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

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first profiles of endoplasmic reticulum can be discerned, but their profiles are bordered by very few ribosomes (Figure 3).

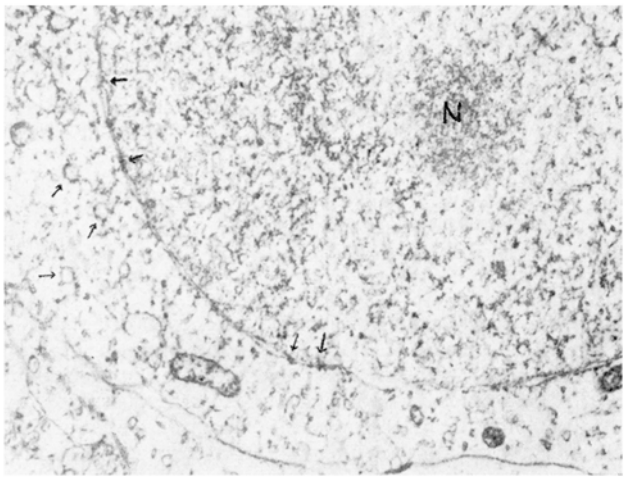

Fig. 1. Nucleus of a neuroblast with scanty cytoplasm. Tubularlike formations of endoplasmic reticulum may be observed in the cytoplasm, although the typicle profiles of endoplasmic reticulum are not yet present. Other arrows indicate pores through which nuclear material is emitted. Mag.: 30000 ×.

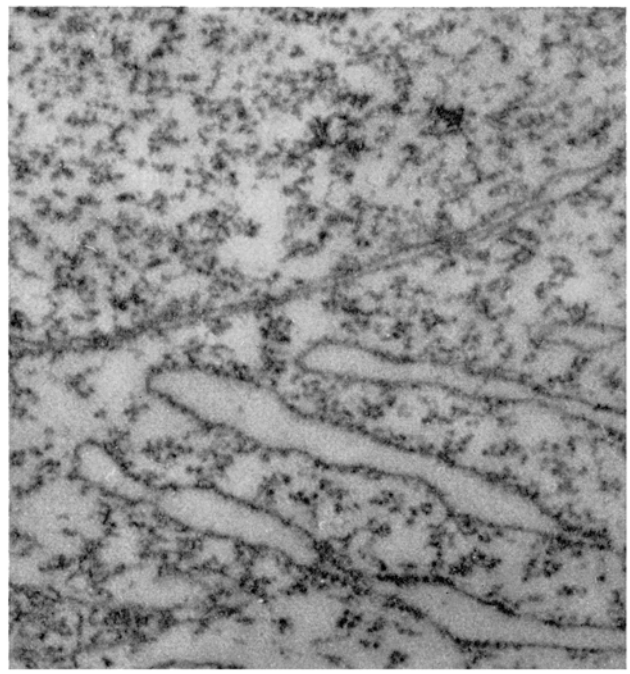

Fig. 3. The first profiles of endoplasmic reticulum appear, here lightly sown with ribosomes. The profiles show dilatation typical for embryonic cells. Mag.: 100000 ×.

