BROYER[8]), in Portionen von 0,5 g für Zeiten von 3–18 min einem Volumen von 200 cm³ einer auf pH 4,5 eingestellten, mit $^{32}P$ markierten, durchlüfteten m/1000-$KH_2PO_4$-Lösung ausgesetzt, gewaschen (Verfahrensdetails bei NOGGLE und FRIED[5]) und verascht. Durch Messung ihrer Radioaktivität wurde die aufgenommene Phosphatmenge ermittelt. In fünf unabhängigen Versuchsreihen wurden im wesentlichen dieselben Resultate erhalten. Die hier dargestellten Kurven sind derjenigen Versuchsreihe entnommen worden, deren Werte in der Mitte der fünf Reihen liegen. Nach dem eingangs erwähnten Verfahren wurden aus Figur 1 die folgenden $k_2$-Werte berechnet:

$$k_2^{I} = 1{,}7 \cdot 10^{-3}\,[\text{sec}^{-1}];\quad k_2^{II} = 5 \cdot 10^{-3}\,[\text{sec}^{-1}];$$
$$k_2^{III} = 12{,}7 \cdot 10^{-3}\,[\text{sec}^{-1}];\quad k_2^{IV} = 25{,}5 \cdot 10^{-3}\,[\text{sec}^{-1}].$$

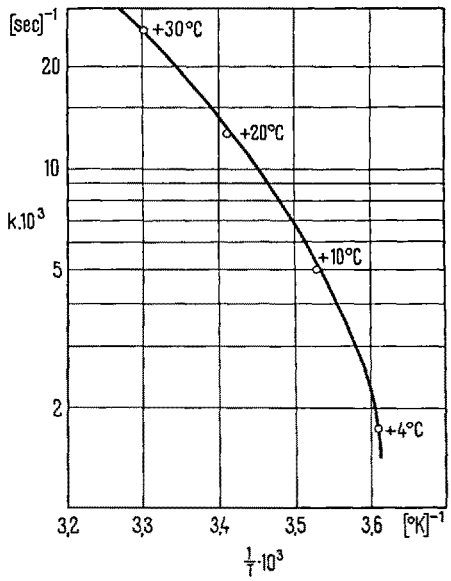


Fig. 2. Temperaturabhängigkeit der Aufnahmegeschwindigkeit von Phospationen in abgetrennten Gerstenwurzeln

Es sei erwähnt, dass NOGGLE und FRIED[5] für $k_2^{IV}$ die Werte 12,6 bzw. $16{,}6 \cdot 10^{-3}\,[\text{sec}^{-1}]$ für die beiden sich überlagernden Teilmechanismen fanden.

Aus Figur 2 erhellt, dass $\ln k_2 = \varphi(1/T)$ nur annähernd eine Gerade darstellt. Daher wurde $\Delta H^*$ nur für das Intervall $T^{III} \ldots T^{IV}$ aus (5), $\Delta S^*$ für $T^{III}$ (mit $\Delta H^*_{III,\,IV}$) aus (4) und die freie Aktivierungsenthalpie $\Delta G^*$ aus der bekannten Beziehung errechnet:

$$\Delta G^* = \Delta H^* - T\,\Delta S^*. \qquad (6)$$

Es ergibt sich:
$\Delta H^* = 11\,700\,[\text{cal} \cdot \text{Mol}^{-1}]$
$\Delta S^* = -28\,[\text{cal} \cdot \text{Grad}^{-1} \cdot \text{Mol}^{-1}]$
$\Delta G^* = 19\,900\,[\text{cal} \cdot \text{Mol}^{-1}]$

Ähnliche Werte für die thermodynamischen Grössen der Aktivierung von Reaktionen sind üblicherweise beim Zerfall von Enzym-Substratkomplexen anzutreffen[9,10].

*Summary.* From experiments on the uptake of phosphorus by excised barley roots, the temperature function of the rate-constant of this uptake reaction was determined and the enthalpy, free enthalpy and entropy of activation were calculated.

