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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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),

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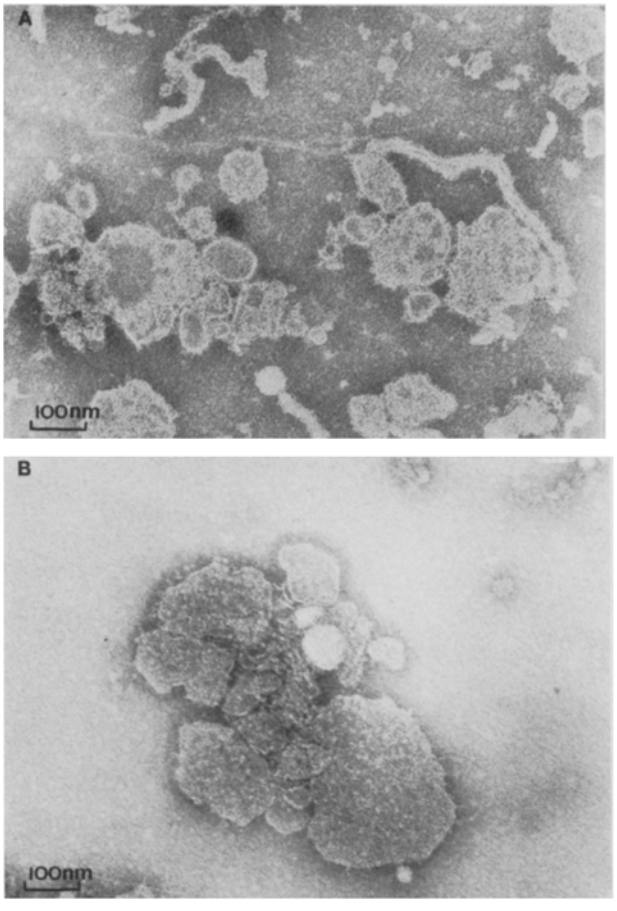

Fig. 1. Electron micrographs of negatively stained dispersates of A) rat skeletal, and B) rat cardiac sarcoplasmic reticular vesicles.

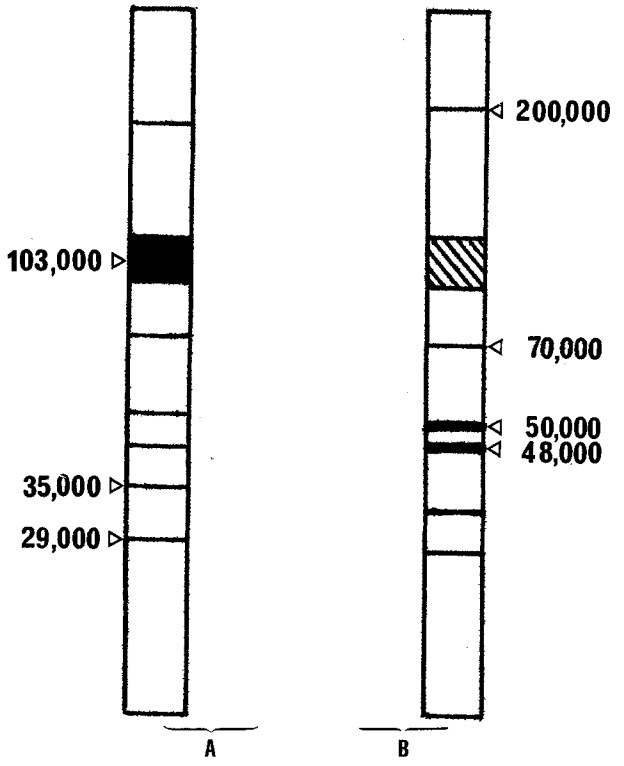

Fig. 2. Polyacrylamide Gel electrophoresis. Profiles of A) rat skeletal SR, and B) rat cardiac SR.