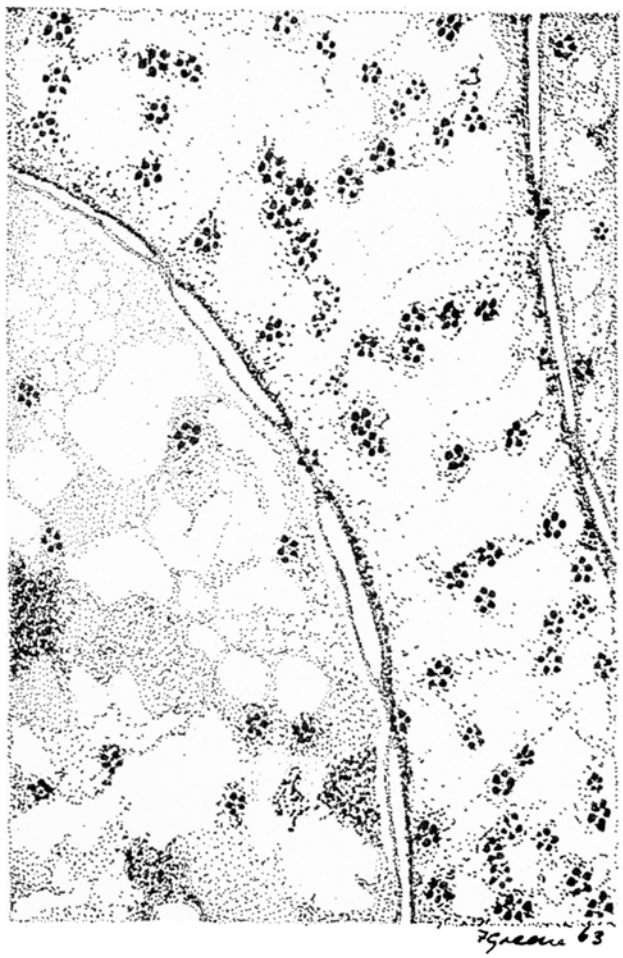

Fig. 2. Diagrammatic presentation of the emission of ribosomes through the Stomata of the nucleus of a neuroblast. Mag.: 30000 × (drawing by F. GREENE)[21].

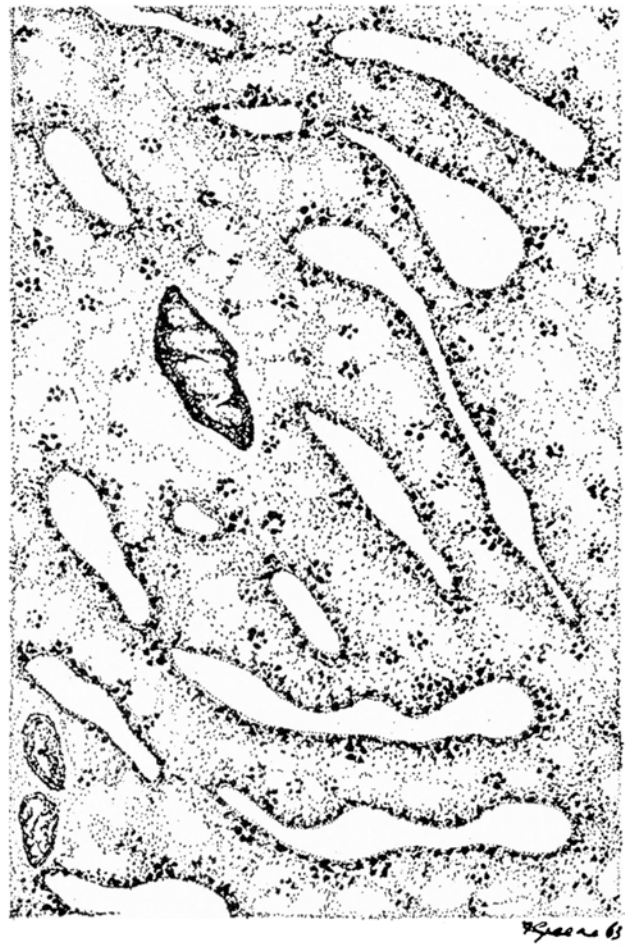

Fig. 4. Diagrammatic presentation of the cytoplasm of a maturing neuron. Note the free ribosomes. Mag.: 44000 × (drawing by F. GREENE)[21].

The free ribosomes, arranged in rosettes, are still present in great numbers. With progressive maturation of neurons, more profiles appear in the cytoplasm, and an accumulation of ribosome granules can be observed as if queuing up to pass through the nuclear pores. In the final stage of neuronal development, there appears to be a steady state between free and membrane-bound ribosomes. Concerning mitochondria, the great number of mitochondria in embryonic nerve cells must be emphasized. The mitochondria can reach considerable size and their structure is of the crista type. Similar large mitochondria have been reported by TENNYSON[12] for the cord of rabbit embryos.

