flushed out with 0.9% saline solution while the rat was under ether anaesthesia, the entire intestine then quickly removed and everted over a slender steel rod. Only the jejunum and distal ileum were used. Rings were prepared by cutting the gut into segments, each approximately 1 mm long. A suspension of mucosal epithelial cells was obtained by 10 min low-amplitude, high frequency vibration of a 10–15 cm segment of everted intestine using the technique of LEVINE and WEINTRAUB[4] except the saline in the original method was replaced by a 10 m*M* solution of EDTA in physiological saline, for cell recovery without the EDTA was poor. Cell yield from animals infected for 7 days was poor and several animals were discarded as insufficient quantities of cells were recovered to measure oxygen consumption. Oxygen uptake by isolated mucosal epithelial cells (4–8 mg protein) and rings (approximately 100 mg wet wt) was measured with a Clark oxygen electrode. The material was incubated at 37 °C in about 4 ml Krebs-Ringer bicarbonate solution containing 10 m*M* D-glucose oxygenated with 5% CO_2 in oxygen. Protein assays[5] were made on the isolated cells after each experiment. The data were analyzed by Student's *t*-test.

Table 1. Oxygen uptake (µl/mg protein/min) by mucosal epithelial cells isolated from the jejunum or ileum of uninfected rats and rats at various times after infection with 4000 larvae of *N. dubius*

Experimental group	Jejunum	Ileum
Control	0.662 ± 0.069 (13)	0.460 ± 0.025 (10)
7 days	0.873 ± 0.064[a] (12)	0.520 ± 0.083 (10)
29–36 days	0.680 ± 0.050 (16)	0.474 ± 0.032 (14)

Means ± S.E.M. (*n*). [a] Significantly different from control and 29–36 day groups at 5% level.

Table 2. Oxygen uptake by jejunal rings of uninfected rats and rats 7 days after infection with 4000 larvae of *N. dubius*

Experimental group	Q_{O_2} ± S.E.M. (µl/mg dry mass/h)(*n*)
Control	15.60 ± 1.40 (16)
7 days	15.43 ± 0.85 (10)

Results and discussion. Table 1 shows Q_{O_2} of isolated mucosal epithelial cells. In each of the three groups Q_{O_2} of cells from the jejunum was significantly greater than cells from the ileum ($p < 0.01$), as previously noted by others for normal rats[6,7]. There were no significant differences in Q_{O_2} between experimental groups for the ileum, which was largely uninfected, but Q_{O_2} of cells from the jejunum of rats infected for 7 days was significantly greater than controls or animals 29–36 days after infection. In the latter group intestinal absorption, which was significantly depressed by 7 days, had returned to normal and the gut was free of parasites[2,3]. The poor recovery of epithelial cells from 7-day infected jejunum may be associated with the villous atrophy that often accompanies heavy infections[8]. Amongst the cells that were recovered were probably many immature crypt-like cells, which are present on the villi during infection with *Nippostrongylus brasiliensis*[9], a gastrointestinal nematode which also causes malabsorption[1]. The higher Q_{O_2} of the cells from infected intestines may reflect the activity of these immature cells as the Q_{O_2} of the cells from the base of the villus is approximately twice that of cells from the villous tip[10].

These results contrast with those obtained from rats infected with *N. brasiliensis* in which the respiratory activity of jejunal epithelial cells or mitochondria differed little from the normal jejunum[9,11]. This difference may either reflect the differing methods used for obtaining the cells or a more pronounced effect of the parasite on the host; the malabsorption associated with *N. dubius* infection is more severe than with *N. brasiliensis* infections[2,3,12].

There was no significant difference in Q_{O_2} of jejunal rings between control and infected animals (Table 2), which suggests that oxygen consumption by the bulk of intestinal tissue is not affected by infection.

[4] P. H. LEVINE and L. R. WEINTRAUB, J. Lab. clin. Med. *75*, 1026 (1970).
[5] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[6] J. R. BRONK and D. S. PARSONS, Biochim. Biophys. Acta *107*, 397 (1965).
[7] H. S. A. SHERRAT, Comp. Biochem. Physiol. *24*, 745 (1968).
[8] A. M. SCOFIELD (unpublished data).
[9] L. E. A. SYMONS, J. R. GIBBINS and W. O. JONES, Int. J. Parasit. *1*, 179 (1971).
[10] L. HAMBERGER and O. LUNDGREN, Experientia *27*, 56 (1971).
[11] C. H. GALLAGHER and L. E. A. SYMONS, Aust. J. biol. Sci. *37*, 421 (1959).
[12] A. M. SCOFIELD, Int. J. Parasit. (in press).

