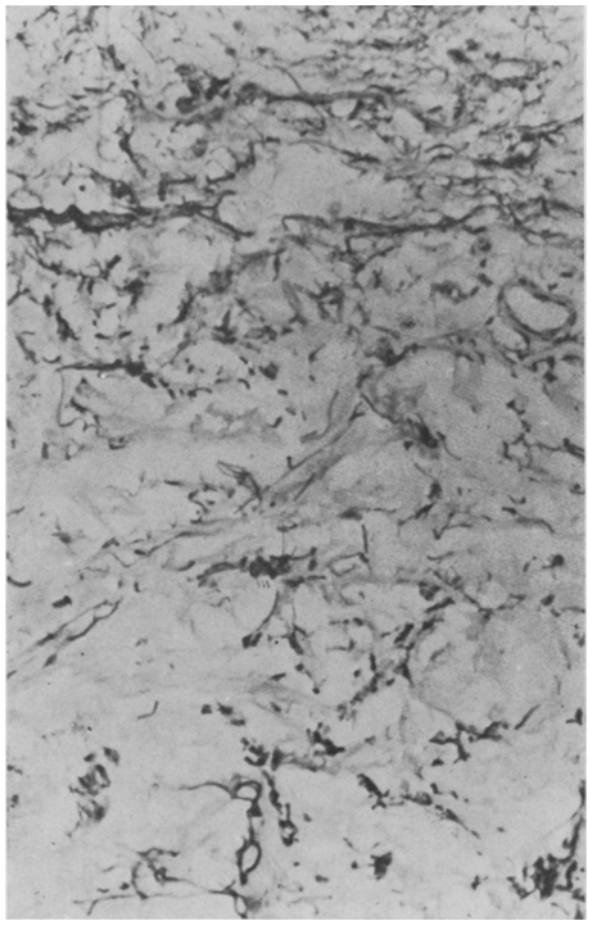

Fig. 3. Emphysema. 24-year-old patient. In the upper dermis, some enlarged elastic fibres can be seen (elastosis). In the middle dermis, there is decrease in the number of elastic fibres (elastolysis) Staining: Gomori's fuchsin-resorcinol.

Contingency analyses

	Emphysema	Asthma	
Normal skin	3	109	112
Xeroderma	40	6	46[a]
Total	43	115	158

[a] X^2 117.2 ($P \ll 0.001$) shows a close relationship between xeroderma and emphysema on the one hand and normal skin and asthma on the other. (Computation Department of the Neurobiological Institute)

age limits 12 and 64 years. In 40 cases histological changes were found in skin taken from the inner surface of the forearm where the skin is protected from external influences that could change its structure. The following techniques were used: staining with haematoxilin-eosin, McManu's PAS technique for collagen fibres and Gomori's fuchsin-resorcinol for the elastic fibers.

The changes found were hyperkeratosis, diffuse orthokeratosis of Malpighi's mucous body, and pilose infundibula. The mucous body showed atrophy of the crests and of intercrestal sectors, and less common acantosis of the interpapillary crests with fusion of neighbouring crests. The thickness of the granulous layer was diminished, and in some cases completely absent, imitating a true ichthyosiform picture. The pilosebaceous skin adnexa were hypotrophic and the sudoriparous glands hypertrophic. There were no changes in the collagen fibers. Finally, it can be said that histopathology of the skin of patients suffering from emphysema showed none of the follicular plugs that are found in patients suffering from ichthyosis and that the elastosis is different from that found in senile patients.

A comparative study was carried out simulataneously on 109 patients suffering from allergic asthma, who had no signs of clinical roentgenological or functional emphysema. Hyperkeratosis was found in three asthmatic patients, but in the rest the skin was normal.

Contingency analyses were done for asthma and emphysema on the one hand normal skin and xeroderma on the other. The $X^2 = 11.2$ was very highly significant ($P \ll 0.001$), indicating a very close association between xeroderma and emphysema on the one hand and normal skin and asthma on the other. The histological changes found in emphysematous patients could possibily be related to the similar process in pulmonary tissue where degeneration phemonema, alveolos and alveolar ducts can be seen[1].

