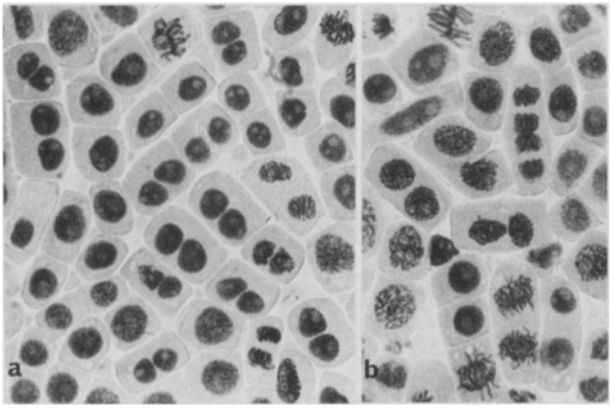
These percentages give us a better estimate of cytokinesis inhibition, in case mitotic index should be lowered.

The concentration of 10^{-7} M/ml (2.76/100 ml) is ineffective and cytokinesis inhibition is seen only with higher concentrations. From 3.7×10^{-7} M/ml to 3×10^{-6} M/ml, the growth of roots is normal within 48 h but on the 4th day their length is about 60% of the control and the roots have swelled.

Results obtained during the first 24 h are shown in the Table. The optimal concentration inhibiting cytokinesis is about 2×10^{-6} M/ml. For all coneentrations we can observe: partial Cytokinesis, a slight chromatoclasic effect (anaphase bridges and breaks), numerous bimitosis after 48 or 72 h in deoxyguanosine. And with the highest concentration: nuclear diapedeses, distorted nuclei, and a strong mitodepressive effect after 48 h.



2′ Deoxyguanosine $7{,}5 \times 10^{-7}$ M/ml: a) 24 h, numerous binucleate cells; b) 72 h, binucleate cells enter mitosis.

A short treatment (1 h) using 1.5×10^{6} M/ml has been applied: at the end of this treatment there is no telophase without phragmoplast but, after recovery in Knop medium $^1/_2$ for 2 h, such abnormal mitosis does appear, showing a delayed effect of deoxyguanosine. Comparatively, the study of deoxyadenosine showed that it has not the same property as deoxyguanosine: the concentration of 10^{-8} M/ml is ineffective and with 2×10^{-8} M/ml there is no mitosis within 24 h.

We are continuing to study the action of deoxyguanosine (and related chemicals) on cytokinesis; Indeed, we think that, as deoxyguanosine is a physiological compound, discovering its mechanism of action could help to understand the metabolism of normal cytokinesis.

Résumé. La désoxyguanosine, à partir d'une concentration seuil de $3{,}7 \times 10^{-7}$ M/ml, inhibe fortement la cytodiérèse des cellules méristématiques d'*Allium sativum* L. L'activité mitotique n'est que faiblement touchée.

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[1] A. Brulfert and G. Deysson, Annls pharm. fr. *31*, 19 (1973).
[2] A. Brulfert, E. Clain and G. Deysson, C.r. Soc. Biol., *167*, 1740 (1973).

Electrophoretic Characterization of Melanosomal Proteins Extracted from Normal and Malignant Tissues

One approach to the study of pigment granule structure and development has been the recent development of techniques which extract the protein constituents of the melanin granules under conditions which preserve protein integrity[1,2]. In avian and mammalian melanocytes, melanin granules are ovoid and the melanin is deposited upon a filamentous matrix[3,4]; however, the matrix of premelanosomes from oocytes of the frog *Xenopus laevis* is granular and sometimes paracrystalline in appearance[5] and mature melanin granules are spherical. Further, the melanosomes of the malignant mouse melanoma are very atypical with disorganized fibres and incomplete melanization[6]. The study reported here was initiated in order to ascertain whether these fine structural variations could be demonstrated at the biochemical level by gel electrophoresis.