Resolution of Ca^{++}-ATPase of sarcoplasmic reticulum into subunits

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Summary. The Ca^{++}-ATPase system of sarcoplasmic reticulum (SR) was resolved into several subunits by isoelectric focusing and isotachophoresis in acrylamide gels. The results obtained support the concept that the ATPase system of SR is oligomeric forming a tetramer of 100,000 mol.wt subunits.

The biochemical functions of the Ca^{++}-pump system of SR have been extensively characterized[2–4]. However, the molecular arrangement of the Ca^{++}-pump in SR membranes has not yet been characterized. The ATPase system within the lipid phase of the membrane transduces chemical into osmotic energy, probably through its ionophoretic activity[5], which cannot be explained in terms of a rotatory diffusion carrier[4]. Recent detailed biochemical and structural analysis of SR[6] and the results of this work support the concept that the ATPase enzyme may be arranged in the membrane as an oligomeric system forming a hydrophylic channel specific for the transport of Ca^{++}.

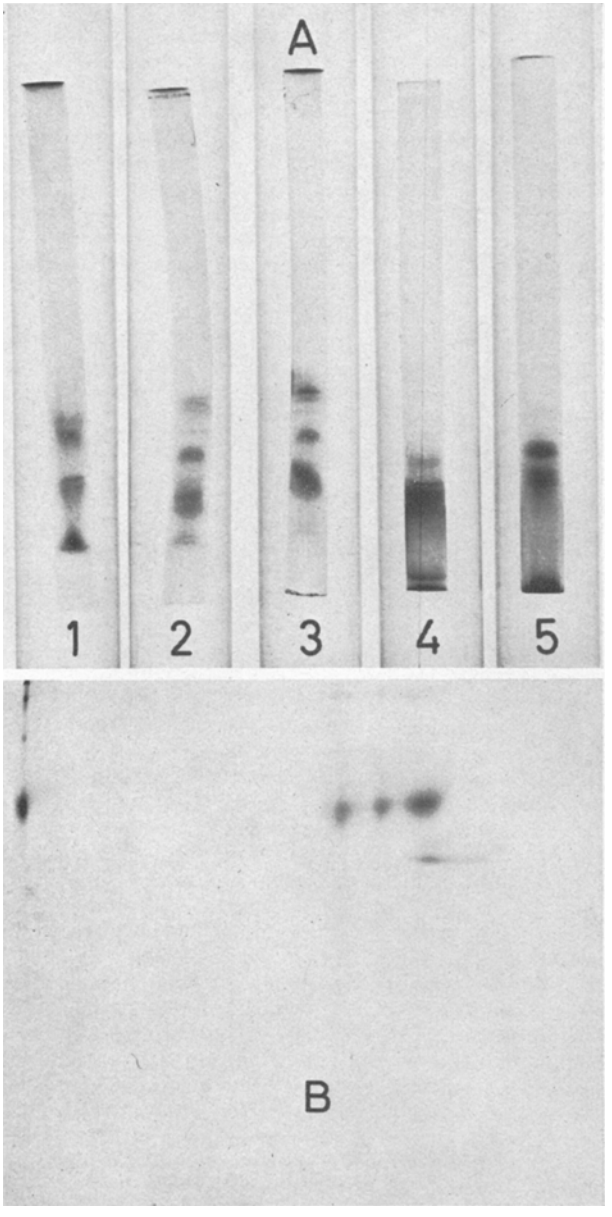

Fig. 1. *A* Isoelectric focusing of SR. All the gels, except the gels 1 and 4, have 1% Triton. The protein (80 µg) was loaded at the alkaline cathodal terminal (top) in gels 1, 2 and 3, and it was loaded at the acid anodal terminal (bottom) in gels 4 and 5. The isoelectric points of the bands range from about 6 (upper bands) to about 5 (lower bands of gels 1, 2 and 3) or to about 4.5 (lower bands of gels 4 and 5). *B* Second dimension electrophoresis in SDS slabs of gel 3. The dense bands correspond to a mol.wt of 100,000 daltons. The heavier band corresponds to the 2 polypeptides not sharply separated in gel 3 (heavier mark). Faint bands of mol.wt 60,000 correspond to the minor proteins of SR.

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2 A. Martonosi, in: Current Topics in Bioenergetics. Ed. F. Bronner and A. Kleinzeller. Academic Press, New York 1972.
3 D. H. Mac-Lennan, Can. J. Biochem. *53*, 251 (1975).
4 A. N. Martonosi, in: Calcium Transport in Contraction and Secretion. Ed. E. Carafoli, F. Clementi, W. Drabikowsky and A. Margreth. North-Holland, Amsterdam 1975.
5 A. E. Shamoo and D. H. MacLennan, Proc. Nat. Acad. Sci. USA *71*, 3522 (1974).
6 R. L. Jilka and A. N. Martonosi, J. biol. Chem. *250*, 7511 (1975).