β-galactosidase (130,000), and myosin (220,000). When the mobility of these standards was plotted against the logarithm of their respective molecular weights, an inverse linear relationship was obtained. After electrophoresis, gels were stained for 30 min in 1% amido black in 7% acetic acid containing 2% trichloroacetic acid, and subsequently destained in methanol : acetic acid : glycerol : water, 1000 : 125 : 62 : 1312. Samples for electron microscopic examination were prepared by dispersing a small amount of the 28,000 *g* pellet with 1% phosphotungstic acid at pH 7.4, onto carboncoated copper grids. The preparations were examined in an A.E.I. E.M. 801 electron microscope at 80 kv accelerating voltage.

Results. Figures 1a and b show negatively stained dispersates of the 28,000 *g* fraction from rat skeletal and cardiac muscle respectively. The micrographs show an overall similarity in appearance of the vesicles except that fewer 'tadpole' conformations are found amongst the cardiac vesicles, which vary in size from 60–300 nm (BASKIN and DEAMER[8]). Some of the cardiac vesicles exhibit a fringe of 40 Å particles and many show the presence of variable amounts of irregularly-spaced particles of slightly larger size.

Figure 2 shows the results of polyacrylamide gel electrophoresis of the 2 types of vesicles. The findings indicate that the major band in skeletal vesicles corresponds to a molecular weight of approximately 103,000, as previously reported[4,5]. In addition there are bands at 50,000, 35,000 and 29,000 daltons which are in fairly good agreement with the values obtained by MACLENNAN et al.[4] for the calsequestrin and acidic proteins of skeletal SR.

The cardiac SR (Figure 2b), however, shows a consistently different electrophoretic profile. The 103,000 dalton component is reduced to a very faint band of stain whilst the major constituent is represented by 2 prominent discrete bands whose molecular weight is calculated at 50,000 and 48,000. Also the 35,000 and 29,000 components are present in relatively greater amounts in the cardiac material.

Discussion. The preparation of cardiac sarcoplasmic reticulum vesicles has for some time been the subject of considerable discussion. It has been shown[8] that cardiac microsomes prepared by the method of FANBURG and GERGELY[9], are considerably contaminated by mitochondrial fragments and possibly by remnants of the T-tubule system. Mitochondrial fragments are easily distinguishable by the presence of 80–90 Å membrane bound particles regularly spaced at 100 Å intervals[8].

The method of IKEMOTO et al.[3] which involves the use of a 'muscle press' and pestle homogenizer in place of a blender with sharp cutting blades produces a much 'cleaner' microsomal preparation, and contamination by mitochondrial fragments is largely precluded as they remain intact and are sedimented at 8000 *g*. This latter isolation procedure was used for all the preparations in this study. We consider that our method of preparation of the microsomal fraction and the subsequent ultrastructural inspection ensures that our sarcoplasmic reticulum is reasonably uncontaminated.

It is noticable on our preparations that at certain stain densities irregularly spaced particles are apparent both on the body of the vesicles and at the surface (Figure 1b). Since these do not resemble mitochondrial particles it is likely that they represent the type of particle seen in freeze etched preparations[8].

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The electrophoretic studies have revealed a major difference in the distribution of the 103,000 dalton band, generally acknowledged to be the ATPase[4]. In skeletal muscle microsomes this band represents the major protein constituent, whilst in cardiac vesicles the major components occur at 50,000 and 48,000 daltons; the 103,000 dalton band being considerably reduced.

There are two possibilities which could explain this apparent difference in protein distribution. Either the proportion of ATPase to other protein constituents in cardiac SR is much lower than in skeletal SR, or there is a compositional difference in the cardiac vesicular ATPase resulting in an altered electrophoretic profile. It is interesting to note that STEWART and MCLENNAN[10] have recently reported that brief tryptic digestion of skeletal muscle SR leads to dissociation of the ATPase molecule into 2 peptide fractions of molecular weights 45,000 and 55,000, without loss of ATPase activity.

Investigations are now proceeding to determine the ATPase activity of the individual isolated proteins from cardiac muscle SR with a view to establishing their functions.

Zusammenfassung. Die Protein-Zusammensetzung des sarcoplasmatischen Reticulums vom Herzmuskel wurde mittels Polyacrylamid-Gel-Elektrophorese untersucht und ihr Unterschied in der hauptsächlichen Proteinkomponente zum Skelettmuskel SR gefunden.