Materials and methods. Melanogenic tissues used in this study were obtained from: 1. 5-day-old black (C57B1) mouse eyes, 2. an actively growing S-91 mouse melanoma, 3. 15-day embryonic chick eye (White Leghorn), and 4. *Xenopus laevis* oocytes. Melanin granules were isolated and purified from the murine and avian tissues by the procedure of Hearing and Lutzner[1]; those from frog oocytes were obtained by the method of Eppig and Dumont[7]. Protein solubilization techniques are detailed in the legend to Table I. Gel electrophoresis (PAGE)[8] was carried out on these extracts in 7.5% gels at 2 mA/tube at 25 °C, using the *Tris*-glycine buffer system with either SDS or urea[9]. Proteins were stained with Fast green[10]; the relative mobilities were calculated against the migration of pyronin Y tracking dye.

Results and discussion. The relative mobility (Rm) of several protein bands extracted from melanin granules of diverse origin are very similar (Tables I and II). This similarity was especially striking with respect to extract

[1] V. J. Hearing and M. A. Lutzner, Yale J. Biol. Med. *46*, 553 (1973).
[2] P. Doezema, J. Cell Physiol. *82*, 65 (1973).
[3] M. A. Lutzner and C. T. Lowrie, in *Pigmentation: Its Genesis and Biologic Control* (Appleton-Century-Crofts, New York 1972), p. 89.
[4] V. J. Hearing and M. A. Lutzner, J. Ultrastruct. Res. *43*, 88 (1973).
[5] J. N. Dumont and J. J. Eppig jr., manuscript in preparation.
[6] F. H. Moyer, Ann. N. Y. Acad. Sci. *100*, 584 (1963).
[7] J. J. Eppig jr. and J. N. Dumont, Devel. Biol. *36*, 330 (1974).
[8] Abbreviations used: PAGE, polyacrylamide gel electrophoresis; SDS, sodium decyl sulfate; Rm, relative mobility.
[9] A. R. Ugel, A. Chrambach and D. Rodbard, Analyt. Biochem. *43*, 410 (1971).
[10] M. A. Gorovsky, K. Carlson and J. G. Rosenbaum, Analyt. Biochem. *35*, 359 (1970).

Table I. Melanosomal proteins isolated from various tissues and resolved by PAGE with 0.02 *M* SDS (7.5% gels)

Band	Rm	Xenopus No. 1 [a]	No. 2	Chick No. 1	No. 2	Mouse No. 1	No. 2	Melanoma No. 1	No. 2
1	0.565					+			
2	0.520							+++	+++
3	0.440	+++	+++	+++	+++	+++	+++		
4	0.425	+		+		++			
5	0.400	+		+		+			
6	0.365							+	
7	0.350			+				+	
8	0.330	+		++		+		++	
9	0.300			+				+	
10	0.155	+		+		+		+	
11	0.120			+		++		+	
12	0.065	+		+		+		+	
13	0.040	++		+		++		+	
14	0.030					++		+	

[a] No. 1 refers to the initial extraction of melanin granules in either 1% SDS (Table I) or 8 *M* urea (Table II) for 3 h at 70°C. Samples were then centrifuged at 10,000 *g* for 30 min; the pellet was then reextracted overnight in the respective solvent at 70°C, then centrifuged, and the supernatant referred to as extract No. 2.

No. 2 protein. Although identical Rm's in PAGE do not establish protein identity, similar migration in the presence of SDS[8] indicates comparable molecular weights between the 2 polypeptides, whereas migration in the presence of urea is a function of charge density. Urea treatment eventually results in the solubilization of the entire melanin granule. After the initial extraction, which removes the limiting membrane, sequential solubilizing treatments and electrophoresis reveal only the single protein band (Table II). It seems certain, therefore, that the material in this band constitutes internal melanin granule protein, and since only a single band is resolved after electrophoresis in either SDS or urea, it is probable that this band consists a of single protein. It has also been shown that PAGE of this protein from murine sources over a range of acrylamide concentrations reveals only a single band[1], reinforcing the conclusion that it is a single homogeneous protein species.