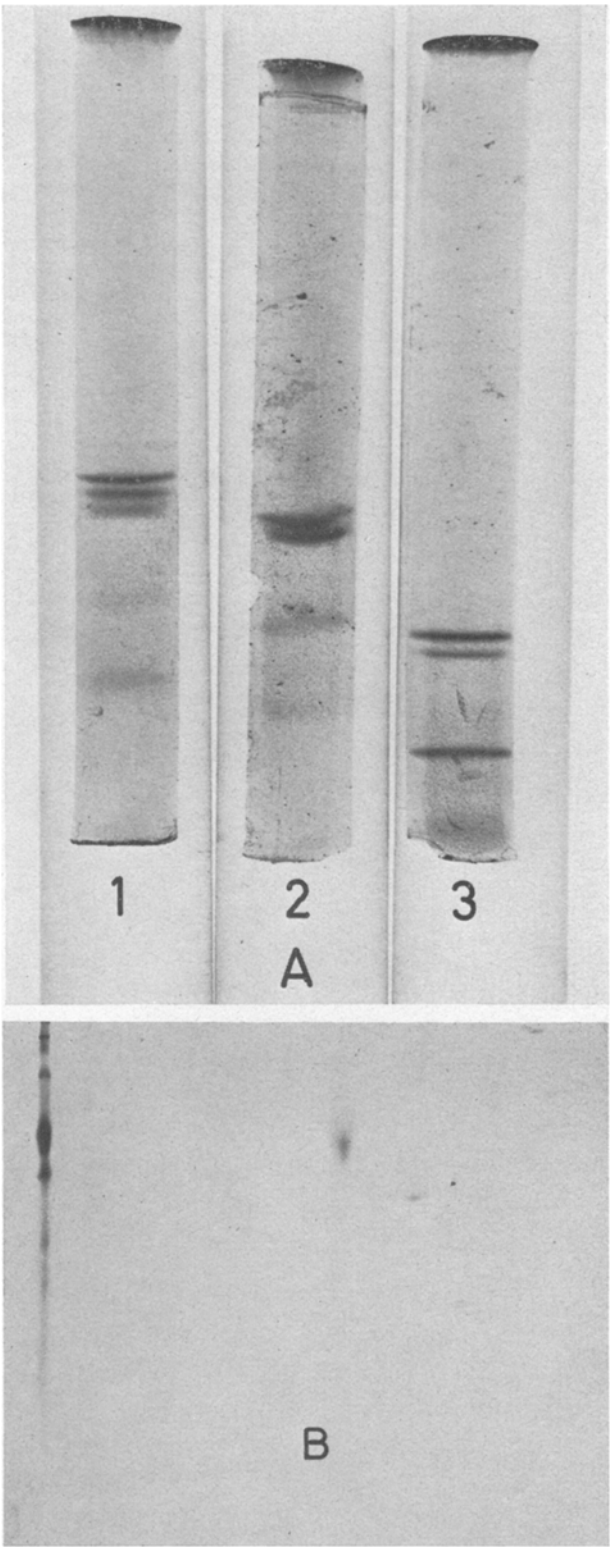

Fig. 2. *A* Isotachophoresis of SR. Gels 2 and 3 differ from gel 1 since they have 1% Triton. Migration in gel 1 was allowed for 90 min (2 mA) and that of gels 2 and 3 was allowed for 90 and 105 min, respectively (2 mA). *B* Second dimension electrophoresis in SDS slab of a thin diameter gel (0.3 cm) similar to the large diameter gel 3 (0.6 cm). The heavier spot in the middle (mol.wt 100,000) corresponds to the 2 upper bands of gel 3, and the lighter spot (mol.wt 60,000) corresponds to the lower band of gel 3. Note the overloaded pattern at the left side of the gel, due to the protein which did not penetrate in the first dimension gel.

Materials and methods. Fragmented SR was isolated as described elsewhere[7], and the Ca^{++}-pump enzyme was purified according to Warren et al.[8]. The relative amount of Ca^{++}-ATPase (mol.wt. 100,000) was about 80% and 96% of the total protein for crude SR and purified enzyme, respectively, as estimated from densitometric traces of SDS polyacrylamide electrophoregrams.