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[8] D. R. HOAGLAND und T. C. BROYER, Plant Physiol. *11*, 471 (1936).
[9] J. S. FRUTON und S. SIMMONDS, *General Biochemistry*, 2nd ed. (J. Wiley & Sons, Inc., New York 1958), p. 267.
[10] Herrn Dr. M. FRIED (Internationale Atomenergiebehörde, Wien) bin ich für viele anregende und klärende Diskussionen zu Dank verpflichtet. – Frl. E. HOFMEISTER und Frl. I. STADLER unterstützten mich eifrig bei der Ausführung der Arbeit.

## The Amino Acid Composition of Human and Bovine Lactotransferrins

It is well known that proteins with identical function originating from different species may have some difference in amino acid composition. Thus the residues number 8, 9 and 10 of the A chain of insulin vary according to the species, whereas the rest of the molecule remains unchanged[1]. Serum albumins of different species consist of a continuous polypeptide chain with little substitutions at the extremities of the molecule, for example: human serumalbumin (HSA) presents one residue of alanine as N-terminal group while bovine serumalbumin (BSA) possesses at the same place a residue of threonine; at the C-terminal end an interchange between -alanyl-leucine for HSA and -leucyl-alanine for BSA was noted[2]. Other examples of this kind are known and show that limited variations in the amino acid sequence in specific parts of the molecule are compatible with unimpaired function and that even these minor variations occur not at random but are genetically determined.

Having isolated a salmon-coloured iron-containing glycoprotein, called lactotransferrin, from human milk[3], we were interested in comparing its amino acid composition with that of the analogous protein from cow's milk.

*Material and Methods.* Lactotransferrins: The preparation of human lactotransferrin and its principal physicochemical and immuno-chemical properties were described elsewhere[3]. Bovine lactotransferrin was a sample isolated by GROVES[4] and kindly given to us by Dr. MCMEEKIN[5]. The purity of both proteins used for amino acid analysis was checked by immunoelectrophoresis[6] and gave a

[1] A. P. RYLE and F. SANGER, Biochem. J. *60*, 535 (1955).—A. P. RYLE, F. SANGER, L. F. SMITH, and R. KITAI, Biochem. J. *60*, 541 (1955).—H. BROWN, F. SANGER, and R. KITAI, Biochem. J. *60*, 556 (1955).—J. I. HARVIS, F. SANGER, and M. A. NAUGHTON, Arch. Biochem. Biophys. *65*, 927 (1956).
[2] E. O. P. THOMPSON, J. biol. Chem. *208*, 565 (1954).
[3] B. BLANC and H. ISLIKER, Bull. Soc. Chim. Biol. (Fr.) *43*, 929 (1961).
[4] M. L. GROVES, J. Amer. chem. Soc. *82*, 3345 (1960).
[5] Prof. Dr. T. L. MCMEEKIN, Eastern Research Laboratory, Philadelphia (Penn., USA).
[6] P. GRABAR and C. A. WILLIAMS, Biochim. biophys. Acta *10*, 193 (1953); *17*, 67 (1955).

single line of precipitation when run against polyvalent rabbit antisera containing high titers of homologous antibody.

Hydrolysis: 5 mg lactotransferrin were refluxed with 30 ml 6*N* HCl in a stream of nitrogen for 24 h. This great dilution of the sample in HCl keeps amino acid destruction at a minimum[7]. The hydrolyzate was evaporated under reduced pressure in a rotating evaporator of the type described by CRAIG[8]. Care was taken to maintain the temperature below 35°C.

Amino acid chromatography: The amino acid analysis was performed according to SPACKMAN, STEIN, and MOORE[9] on a Beckman–Spinco Analyzer. β-2-thienyl-DL-alanine was carried with each analysis as internal standard[10].

Determination of tryptophane: This amino acid being destroyed by acid hydrolysis, it was determined colorimetrically in the intact protein according to SPIES and CHAMBERS[11]. 2 mg lactotransferrin were used for analysis and reaction I was allowed to proceed for 14 h instead of 1 h in the original method.

*Results and Discussion.* The amino acid composition of the two lactotransferrins is given in Tables I and II. In the first column the percentage of amino acid residues found in the sample as such is given with the standard error of each value. In the next column, the percentage nitrogen supplied by each amino acid is mentioned. In the third column amino acid residues are represented as fractions of a total of 100 residues. The last column gives the number of residues per molecule.