The protein in urea extract No. 2 from the melanin granules from each type of tissue has the same Rm, establishing the similarity of the charge density of each. However, in the presence of SDS, the Rm of this protein (band 2, Table I) from the S-91 melanoma is greater than the Rm of the analogous melanosomal protein from the other tissues (band 3, Table I). The weight of the protein from the melanoma melanosome is about 50,000 daltons (based on known protein markers) as compared to about 70,000 for the protein from the other types of granules. The atypical nature of this protein in the melanoma melanosoma may be a factor contributing to the disorganization and/or incomplete melanization of the granule found in the malignant tissue.

In the first SDS treatment, 6 to 10 proteins are extracted in concentrations high enough to be visualized in polyacrylamide gels. Since the first extraction disrupts and solubilizes the limiting membrane of the granule, undoubtedly some of these bands are membrane proteins. Although we cannot exclude the possibility that some of these bands may be cytoplasmic proteins bound to the surface of the isolated melanin granule, our evidence suggests that such contamination is absent: a) repeated washings in buffer and sucrose failed to remove any of these proteins, b) when homogenates were preincubated with isotopically labelled proteins, the radioactivity was completely washed off during the melanosome isolation procedure. Our results show that at least 4 proteins (No. 8, 10, 12, 13) present in the melanin granules isolated from embryologically and phylogenetically diverse

Table II. Melanosomal proteins as resolved by PAGE in the presence of 8 *M* urea (7.5% gels)

Band	Rm	Xenopus No. 1 [a]	No. 2	Chick No. 1	No. 2	Mouse No. 1	No. 2	Melanoma No. 1	No. 2
A	0.885	+++	+++	+++	+++	+++	+++	+++	+++
B	0.570			+		+		+	
C	0.495					+		+	
D	0.455					+		+	
E	0.430					+		+	
F	0.390			+		+		+	
G	0.305					+		+	
H	0.265			+					
I	0.215			+					
J	0.190			+		++		++	
K	0.160			+		++		++	
L	0.140			+		++		++	

[a] See footnote to Table I.

sources are similar in molecular weight and charge density. We are currently studying the functional identity of these proteins.

Several workers have attempted to study melanoproteins from various tissues, but their isolation procedure called for extraction in the presence of strong alkali, which precludes further study of these proteins[11,12]. DOEZEMA[2] has studied melanosomal proteins, solubilized with hot SDS, from chick eye, murine skin and the B-16 melanoma. He found that there were many proteins with similar electrophoretic migration among the 3 systems. In addition, there were several different protein bands between the murine melanoma and skin tissues. Our work using urea as a solvent shows that these proteins, although altered in respect to their molecular weight, maintain the normal charge density, and thus they probably differ as a result of the shortening of the polypeptide chain. This would allow the protein to keep at least some of its functional arrangement, and might explain why the melanosome in the melanoma tissues is present but atypical.

A similar study concerning the alteration of proteins in malignancy has been made by other workers[13,14]. These investigators compared the proteins extracted from the nucleoli of normal rat liver and the Novikoff hepatoma by two-dimensional PAGE. They have shown that not only are different amounts of proteins synthesized in the 2 tissues but that some unique proteins are found in each tissue as well. The similarity of our findings and those of these workers, which concern different tissues in different animals, both in normal and malignant conditions, serves to point out the rather basic level of disruption of the normal cellular metabolism in malignancy[15].

Résumé. Des granules de mélanine furent extraites des yeux d'embryons de poussins et de souris noires venant de naître ainsi que d'œufs de *Xenopus laevis* et du mélanome S-91. Après que des purifications extensives de granules de mélanine furent mises en solution soit dans l'urée de 8 M ou 1% de SDS et caractérisées par électrophorèse en gel de polyacrylamide. Le résultat indique que plusieurs protéines de même comportement en électrophorèse son présents dans les granules de ces diverses provenances. En plus, il semble encore plus significatif qu'il y ait plusieurs différences entre les protéines mélanosomales des mélanocytes normaux et nocifs.

