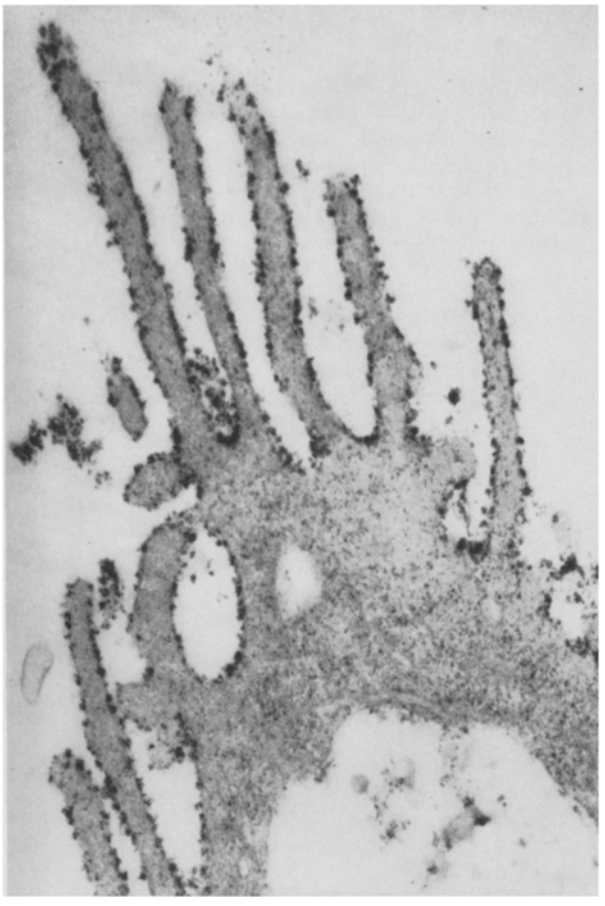

Fig. 1. ATPase reaction in the pseudopodia of an alveolar macrophage. Incubation time 60 min. ×38,000.

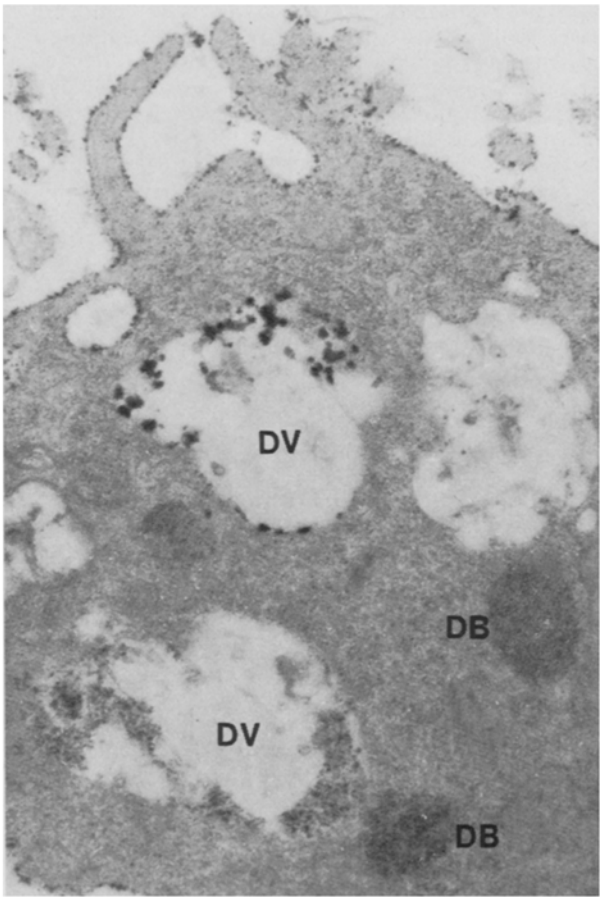

Fig. 2. ATPase reaction in the digestive vacuoles (DV) and dense bodies (DB) of an alveolar macrophage. Incubation time 60 min. ×50,000.

ed within alveolar macrophages is responsible for the active movements of the surface membranes which occur when the cells migrate or engulf foreign material.

Zusammenfassung. Durch eine elektronenmikroskopische Technik konnte bei Alveolarmakrophagen der Hamsterlunge die intrazelluläre Lokalisation der ATPase-Aktivität bestimmt werden. Dabei zeigte die Plasmamembran der Makrophagen eine hohe ATPase-Aktivität, während in den Vakuolen und in den elektronendichten Körperchen der Phagozyten eine geringere Reaktion vorgefunden wurde.

