Table I. Activity of acid phosphatases

No. of experiments	Total activity	Free activity			
		Control	cAMP ($10^{-3}M$)	Theophyllin ($10^{-4}M$)	cAMP+Theophyllin
	(phosphatase/ml of resuspended sediment μmol P/ml/10 min)				
12	181.3 ± 6.3	29.4 ± 3.1	28.4 ± 4.3	33.6 ± 3.1	62.8 ± 5.9[a]

Table II. Activity of β-glucuronidases

No. of experiments	Total activity	Free activity			
		Control	cAMP ($10^{-3}M$)	Theophyllin ($10^{-4}M$)	cAMP+Theophyllin
	(β-glucuronidase/ml of resuspended sediment umol phenolphtalein/ml/10 min)				
11	33.0 ± 2.5	18.1 ± 2.0	18.0 ± 2.3	18.5 ± 1.7	22.6 ± 0.5[a]

[a] $p < 0.01$.

The second sample made up for the determination of control free activity of lysosomal enzymes was composed of 2.0 ml of the suspension and 1.0 ml of the buffer. The third sample was made up for the determination of the changed free activity of lysosomal enzymes in consequence of in vitro administered agents and was composed as the second sample. The agents were resolved in 1.0 ml of buffer solution.

All samples were incubated at 37°C for 45 min. After incubation they were cooled in ice-cold water and then centrifuged at 4°C for 20 min at 15.000 *g*. 0.1 ml of the supernatants was then incubated for 10 min at 37°C in 1.9 ml of acetate buffer (0.05 *M*, pH 5.4) with β-glycerophosphate and phenolphtalein glucuronide as substrates to determine the acid phosphatase[4] and β-glucuronidase activity[6].

Results and discussion. The 3′–5′ cAMP ($10^{-3}M$) and theophyllin ($10^{-4}M$) increased the permeability of lysosome membrane prepared from rat liver tissue with respect to acid phosphatase (Table I) and to a certain extent β-glucuronidase (Table II) enzymes and this change in permeability indirectly suggests that the micellar organisation, as a more labile structural arrangement, prevails. The 3′–5′-cAMP and theophyllin, alone, could not produce this effect, their remarkable common effect, we think depends on the inhibition of cAMP phosphodiesterase enzyme by theophyllin.

There are, however, two questions to be discussed concerning the results of experiments: At the administration of cAMP + theophyllin is it a disruption or increased permeability of lysosome membrane that took place? b) Why does the percent-value of the increased free activity of acid phosphatase and β-glucuronidase enzymes differ under the same effect? (40%, respectively only 30% increase).

a) As to the first question the change of permeability seems more probable: This is supported by the presence of intact lysosomes in lysosome fraction at electronmicroscopical control and by the insignificant increase of supernatant protein concentration (the increase is less than 10%). b) The difference in the release of the two enzymes also rather suggests specific change of membrane permeability than lysosome membrane disruption.

It was observed that exogenous lysolecithin[7] and Na-desoxycholate (this latter is a known activator of phospholipase A enzyme)[8] increase the proportion of the micellar state within membranes and therefore increase the permeability and facilitate the fusion between adjacent membranes. However, these materials are exogenous agents and serve only as a model to investigate a physiological mechanism. The above results indirectly suggest the possibility that the cAMP would be an endogenous, physiological material, which may start an ultrastructural rearrangement of the lysosomal membrane.

Zusammenfassung. Es wird gezeigt, dass 3′–5′-cAMP ($10^{-3}M$) und Theophyllin ($10^{-4}M$) die Permeabilität derjenigen Lysosmen-Membranen steigern, die aus Rattenleberzellen in bezug auf saure Phosphatase and in bezug auf β-Glucuronidase-Enzyme präpariert wurden.

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Lack of Major Cytoplasmic Protein Contamination of Rat Liver Nuclear Chromatin

It was suggested by JOHNS and FORRESTER[1] that non-specific contaminating proteins, possibly of cytoplasmic origin, could be removed from calf thymus chromatin by washing in 0.3–0.35 *M* NaCl. In recent studies, we have observed that many different nonhistone proteins are solubilized from both rat liver and rat kidney chromatin by treatment with 0.3 *M* NaCl[2,3]. In order to determine if these proteins represent cytoplasmic contamination of nuclear chromatin, the following experiment was performed.

Rat liver nuclei were prepared from frozen tissue (25 g) by the procedure of CHAUVEAU et al.[4], with slight modifications[5]. The supernatants from the first two homogenizations were combined, centrifuged at 10,000 *g* for 10 min and then at 100,000 *g* for 2 h. This soluble cytoplasmic protein preparation was dialyzed against two changes of 100 vol of 0.14 *M* NaCl. No precipitate formed under these conditions which gave a concentrated (20 mg/ml) total cytoplasmic protein fraction.