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'Cytosome', an Addition to the Cytoplasmic Organelles of *Taphrina maculans* Butler

Taphrina maculans Butler, an incitant of leaf spot of turmeric (*Curcuma longa* L.) in India, was first isolated in artificial culture by PAVGI and UPADHYAYA[1], who obtained 2 types of colonies, namely, 'salmon red' and 'creamy white', from a single infection spot. Both the colonies composed of unicellular blastospores, multiply by budding. Creamy white strain is long lived while salmon red dies gradually. The senescent colony becomes white.

Inspite of the fact that some of the earlier light microscopic findings have recently been corroborated by electron microscopic studies, much of the ultrastructural details in microorganisms are awaiting further investigation with the gradual improvement of the fixation techniques. Spores of *T. maculans*, after being exposed under the electron microscope, exhibited a novel type of organelle only in salmon red strain, whose morphology, chemical nature and a comparative account with regard to the previously described cellular components are described here.

Materials and methods. Both the strains were fixed for 2 h in a mixture of 3.5% glutaraldehyde and 2% paraformaldehyde prepared in phosphate buffer (pH 7.4) and post-fixed with 1% aqueous osmium tetraoxide at pH 7.3. Materials were dehydrated by passing through an ascending series of ethanol and embedded in epoxy resin. Ultra-thin sections were cut with Porter-Blum microtome I and stained with lead acetate for examination in an electron microscope JEM 7.

PICKETT-HEAPS's method[2] was used to determine the chemical nature of the new organelle. The material was treated with 1% aqueous periodic acid for 45 min at room temperature, preceded by 2% sodium bisulphite for 1.5 h at 60 °C. The material was transferred to 1% borate buffered hexamine (pH 9.2) containing 0.1% silver nitrate for 45 min at 50 °C. The sections were carefully watched during the later stages of incubation to prevent over-staining. Further treatment with 2% sodium thiosulphate for 15 min was followed by mounting the sections on copper grids for observation.

Results and discussion. Several round to spherical electron dense particulate bodies, named here as 'cytosomes' (cytoplasmic bodies), appeared extra-nuclear in the cytoplasm. They varied from few to about 35 in number in each section, possibly representing the approximate number in a cell and measured 250 × 200 nm in dimension. The wall was smooth, occasionally irregular and 7–10 nm thick. Small round to irregular electron dense material surrounded by white area was visible inside the cytosome (Figure 1). Such bodies were discer-

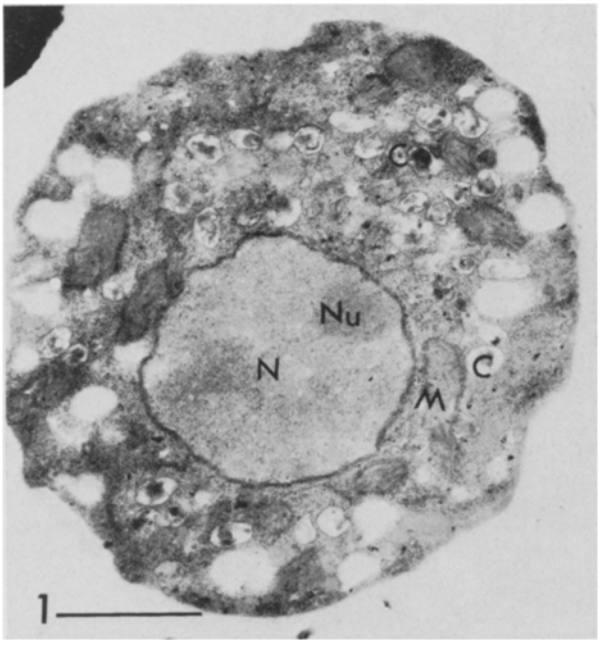

Fig. 1. Salmon red cell of *Taphrina maculans* Butler showing: C, cytosomes; M, mitochondrion; N, nucleus; Nu, nucleolus. Scale 1 μm.

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