Discussion. The marked basophilia of neurons seen in light microscopy and referred to as Nissl substance has in electron microscopy its counterpart in an accumulation of profiles densely sown with ribosomes[13–17]. Apart from these membrane-bound ribosomes, the cytoplasm of neurons shows a fair number of ribosomes not attached to membranes which may be called free ribosomes, often arranged in rosettes. It seems reasonable to suggest from our embryo studies reported in this paper that the function of these free ribosomes is the protein synthesis necessary for cell development and maintenance, and that this synthesis is carried out provided messenger RNS is organized to form a rosette of ribosomes[18]. Taking this view into account, it is probable that free ribosomes have the task of synthesizing proteins necessary for the build-up of the neuron which, due to its axonic and dendritic expansion, reaches considerable size in comparison with other body cells. It is not surprising, therefore, to find a continuous expulsion of ribosomes from the nucleus into the cytoplasm taking place while simultaneously mediating the genetic coding necessary for the future structural or functional organization of a particular neuron. The problem, therefore, arises as to what is the functional significance of the progressive increase of membrane-bound ribosomes in the final development stages of neurons. Some neurons show a preponderance of membrane-bound ribosomes (Nissl bodies) in a distinct way. This stage is reached when functional connections of motoneurons have been established with skeletal muscles on the one hand and with receptors on the other.

We would like to suggest as a possible task for these membrane-bound ribosomes the morphological coding of information resulting from the functional interconnection of receptor activity and motor discharge muscular contraction. We tend, therefore, to postulate the preservation of a functional pattern in or around the membrane-bound ribosomal organization capable of preserving a functional pattern of afferent discharges and enabling the cell to discharge in a particular sequence. For this morphological type of coding the same material is being used, namely membrane-bound ribosomes in contrast to free ribosomes carrying out 'genetic orders' and establishing neuronal connections. This speculative view finds some support when we relate our morphological findings to the observations of KUO[19], who finds a rhythmic discharge of chicken neurons after 96 h incubation. In this stage of development a motoneuron has only efferent connections, while afferent stimuli reach the neuron only after 126 h. Examination of cords stained for fibre connections seem to confirm these functional observations, namely that afferent connections follow efferent ones. With progressive establishment of afferent connections, synchronized discharges become more frequent. It is in these stages that the previously discussed appearance of membrane-bound ribosomes becomes more marked[20].

Zusammenfassung. Die Histogenese sowie die Anordnung und Verteilung der Nissl-Substanz wurde an ausreifenden Neuronen des Hühnchenrückenmarks licht- und elektronenmikroskopisch untersucht. In den ersten Phasen der Neurogenese wurden nur freie Ribosome im Cytoplasma aufgefunden. Mit fortschreitender Reifung bilden sich erste Profile des endoplasmatischen Retikulums aus, die spärlich mit Ribosomen besetzt sind. Diese Befunde werden funktionell ausgedeutet.

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[20] *Acknowledgments.* Our thanks are due to Miss I. SCHAAF for technical assistance with the work in light microscopy, to Miss I. MENDE and Mr. B. SHEPPARD for the electron microscopical preparations, and to Miss F. GREENE, who contributed the illustrations. We are especially grateful to the Welcome Trustees, London, for the generous loan of an electron microscope.
[21] All illustrations are derived from chick material of 7 days incubation. The two diagrams are based on original electron microscopical photographs.

Experiments on the Formation of Combs in the Ctenophores

According to FISCHEL[1], each blastomere of the Ctenophore egg at the 8-cell stage has the potential of forming one row of combs in the larva.

The results obtained by YATSU[2] confirm this statement. SPEK[3] maintains that in *Beroë ovata* this potential is connected with a peculiar green luminescent plasma; this plasma, according to REVERBERI[4], would consist mainly of mitochondria. At the 16-cell stage the green plasma is segregated into the 8 micromeres; these are thought to give rise to the 8 rows of combs: each micromere giving rise to one row.

YATSU[5], who described the cell-lineage in *Beroë ovata*, *Beroë forskalij* and *Callianira bialata*, found differences in the segmentative behaviour of the first 8 cells; the end-cells (E) would behave in a different way to the middle-cells (M).

YATSU did not thoroughly analyse the meaning of these differences, but the results he obtained from development

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