For bovine lactotransferrin the molecular weight of 86,100 determined by GROVES[4] was used, from which 7.2% carbohydrates were deduced. For human lactotransferrin the calculation was based upon the value of MONTREUIL et al.[12] of 95,000.

The addition of the amino acid residues in the sample does not reach 100% because both proteins contain 7.2% carbohydrates and are contaminated with inorganic salts and some moisture. The high values for ammonia are due to the destruction of nitrogen containing organic compounds during acid hydrolysis and to the possible presence of traces of inorganic ammonium salts in the samples. Ammonia was therefore not included in the final calculation of amino acid residues.

MONTREUIL et al.[12] have published a partial amino acid analysis of human lactotransferrin, made by paper chromatography of the DNP-amino acids. The correspondence between his values and ours is fair, except for alanine and tyrosine. For the latter amino acid MONTREUIL's value is low (9 residues per molecule), due probably to the different methods used.

The high tryptophane value we found in bovine lactotransferrin is in good agreement with the value by GORDON and BASH[13].

If we compare the amino acid composition of the two lactotransferrins of human and bovine origin we observe that the general pattern of amino acid distribution is similar. The amount of the 4 amino acids which are most likely to bind iron: histidine, threonine, cystine (as cysteine) and tyrosine, is practically the same—the difference does not exceed one residue—suggesting a similar disposition of the iron binding sites of the two lactotransferrins.

The greatest difference between the human and bovine protein is in the tryptophane content, high in the bovine lactotransferrin and below the sensitivity of the method[11] for human lactotransferrin. The methionine is twice as abundant in the human as in the bovine protein. Notable differences are also found in the amount of arginine, aspartic acid, glycine and isoleucine.

Table I. Amino acid composition of bovine lactotransferrin

| | % Amino acid residue in sample | % Nitrogen | Residue for 100 amino acid residue | Number of residues per molecule [a] |
|---|---|---|---|---|
| Lysine | 8.09 ± 0.135 | 1.77 | 9.61 | 60 |
| Histidine | 1.58 ± 0.055 | 0.49 | 1.88 | 11 |
| Ammonia | 2.53 | 2.22 | – | – |
| Arginine | 6.43 ± 0.070 | 2.31 | 7.64 | 39 |
| Aspartic acid | 8.26 ± 0.035 | 1.01 | 9.81 | 68 |
| Threonine | 3.76 ± 0.030 | 0.52 | 4.47 | 35 |
| Serine | 4.02 ± 0.007 | 0.65 | 4.78 | 44 |
| Glutamic acid | 10.12 ± 0.065 | 1.10 | 12.02 | 74 |
| Proline | 3.59 ± 0.026 | 0.52 | 4.26 | 35 |
| Glycine | 3.01 ± 0.035 | 0.74 | 3.58 | 50 |
| Alanine | 4.94 ± 0.055 | 0.97 | 5.87 | 66 |
| Cystine | 3.98 ± 0.110 | 0.50 | 4.73 | 17 |
| Valine | 4.23 ± 0.007 | 0.60 | 5.03 | 41 |
| Methionine | 0.45 ± 0.020 | 0.05 | 0.53 | 3 |
| Isoleucine | 1.66 ± 0.050 | 0.21 | 1.97 | 14 |
| Leucine | 7.66 ± 0.170 | 0.95 | 9.10 | 64 |
| Tyrosine | 4.82 ± 0.340 | 0.41 | 5.73 | 28 |
| Phenylalanine | 4.61 ± 0.205 | 0.44 | 5.48 | 30 |
| Tryptophane | 2.94 | 0.44 | 3.49 | 15 |
| Total with $NH_3$ | 86.68 | 15.90 | | |
| Total without $NH_3$ | 84.15 | 13.68 | | |

[a] Calculated for a molecular weight of the protein part of 80000.