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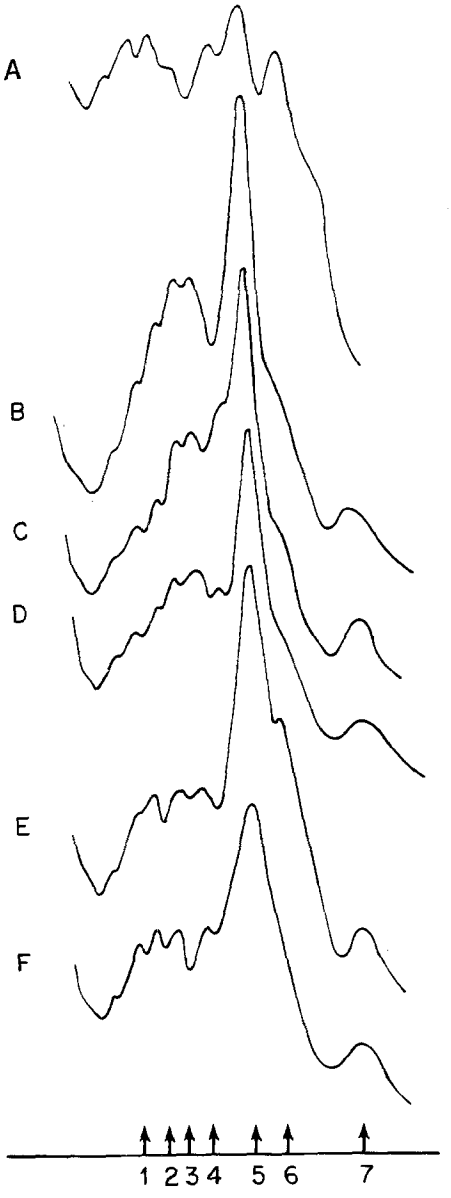
Presence of the Same Types of Nonhistone Chromosomal Proteins in Different Tissues

It has been proposed that nonhistone chromosomal proteins (NHC proteins) act as the specific regulators of gene expression (for review see [1]). In support of this proposal a considerable tissue specificity in the electrophoretic patterns of NHC proteins has been reported by many authors[2–9]. Since only a small fraction of the genome is active in any given type of cells[10–13], such markedly expressed tissue specificity of NHC proteins was surprising. Therefore, we decided to reexamine the question of the tissue specificity of the total NHC proteins.

Material and methods. Chromatin was prepared from isolated pure nuclei and the total chromosomal proteins were analyzed by polyacrylamide gel electrophoresis in a system which separated histones and NHC proteins on the same gel. The nuclei were isolated from liver, cerebral cortex, cerebellum and a mixture of midbrain and brainstem of female albino rats derived from Holtzman strain. In addition nuclei were also prepared from white leghorn chicken embryos in developmental stage 18 and from CHANG's rat ascites hepatoma[14]. From rat organs and

chicken embryos the nuclei were prepared directly by the CHAUVEAU procedure[15]. From hepatoma, the nuclei were prepared by the same procedure after the disruption of cells by osmotic shock with distilled water. Before the preparation of chromatin, the nuclear sap proteins were first removed by 2 extractions with standard saline citrate and 2 extractions with 0.1 *M Tris*-HCl buffer, pH 7.6. The chromatin was solubilized in 1 *M* NaCl (containing 0.01 *M* sodium citrate) precipitated by ethanol (66% final concentration) and redissolved in lysine-urea buffer (0.01 *M* L-lysine, 4 *M* urea, 2 m*M* Na_2EDTA, 50 m*M* β-mercaptoethanol, pH 11.6). To the solution was added CsCl and urea to the final concentration of 4.0 *M* urea and 3.38 *M* CsCl. The resulting solution with a density of 1.49 was centrifuged in a SW 39 rotor in a Spinco preparative ultracentrifuge at 125,000 $\times g$ for 48 h. The DNA together with 3 to 5% of the protein sedimented and chromosomal proteins stayed in the supernatant. For electrophoresis the CsCl was removed from the protein solution by overnight dialyses against lysine-urea buffer and the proteins concentrated by dehydration against Aquacide (Calbiochem). To separate all chromosomal proteins on 1 gel, a modification of the electrophoretic system described by FAMBROUGH, FUJIMURA and BONNER[16] was used. The solution of chromosomal proteins in lysine-urea buffer was acidified by HCl to pH 3.0. 100 μg of proteins were applied onto 7.5% Cyanogum-41 (Fisher Sci. Co.) gel and electrophoresis conducted at 4 mA per tube for 3 h in 0.35 *M* β-alanine tray buffer, pH 4.5. The gels were stained with 1% solution of amido black. The same electrophoretic pattern of analyzed proteins was observed regardless whether all isolation procedures were performed in the presence of the 100 μM of phenylmethylsulfonyl fluoride (Calbiochem) or not.



Densitometric tracings of the electrophoretic patterns of nonhistone chromosomal proteins from A) CHANG's rat ascites hepatoma; B) rat cerebral cortex; C) rat cerebellum; D) a mixture of rat midbrain and brainstem; E) rat liver and F) chicken embryo – developmental stage 18.