Résumé. On a étudié l'histopathologie de la peau chez 43 sujets ayant un emphysème pulmonaire chronique. Chez la plupart de ces malades, on a observé des lésions cutanées de hyperkératose ortokératosique diffuse du corps muqueux de Malpighi, l'épaisseur de la couche granuleuse diminuée ou absente et des phénomènes dégénératifs des fibres élastiques. L'analyse de contingence a montré des différences hautement significatives ($P \ll 0.001$) par rapport aux malades asthmatiques sans emphysème pris comme témoins.

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Decondensation of Nuclear Chromatin under the Influence of *Tris* Buffer

A characteristics feature of the interphase nuclei of Eucariota is the occurrence of chromatin as well in a condensed as in dispersed form, the latter being especially involved in RNA synthesis, while the former remains more inert[1]. So experimental conditions which provoke any changes in chromatin pattern are of great interest from the biological point of view.

Materials and methods. The experiments were performed on liver of Wistar rats. Immediately after removing, the tissue was cut into thin slices and immersed in the exper-

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imental medium. Two kinds of solutions were used: 1.2 m*M* *tris* HCl, pH 7.4; and 2. 2 m*M* *tris* HCl containing 0.25 *M* sucrose, pH 7.4. Incubation was carried out at 37 °C for 30 to 150 min. For the last 30 min the medium was supplemented with H^3-5-uridine in concentration 20 μCi/ml; specific activity 23, 24 Ci/m*M*.

For the electron microscope study, the samples were fixed in buffered 2% glutharaldehyde followed with 1% buffered osmium tetroxide and embedded in Epon 812[2]. Sections were cut on Tesla ultramicrotome, stained after the routine procedure[3] and examined in JEM-7A.

The light microscope autoradiography was performed on samples fixed in ethanol acetic acid mixture, covered with liquid emulsion (Ilford L-4) and exposed for 10 days.

Results. Figure 1 presents the nucleus of the tissue fragment incubated for 30 min in *tris* HCl solution. The general pattern of chromatin is like that usually observed in the interphase nuclei. After a 60 min incubation some of the nuclei became homogenous, probably in consequence of better penetration of the medium into the external parts of the incubated tissue fragments. Prolonged incubation makes all nuclei homogeneous. There is no compact chromatin to be seen either close to the nucleolus (Figure 4) or to the nuclear membrane (Figure 2). Around the nuclear membrane, there are rows of particles. These are probably preribosomal particles (Figure 3). Diameter of these particles varied between 140–300 Å, depending, as we suppose, on the section level. The ultrastructure of nucleolus (Figure 4), though loosened significantly, still preserves its compact fragments.

The addition of sucrose to the medium at the final concentration 0.25 *M*, does not prevent the decondensation of chromatin. However, in this case, the action of medium is significantly retarded. After 60 min, the chromatin pattern resembles that usually observed in normal rat liver and that incubated for 30 min in *tris* HCl; however, after a 150 min incubation, only the residual condensed chromatin is observed (Figure 5). So, the presence of 0.25 *M* sucrose in 2 m*M* *tris* HCl delays the dispersion of chromatin.

The maintenance of the tissue in *tris* HCl for a period sufficient for chromatin decondensation, provokes an increase of uridine incorporation. However, these differences are statistically insignificant (Table).

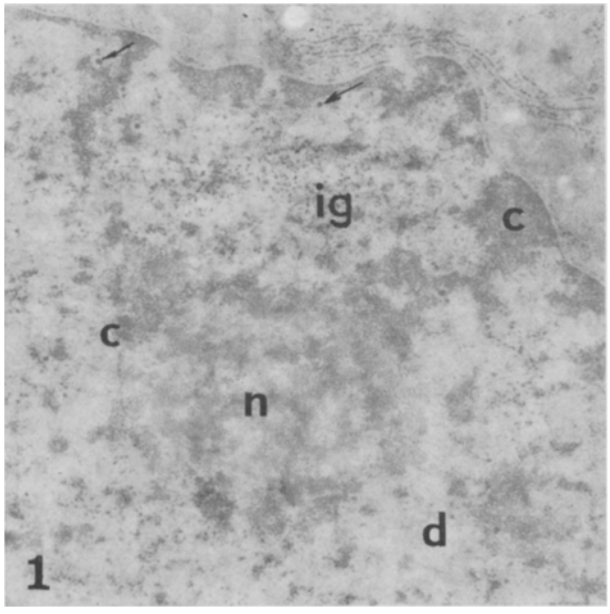

Fig. 1. Rat liver tissue after 30 min incubation with *tris* HCl buffer. ×16800. c, compact chromatin; d, dispersed chromatin; n, nucleolus; ig, interchromatin granules, perichromatin granules marked with arrows.