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[11] A. G. BOLT, Life Sci. *6*, 1277 (1967).
[12] S. BRATOSIN, J. Invest. Derm. *60*, 224 (1973).
[13] L. R. ORRICK, M. O. J. OLSON and J. BUSCH, Proc. natn. Acad. Sci., USA *70*, 1316 (1973).
[14] L. C. YEOMAN, C. W. TAYLOR, J. J. JORDAN and J. BUSCH, Biochem. biophys. Res. Commun. *53*, 1067 (1973).
[15] Acknowledgments: The authors appreciate the able assistance of Mr. THOMAS EKEL, Mrs. GERMAINE CASSOU and Mrs. ALVERDA PATTERSON.
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Comparison of the Protein Constituents of Sarcoplasmic Reticulum Isolated from Rat Skeletal and Cardiac Muscle

Determinations of the molecular weights of the protein constituents of skeletal muscle sarcoplasmic reticulum (SR), from several animal species have recently been carried out using polyacrylamide gel electrophoresis[1-4]. This work has shown that the major protein constituent of the SR vesicles has a mobility on the gels consistent with a moiety of molecular weight of approximately 103,000 daltons[4,5]. This communication reports results obtained from investigations of the protein content of cardiac SR vesicles, and indicates a characteristic difference in the electrophoretic behaviour of cardiac and skeletal muscle sarcoplasmic reticular proteins.

Materials and methods. SR from rat skeletal and cardiac muscle was prepared by an adaptation of the method of IKEMOTO et al.[6]. Freshly excised muscle was extruded through a muscle press with 1 mm holes, suspended in 100 m*M* KCl + 1.0 m*M* imidazole and homogenized in a conventional co-axial teflon pestle homogenizer. The homogenate was centrifuged at 1000 *g* for 20 min to remove cell debris; the supernatant being then filtered through glass wool to remove free lipids, and the filtrate recentrifuged at 8000 *g* for 30 min to sediment the mitochondrial fraction. After further filtration of the supernatant through glass wool, the SR vesicular pellet was obtained by re-centrifugation at 28,000 *g* for 1 h. The material sedimented from this spin was retained for polyacrylamide gel electrophoresis and ultrastructural examination. Samples for electrophoresis contained between 0.05 and 0.1 g (wet weight) of microsomal pellet in 1 ml of 0.01 *M* sodium phosphate buffer pH 7.4. Samples were incubated at 35 °C for 24 h in this buffer plus 1% sodium dodecyl sulphate (SDS), and 8 *M* urea. Aliquots (0.25 ml) of the incubated material were mixed with 0.1 ml of bromophenol blue tracking dye and 1 drop of glycerol and a 50 µl sample of this mixture was loaded onto each gel. Phosphate buffers were made up according to WEBER and OSBORN[7] and 5% gels were prepared using half the concentrations of acrylamide and methylene *bis*-acrylamide stated.

Electrophoresis was performed at 8 mA per gel for a period sufficient for the tracker dye to move 6 cm. Gels were calibrated using a number of proteins with molecular weights ranging from 10^4 — 2×10^5 daltons. These were, cytochrome C (11,700), lysozyme (14,300), pepsin (35,000), ovalbumen (43,000), serum albumen (68,000),

[1] A. MARTONOSI, Biochem. biophys. Res. Commun. *36*, 1039 (1969).
[2] D. H. MACLENNAN and P. T. S. WONG, Proc. natn. Acad. Sci., USA *68*, 1231 (1971).
[3] N. IKEMOTO, G. M. BHATNAGOR and J. GERGELY, Biochem. biophys. Res. Commun. *44*, 1510 (1971).
[4] D. H. MACLENNAN, C. C. YIP, G. H. ILES and P. SEEMAN, Symp. quant. Biol. *37*, 469 (1972).
[5] D. H. MACLENNAN, J. biol. Chem. *245*, 4508 (1970).
[6] N. IKEMOTO, F. A. SRETER, A. NAKAMURA and J. GERGELY, J. Ultrastruct. Res. *23*, 216 (1968).
[7] K. WEBER and M. OSBORN, J. biol. Chem. *244*, 4406 (1969).