Nuclear chromatin was prepared as previously described[6]. It was extracted twice in 70 ml of 0.3 *M* NaCl by gentle homogenization (Dounce loose pestle). The chromatin was washed in 120 ml of 0.14 *M* NaCl, centrifuged (2000 *g*, 10 min) and resuspended in 40 ml of the total cytoplasmic protein fraction (also in 0.14 *M* NaCl) by gentle homogenization. After 5 min the chromatin was centrifuged, washed in 120 ml of 0.14 *M* NaCl and re-extracted with 0.3 *M* NaCl to remove any cytoplasmic proteins which may have bound to the chromatin in 0.14 *M* NaCl, as used in most chromatin isolation procedures[1,7].

The 0.3 *M* NaCl soluble chromatin protein fraction was centrifuged at 100,000 *g* for 2 h to remove any remaining small bits of chromatin, dialyzed vs. two changes of 20 vols of 0.02% SDS (sodium dodecyl sulfate), 0.02% 2-mercaptoethanol, 0.2 m*M* $Na\text{-}PO_4$, pH 7, and lyophilized.

Calf thymus nuclei and chromatin and 0.3 *M* NaCl soluble proteins were also prepared as described above. In addition, for comparative purposes, 10 ml of the total rat liver soluble cytoplasmic protein fraction was also dialyzed and lyophilized.

Quantitatively, it was estimated[8] that 10% of the total rat liver chromatin protein was removed by 0.3 *M* NaCl. The amount of protein solubilized by 0.3 *M* NaCl after exposure of the chromatin to the cytoplasmic protein was very small (about 2% of the total). We also found that 0.3 *M* NaCl removed very little protein (about 1%) from the calf thymus chromatin prepared by this method.

To compare the extracted proteins qualitatively, they were electrophoresed on acrylamide gels containing SDS and urea[9] as previously described[2]. It is apparent from the photograph (Figure 1) that the protein patterns of 0.3 *M* NaCl soluble chromatin proteins and the total soluble cytoplasmic proteins are quite dissimilar. Only two main proteins are extracted in 0.3 *M* NaCl from calf thymus chromatin, and these are not histones nor do they resemble the proteins solubilized from rat liver chromatin. As well, it should be noted that no histone is solubilized by 0.3 *M* NaCl from rat liver chromatin.

The comparison (Figure 1) of the rat liver chromatin protein extracted in 0.3 *M* NaCl before and after exposure to the cytoplasmic protein is easier by superimposing the optical profiles of the stained gels (Figure 2). It is apparent that almost every protein band present in the second 0.3 *M* NaCl extract (after cytoplasmic protein exposure) also appears in the original 0.3 *M* NaCl extracted. Thus, the two NaCl chromatin extracts give qualitatively similar patterns, whereas the cytoplasmic fraction is qualitatively much different as compared by acrylamide gels electrophoresis.

Rat liver chromatin was homogenized in a solution containing about a 40 fold excess of cytoplasmic protein to chromatin protein. The protein that was subsequently removed by a second 0.3 *M* NaCl wash was only about 2% of the total chromatin protein, however, and gave a gel pattern similar to the original 0.3 *M* NaCl extract and very dissimilar to the cytoplasmic protein fraction. This result strongly suggests that very little, if any, cytoplasmic protein was absorbed to the chromatin and that the small amount of protein in the second NaCl wash represented a portion of the native nuclear chromatin proteins incompletely extracted by the initial 0.3 *M* NaCl wash.

Chromatin prepared from different sources have varying amounts of non-histone protein[10]. The function or source of the 'extra' nonhistone protein is not well characterized, but the protein solubilized in 0.3 *M* NaCl does not alter the genetic restriction of the chromatin[11], nor does it alter its structure[3]. A greater quantity of different proteins is removed by 0.3 *M* NaCl from rat liver compared to calf thymus chromatin (Figure 1), but this study suggests that these proteins are not cytoplasmic contaminants of nuclear chromatin. Why more proteins (presumably of nuclear sap origin) adhere to isolated rat liver than to calf thymus chromatin is unknown. It should not be concluded,