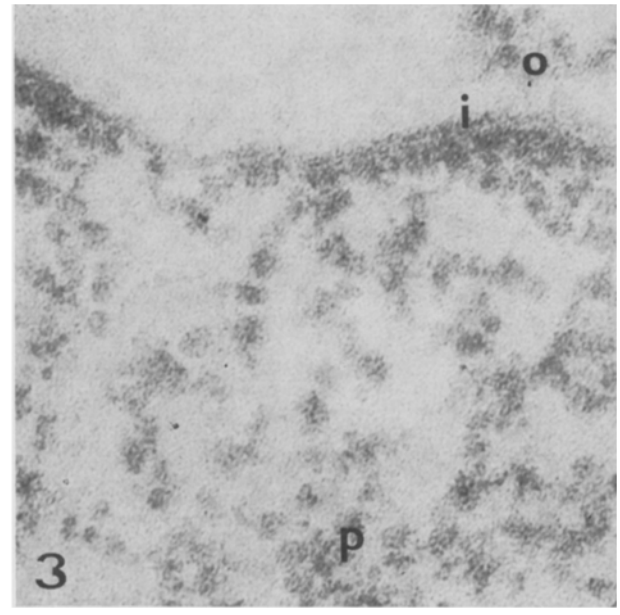

Fig. 3. Inner layer of nuclear envelope with rows of particles attached to its surface after 120 min incubation in *tris* buffer. ×136500. I, inner layer; o, outer layer; p, particle.

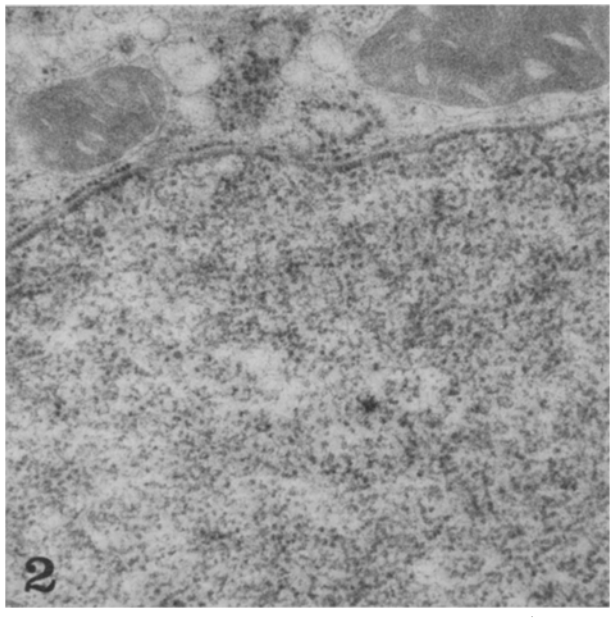

Fig. 2. Nuclei of rat liver after 120 min incubation with *tris* HCl buffer ×25200. Dispersed chromatin is seen only.

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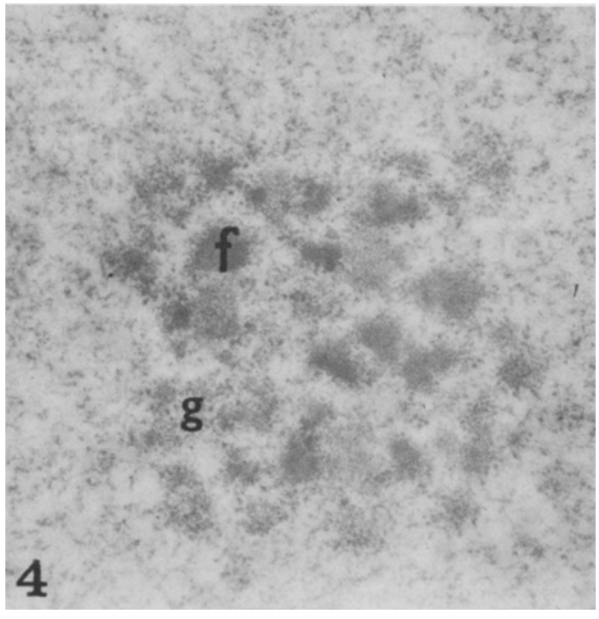

Fig. 4. Nucleolus after 120 min with *tris* HCl. ×30800. g, nucleolar granule; f, nucleolar fibrillar part.