Table II. Amino acid composition of human lactotransferrin

| | % Amino acid residue in sample | % Nitrogen | Residue for 100 amino acid residue | Number of residues per molecule [a] |
|---|---|---|---|---|
| Lysine | 6.49 ± 0.080 | 1.42 | 8.14 | 56 |
| Histidine | 1.50 ± 0.029 | 0.46 | 1.88 | 12 |
| Ammonia | 4.89 | 4.28 | | |
| Arginine | 7.52 ± 0.168 | 2.70 | 9.44 | 53 |
| Aspartic acid | 8.50 ± 0.214 | 1.03 | 10.66 | 81 |
| Threonine | 3.18 ± 0.109 | 0.44 | 3.99 | 35 |
| Serine | 4.09 ± 0.120 | 0.66 | 5.13 | 52 |
| Glutamic acid | 10.23 ± 0.324 | 1.11 | 12.84 | 88 |
| Proline | 3.67 ± 0.052 | 0.53 | 4.60 | 42 |
| Glycine | 3.38 ± 0.125 | 0.83 | 4.24 | 66 |
| Alanine | 4.64 ± 0.155 | 0.91 | 5.82 | 72 |
| Cystine | 3.27 ± 0.125 | 0.41 | 4.10 | 16 |
| Valine | 4.49 ± 0.226 | 0.63 | 5.63 | 50 |
| Methionine | 0.76 ± 0.024 | 0.08 | 0.95 | 6 |
| Isoleucine | 2.07 ± 0.044 | 0.26 | 2.60 | 20 |
| Leucine | 6.81 ± 0.102 | 0.84 | 8.54 | 66 |
| Tyrosine | 4.19 ± 0.116 | 0.36 | 5.26 | 28 |
| Phenylalanine | 4.91 ± 0.240 | 0.47 | 6.16 | 37 |
| Tryptophane | < 0.2 | | | 1 or 0 |
| Total with $NH_3$ | 84.59 | 17.42 | | |
| Total without $NH_3$ | 79.70 | 13.14 | | |

[a] Calculated for a molecular weight of the protein part of 88000.

[7] J. ADRIAN and J. C. FAVIER, Ann. Nutr. Aliment. *15*, 181 (1961).
[8] L. C. CRAIG, J. D. GREGORY, and W. HAUSMAN, Analyt. Chem. *22*, 1462 (1950).
[9] D. H. SPACKMAN, W. H. STEIN, and S. MOORE, Analyt. Chem. *30*, 1190 (1958).
[10] F. L. SIEGEL and M. K. ROACH, Analyt. Chem. *33*, 1628 (1961).
[11] J. R. SPIES and D. C. CHAMBERS, Analyt. Chem. *20*, 30 (1948).
[12] J. MONTREUIL, G. BISERTE, S. MULLET, M. SPIK, and N. LEROY, C. R. Acad. Sci. (Fr.) *252*, 4065 (1961).
[13] W. G. GORDON and J. J. BASH, Biochem. biophys. Acta *48*, 397 (1961).

All these disparities may contribute to the explanation of two facts: the stronger association constant of human lactotransferrin for iron and the absence of the immunochemical cross-reactions between the two lactotransferrins considered.

In order to give precisions about the factors responsible for the iron binding capacity and those implicated in species specificity, it would be necessary to establish the amino acid sequence or to isolate and compare the iron binding sites of human and bovine lactotransferrins.

*Résumé.* La composition en acides aminés des lactotransferrines humaine et bovine a été déterminée. La distribution des acides aminés dans les protéines étudiées est très semblable, sauf en ce qui concerne le tryptophane, la méthionine, l'arginine, l'acide aspartique, la glycine et l'isoleucine.

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## Free and Membrane-Bound Ribosomes in Maturing Neurones of the Chick and their Possible Functional Significance[1]

*Introduction.* The presence of free and membrane-bound ribosomes (RNS or Palade granules) has been proved for all basophilic cells, and it is now well established that this basophilia is related to ribosomes[2,3]. An attempt has been made[4] to suggest possible separate functions for free and membrane-bound ribosomes in nerve cells, for membrane-bound ribosomes seen under the electron microscope are components of the Nissl bodies seen in light microscopy. To give further basis to this view that different functions must be attributed to the two types of ribosomes, a series of studies is in progress to separate riboomes capable of specific protein synthesis intimately related to a specialized cell activity from those which serve a more general reparative or hetero-catalytic function. Koch[5] has drawn attention to the fact that ribosomes are also responsible for mediating genetic information within the framework of histogenesis. In order to gain some knowledge of the variety of the functional significance of ribosomes, the histogenesis of motoneurons of the chick spinal cord has been studied as the time sequence of its maturation can be controlled readily.