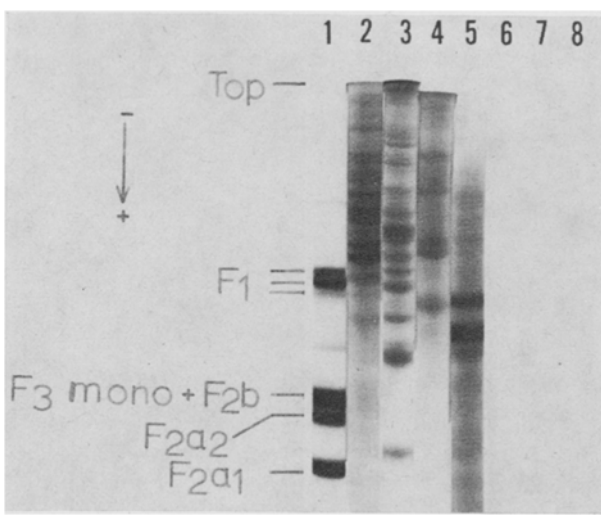

Fig. 1. Acrylamide gel patterns of protein fractions. The gels contain 0.1% SDS, 4 *M* urea, 0.1 *M* $Na\text{-}PO_4$ pH 7, 10% acrylamide and 0.4% *bis*-acrylamide. Electrophoresis at 8 mA per tube was continued for 8 h, the gels fixed in 20% sulfosalicylic acid, stained in 0.02% Coomassie blue (in 12.5% trichloroacetic acid) for 8–12 h and destained by diffusion. The gels were photographed with an orange filter. The histone fractions were identified previously[2]. 'F3 mono' is the monomer (reduced) form of the cysteine containing F3 histone. Gel 1: 40 µg total acid extracted rat liver histone. Gel 2: 100 µg rat liver chromatin 0.3 *M* NaCl soluble proteins. Gel 3: 100µg rat liver cytoplasmic protein soluble in 0.14 *M* NaCl. Gel 4: ~50 µg protein removed from rat liver chromatin after exposure to the soluble cytoplasmic protein. Gel 5: ~30 µg calf thymus chromatin (from nuclei) 0.3 *M* NaCl soluble proteins.

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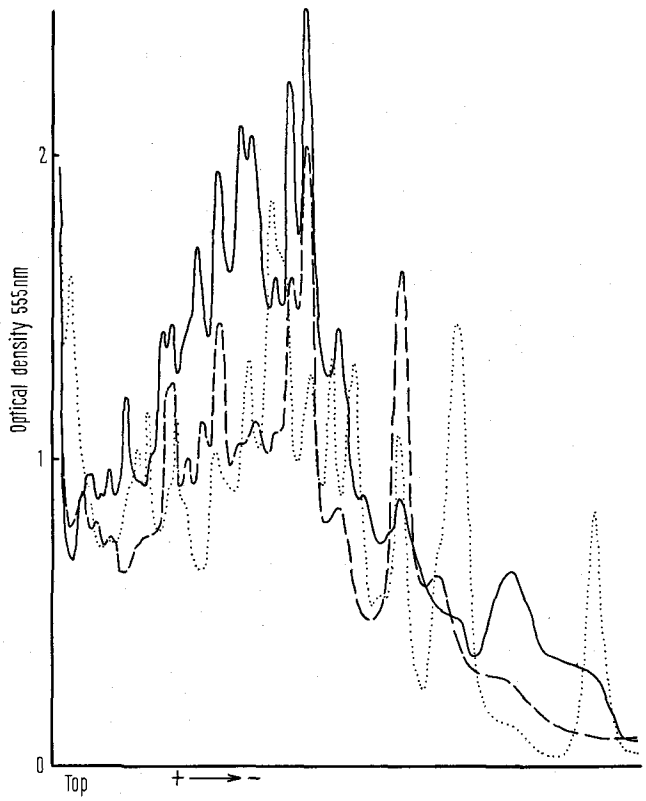

Fig. 2. Optical scan of acrylamide gels. Stained acrylamide gels (see Figure 1 for details) were scanned at 555 nm in a Gilford Spectrohpotometer Gel Scanner and superimposed for this comparison. ——, 0.3 *M* NaCl soluble rat liver chromatin proteins; . . . ., rat liver cytoplasmic soluble protein; – – –, rat liver chromatin 0.3 *M* NaCl soluble proteins after exposure to the cytoplasmic protein.

however, that chromatin prepared from whole cells[1] is not contaminated by non-nuclear material, since membranes, divalent cations, etc., could effect non-specific adsorption[12].

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Résumé. Des mesures quantitatives et des modèles sur gels d'acrilamide ont permi de conclure que les protéines cytoplasmiques préparées de cette manière ne produisent pas de contamination appréciable.

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Localisation des glycosyl-transférases dans les membranes endoplasmiques des splénocytes de rat

Des travaux antérieurs[1,2] ont montré que les glycosyltransférases spléniques, et spécialement la mannosyltransférase, sont localisées au niveau des structures microsomiques. Dans le cadre de ces organites subcellulaires, les membranes endoplasmiques occupent une place prépondérante sous l'angle des activités de transglycosylation[3,4]. Il est possible grâce à une adaptation de la technique de fractionnement cellulaire de GLAUMANN et DALLNER[5], de répartir le long d'un gradient de densité, les activités glycosyl-transférases localisées dans les membranes endoplasmiques préalablement individualisées.

Les rates, obtenues par laparotomie de rats mâles (souche Wistar) de 250 g, sont broyées, à l'aide d'un homogénéiseur de Potter, en saccharose 0,25 *M*, tampon *Tris*-HCl 0.05 *M*, pH 7 (2 g/10 ml). Le surnageant d'une première centrifugation de 20 min à 10,000 *g* est ramené au volume initial par addition de saccharose 0,25 *M*. La suspension obtenue est amenée à 15 m*M* de CsCl. On en dépose 24 ml sur 12 ml de saccharose 1,31 *M*, CsCl 15 m*M* et on centrifuge 7 h à 105 000 *g*. On recueille les membranes du reticulum endoplasmique («smooth microsomes») à l'interface, et on les reprend en suspension dans 20 ml d'eau distillée; 20 ml de cette suspension sont déposés sur

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