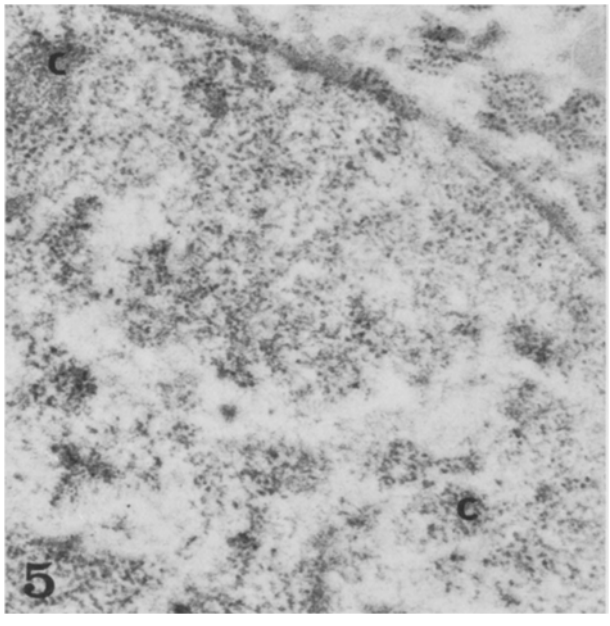

Fig. 5. Liver nucleus after 150 min incubation with *tris* HCl and sucrose. ×22800. c, residual compact chromatin.

Discussion. Three different possibilities may be taken into consideration for explaining this phenomenon: 1. The chelating properties of *tris* buffer, leading in consequence to changes in ionic medium equilibrum of nucleoplasm; 2. the influence of medium used on the permeability of nuclear envelope and on penetration of cytoplasmic proteins into nuclei; 3. the ability of the buffer to dissolve some nuclear proteins.

It is known that *tris* (= *tris*-[hydroxy-methyl]-aminomethane) bounds divalent metales, and the reaction is especially significant in alkaline pH[4]. Under the conditions of our experiment, it may be supposed that Mg^{+2} and Ca^{+2} ions could be removed from chromatin fibres by the action of *tris*. On the other hand, it is a known fact that the presence of these cations is essential for maintainance of ultrastructure of molecular organization of deoxyribonucleoprotein and particularly of native chromatin fibres[5]. NAORA et al.[6] suggested that magnesium ions are associated with DNA, whereas calcium ions are complexed with histones. Thus the dispersion of chromatin may be due to the chelating action of *tris* against calcium ions.

It seems probable that binding of divalent cations by *tris* disturbed ionic medium of nucleoplasm. It is reported by BRASCH[7] that immersion of chicken erythrocytes into water induces a complete decondensation of chromatin. OLINS and OLINS[8] showed that a fall of the potassium ions concentration from 200 m*M* – typical for nucleoplasm[9] – to the 10-fold lower causes homogenous appearance of nuclei. It must be noted that the very delicate balance in ions equilibrum is supposed to be the greatest importance not only for the protection of chromatin structure in the interphase nuclei, but also for its activity[10].

As just mentioned, it cannot be excluded that disturbances in the ionic medium influence the permeability of the nuclear envelope. It is known that proteins may penetrate into cell nuclei involving a partial chromatin decondensation and swelling of the whole nucleus. This is the case observed in chicken erythrocytes activated by hybridiza-

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Incorporation of H^3-uridine in nuclei of rat liver

Medium	Incubation time (min)				
	30	60	90	120	150
Tris HCl, (2 m*M*, pH 7.4)	1.3 ± 0.4	1.0 ± 0.4	1.6 ± 0.5	2.0 ± 0.8	1.5 ± 0.6
Tris HCl, (2 m*M*; sucrose 0.25 *M*, pH 7.4)		1.3 ± 0.5	1.2 ± 0.4	1.0 ± 0.9	1.1 ± 0.4

Number of grains was given from a square unit ± S.E.

tion with other cells[11]. Whether such events may take place in the cell nuclei under our experimental conditions can hardly be decided.

