contained per ml: 10 mg BGH, 0.05 mM NH_3, 0.02 mM $MgCl_2$. Eluted with deionized H_2O at room temperature. Flow rate was 1 ml/min.

[1] F. REUSSER, Acta endocrinol. Copenh. *49*, 578 (1965).

[2] B. GELOTTE, J. Chromat. *3*, 330 (1960).

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.

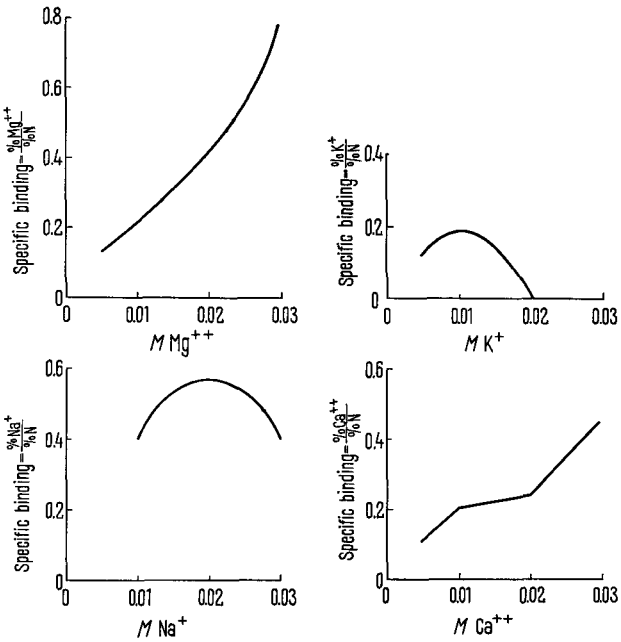

Fig. 2. Specific binding of Mg^{++} (upper left), Ca^{++} (lower right), Na^{+} (lower left) and K^{+} (upper right) by BGH.

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.

F. REUSSER[3]

Department of Microbiology, The Upjohn Company, Kalamazoo (Michigan, USA), February 8, 1966.

[3] The performance of the elementary analyses by R. C. ANDERSON is gratefully acknowledged. Thanks are also due to B. CZUK and N. O. MILKOVICH for technical assistance.

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

[1] F. REUSSER, Archs Biochem. Biophys. *106*, 410 (1964).
[2] C. H. LI and D. CHUNG, J. Biol. Chem. *218*, 33 (1956).
[3] A. J. PARCELLS, Nature *192*, 971 (1961).