*Material and Methods.* Fertilized eggs (White Leghorn) were incubated at 38.7°, and eggs of 7 and 11 days' incubation were used for this particular communication. Parts of the spinal cord were fixed according to Caulfield[6], other portions following the method of North and Pollack[7]. The material was embedded in Vestopal; and the contrast of the sections was increased by means of lead hydroxide following a method by Wolff[8]. Some shrinkage by fixation was unavoidable in view of the high water content of embryonic tissue. For this and other reasons, material such as pancreas, liver and stomach was fixed and processed at the same time to make possible a comparison of cells containing a large number of ribosome granules. The embedded material was cut with an LKB Ultratome and was examined and photographed with an Akashi Tronscope TRS-50.

Paraffin-embedded material from adjacent portions of the cord was stained with methylene blue[9], with the Feulgen technique and with an azan stain. To study neurofibrillae and peripheral connections of the developing cord, a silver technique[10] proved to be very useful for embryonic material.

*Results – Light Microscopy.* Neuroblasts of sections stained with methylene blue or Feulgen showed that the methylene blue-staining portions were deposited around Feulgen-positive particles. Furthermore, an accumulation of nuclear RNS material occurred within the nucleus and was finally channeled into the cytoplasm. Correlated with the progressive diminishing of chromatin material from nuclei initially filled with chromatin is the gradual increase of peri-nuclear cytoplasm, a finding in agreement with that of Gluck and Kulovich[11], who postulated an interdependence of the mass of basophilic substance in the cytoplasm and the increase in volume of the cytoplasm. The emission of methylene blue-positive and Feulgen-negative particles presents the following picture: Chromatin granules appear to be collected on the two opposite poles of the nucleus and to be arranged in long filaments towards the extra-nuclear space. In adjacent parts of the cytoplasm increased basophilia can then be observed. Following this initial phase, a continuous emission of basophilic material from nucleolar pools takes place until finally the scanty contents of the nucleus resembles the familiar stage of a nucleus containing the single distinct nucleolus seen in a fully matured neuron. The cytoplasm shows a progressive formation of Nissl granules which appear in places characterized by uniform patches of basophilia.

*Results – Electron Microscopy.* The electron microscopical pictures prove to be a valuable extension of information seen in light microscopy. In Figure 1, a large nucleus can be seen which is surrounded by a small area of cytoplasm characteristic of an early stage of neuroblast. The nucleus shows an osmophilic centre, the nucleolus, in addition to a further, somewhat diffusely arranged osmophilic area often referred to as an additional nucleolus. The remaining portion of nuclear material is made up of fine granular particles concentrated on certain portions of the double-layered membrane of the nucleus, which shows fine pores through which the osmophilic granules or ribosomes reach the cytoplasm (Figures 2 and 3). The cytoplasm contains some mitochondria (crista type) and a great number of free, loosely arranged ribosomes. Here and there membrane-like structures of a tubular arrangement can be seen. At a later stage of development, the

[1] This paper is based on an investigation in progress which will be submitted by J. Eschner in partial fulfillment of the requirements for the degree of Doctor of Medicine.

[2] A. Claude, J. exp. Med. *84*, 61 (1946).
[3] G. E. Palade and P. Siekevicz, J. biophys. biochem. Cytol. *2*, 171 (1956).
[4] P. Glees, Dtsch. Z. Nervenheilk. *184*, 607 (1963).
[5] G. Koch, Klin. Wschr. *40*, 7 (1962).
[6] J. B. Cailfield, J. biophys. biochem. Cytol. *3*, 827 (1957).
[7] R. J. North and J. K. Pollack, J. Ultrastr. Res. *5*, 497 (1961).
[8] J. Wolff, Elektronenmikroskopisches Institut der FU, Berlin, mündl. Mitteilung.
[9] A. Pischinger, Verh. Anat. Ges. (1930).
[10] P. Glees and L. Marsland, J. Physiol. *118*, 51 (1952).
[11] L. Gluck and M. V. Kulovich, Science *138*, 530 (1962).