An explanation quite contrary to this may be suggested based on the results of experiments in which *tris* buffer enriched by a low concentration of magnesium was used as suitable for extraction of nuclear proteins[12–14]. The lysine-rich histones, probably their phosphorylated form[15–17], are known to be important for maintenance of chromatin condensation as well in interphase nuclei[18] as in the metaphase chromosomes[19]. However, it can hardly be said whether such an extraction might be responsible for the chromatin decondensation observed by us in the electron microscope. The most reasonable explanation of this phenomenon seems to be the chelating action of *tris* buffer followed by disturbances in inonic medium, as discussed above. The lack of significant changes in the chromatin template activity appeared to be quite understandable. It is well known that special loosening of chromatin fibres is necessary, not being, however, a sufficient condition for initiation of RNA synthesis. The other factors, important in this synthesis, were probably not influenced by our experimental conditions.

Résumé. Des petits fragments de foie de rat ont été maintenus dans un tampon 2 m*M tris* HCl avec ou sans saccharose (concentration final 0,25 *M*). L'incubation du tissus pendant au moins 1 h dans le tampon *tris* cause une décondensation de la chromatine, qui peut être retardée par le saccharose. Les changements morphologiques des types de chromatine n'ont pas l'effet d'incorporer l'uridine dans le noyaux interphasiques du foie.

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Electron Microscopic Observations on the Spinal Projections to the Cerebellar Nuclei in the Cat and Rabbit

Recently it has been suggested that spinocerebellar[1,2] and olivocerebellar tracts[1,3] project their collaterals to the cerebellar nuclei in the cat. Collateral projections of the corticipetal fibres to the cerebellar nuclei were also demonstrated by Golgi studies dealing with branching patterns and modes of termination of nuclear afferents[4,5]. While cerebellar corticonuclear fibres inhibit the activities of nuclei cells[6,7], the collaterals of extracerebellar origin are regarded as excitatory sources for these nuclei cells. Our previous studies using the Nauta and the Fink-Heimer methods have shown that the spinocerebellar tracts (SCTs) send many collaterals to the cerebellar nuclei, especially to the medial and the interpositus nuclei of the cat[2], rabbit[8] and rat[8] (see reference 2 for review). On the other hand, electrophysiological studies indicate that these collaterals are not essentially responsible for producing the excitation of the cerebellar nuclei cells[9–11]. The purposes of the present electron microscope study are to confirm the results of our previous Nauta studies and to investigate the mode of termination of these fibres.

Materials and methods. Either ventrolateral cordotomies or hemisections were made at the cervical levels (C4, C5, C6 and C8) in 7 cats and 2 rabbits. On 2–6 days after operation, the animals were perfused under deep pentobarbital anesthesia with the following fixatives; a mixture composed of 4% paraformaldehyde and glutaraldehyde of various concentrations (either 0.5%, 1.25% or 2.5%) buffered with Millonig's phosphate at pH 7.3 or 7.4. Subsequently, the cerebella were dissected free from the brainstem and small blocks were trimmed from the cerebellar nuclei. After a brief rinse in the above buffer solution, the specimens were placed in 1% osmium tetroxide buffered with Millonig's phosphate, and dehydrated in ethanol and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate. 5 cats and 2 rabbits were used as controls.

Results and discussion. The same results were obtained in the medial and the interpositus nuclei of both sides. In cats and rabbits, a fair number of degenerated boutons were observed synapsing with proximal dendrites and cell bodies of medium-sized and large cells. A small number were seen synapsing with peripheral dendrites of various sizes and dendritic spines. Figure 1 shows that an electron-dense, degenerated bouton containing pleomorphic vesicles forms an asymmetrical synapse with the soma of a large cell of the medial nucleus. Figure 2 also shows that a small, electron-dense, degenerated bouton synapses with the soma of a large cell of the medial nucleus. The latter

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