Results and discussion. The histones and NHC proteins separated into distinct nonoverlapping fractions. Densitometric tracing of the electrophoretic patterns of NHC proteins (Figure) has shown the presence of the same 7 main bands of NHC proteins in all analyzed organs. Only quantitative differences in the amount of proteins present in different bands were found.

The presence of the same main fractions of NHC proteins in chromatin of different tissues and species would indicate that the majority of NHC proteins is not involved in a specific regulation of gene expression. Our results are in logical agreement with the findings that in most cells only a few percent of the genome are copied into mRNA[10–13]. They are also in agreement with reports which found only a limited heterogeneity of the NHC proteins[17–20]. The existence of specific regulators of gene expression in the fraction of NHC proteins has been shown by the use

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of artificial chromatin as a template for RNA synthesis[10,21]. But, it is most probable that these specific molecules are present in chromatin only in small quantities not detectable by analytical polyacrylamide gel electrophoresis and that the majority of NHC proteins has nonregulatory functions.

The difference between our findings and the findings reported in the literature could be explained by the following facts. Used procedure eliminated the possibility of contamination of our preparations with cytoplasmic proteins or nuclear sap proteins[22] which could be tissue specific. In addition to working with purified nuclei, our analysis of the total chromosomal proteins by avoiding the different steps required for the subsequent isolation of NHC proteins eliminated additional possibilities for the formation of artefacts.

The most pronounced differences were reported between the electrophoretic patterns of NHC proteins isolated from nucleated erythroid cells and the electrophoretic patterns of NHC proteins isolated from other types of cells[4,7,9]. The NHC proteins isolated from erythroid cells are less heterogeneous with some electrophoretic bands missing. In our opinion, these findings do not support the conclusion that the main electrophoretic bands of NHC proteins are involved in the regulation of gene expression. The nucleus of nucleated erythrocytes is completely inactive and also the reticulocytes are inactive with respect to RNA synthesis[23]. Therefore, it can be expected that the proteins (enzymes) required for DNA synthesis or RNA synthesis and processing are missing in the electrophoretic pattern of NHC proteins isolated from erythroid cells.

Zusammenfassung. Die Analyse mit Polyacrylamide-Gel-Electrophorese von Chromatinproteinen an Hühner-Embryonen, Ratten Ascites Hepatoma, Rattenleber und verschiedenen Teilen des Rattengehirns hat ergeben, dass dieselben Hauptfraktionen der sauren Chromosomenproteine in allen untersuchten Geweben vorhanden sind.

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The Low Calcium Content of Cellular Systems Adapted to Flow

Recently EPSTEIN[1] stressed a few wellknown facts about the phloem and put forward a tentative explanation for their correlation with the observed very low calcium and boron content. In relation to these suggestions – which I would like to endorse – there seem to be a number of other rather interesting points and correlations which could be added to expand the view.

Phloem is not the only living plant tissue adapted to flow. If wounded, the latex system of many plants will show extensive flow. Especially the outflow of latex from *Hevea brasiliensis* has been extensively investigated. This latex is derived from a large drainage area and is extruded by means of osmotic attraction of water[2]. The exuding latex can best be considered as a diluted cytoplasm[3], as it contains both many proteins and plastids and an enormous array of enzymes. The low viscosity of latex is again linked with its very low calcium content, e.g. ca. 2% of the total cation content[4].

This low calcium and high potassium content of both phloem and latex plasm would seem to be appropriate from the point of view of plasmatic viscosity. Monovalent ions cause swelling and lowered viscosity, while divalent ions favour an increased viscosity due to lower water content. The fluidity of latex can be related to a certain amount of swelling. In the ontogeny of the latex vessels in *Hevea* the cytoplasm increases in volume, while the original large vacuoles retract to a multi-disperse system of minute droplets[3,5]. The resulting immense number of lutoids[6] ultimately only occupy a few percent of the volume of exuded latex. Also the electron microscope has produced evidence of the strong dilution of the phloem cytoplasm.

EPSTEIN[1] proposes an exclusion of calcium from the sieve tubes. The question arises as to where we must locate this process of restricting calcium activity. One could conceive of relating it to the process of vein-loading. But there is also a possibility of a low supply towards the phloem from cells with a low calcium activity.

In the conception 'symplasm', the unity of cytoplasmic contents of living cells, connected by their plasmodesmata and including the phloem, is implied. From this point of view of cytoplasmic continuity there is reason to suspect a low calcium activity in the cytoplasm of surrounding cells as well. This would seem to be contrary to the generally moderate to high calcium content of most cells, but much of it is located in cell-walls or vacuoles. That much of the calcium in the plant could be non-essential has also been suggested by WALLACE, FROHLICH and LUNT[7]. Although cell-organelles, e.g. chloroplasts can accumulate calcium, some recent evidence from muscle physiology suggestsvery low calcium activities[8–10]. For *Nitella translucens* a value of 8 mM in the flowing cytoplasm is mentioned[11], while for *Nitella flexilis* a value of 125 mM/l is given for potassium[12].

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