Isoelectric focusing was carried out in gels containing 4% acrylamide, 0.125% bisacrylamide, 8 M urea, 1.12% carrier ampholytes (pH 3.5–10) and 1% Triton X-100 (where noted). The electrolytes were 50 mM H_2SO_4 (anodal) and 30 mM NaOH (cathodal). The gels were loaded with 80 µg of protein in 5% Triton, 8 M urea, 1% 2-mercaptoethanol and 20 mM Tris (pH 7.4). Isoelectric focusing proceeded at 260 V for 5–18 h. Some gel rods were electrophoresed in second dimension which was performed in slabs (10% acrylamide, 0.135% bioacrylamide) containing 0.5% SDS. Separations were carried out for 6 h at 15 mA and pH 8.9 (50 mM Tris-glycine as buffer system). Isotachophoresis was carried out according to Griffith and Catsimpoolas[9], but sometimes 8 M urea and 1% Triton were included in the gels. Sample protein solutions (1 mg total protein) were prepared in the terminating buffer containing 3.1% Triton X-100, 8 M urea and 15.8% carrier ampholytes (pH 4–6). About 140 µg or 70 µg of total protein were applied in each gel rod of 0.6 cm or 0.3 cm diameter, respectively. Separations in second dimension were performed as describe above.

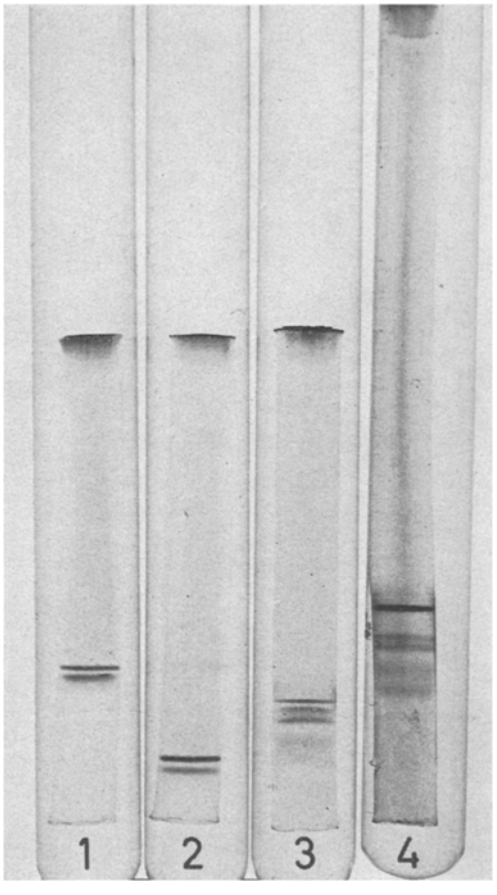

Fig. 3. Isochachophoresis of purified Ca^{++}-ATPase. Gels 1 and 2 are identical and contain 8 M urea. The protein loaded in these gels was dissolved in the normal medium. The migration was allowed for 105 min in gel 1 and for 120 min in gel 2 (2 mA). The gels 3 and 4 and the protein samples did not contain urea. Gel 3 was 8 cm long and migration proceeded for 112 min (2 mA), whereas gel 4 was 13 cm long and migration proceeded for 150 min (2 mA).

Results and discussion. The Ca^{++}-ATPase enzyme of our SR preparations (80% of the total protein) was resolved into 4 main subunits when the solubilized membranes were submitted to isoelectric focusing (figure 1). 4 well-defined bands, with isoelectric points ranging from 6 to 5, were separated (figure 1A). The individual peptides of the ATPase system have mol.wt of about 100,000 daltons as revealed by second dimension electrophoresis in SDS system (figure 1B).

When the protein was loaded at the acid terminal of the gel (figure 1A, gels 4 and 5), a bad resolution was obtained and the results were not reproducible, probably because the protein precipitates at the acid terminal and, thus, free penetration into the gel is not possible. Thus, additional experiments designed to follow the behaviour of the phosphorylated Ca^{++}-ATPase (the phosphorylated complex is only preserved at acidic pH) could not be carried out.

The results obtained agree well with those obtained by Murphy[10] on cross-linking experiments, and both reinforce the conclusion that the ATPase peptides associate in the membrane as a tetramer. Furthermore, it was shown that the smallest fully active Ca^{++}-ATPase particles obtained by detergent treatment contain 3–4 ATPase polypeptides[11]. On the other hand, structural data revealed that the particles assigned to ATPase are arranged in the membrane as regular complexes[6].

Isotachophoresis, which permits the separation of ions into consecutive zones according to their mobilities, was also applied to SR. As far as we know, this technique was not previously applied to membrane proteins, since it is difficult to bring such proteins into solution without modifying their mobility characteristics. The nonionic detergent, Triton X-100, in combination with urea, was used to solubilize SR. It was possible to carry out very reproducible separations which, depending on experimental conditions, gave 2–3 main individual peptides belonging to the ATPase system (figure 2). The only drawback is that the penetration of the protein into the gels is somewhat difficult, and this effect is more pronounced when thin gel rods (3 mm diameter) are used in order to perform additional second dimension electrophoresis in SDS system (figure 2 B).

The purified ATPase (96% of the total protein) when submitted to isotachophoresis gave also 2–3 main bands depending on the experimental conditions (figure 3). The results obtained support the concept that the Ca^{++}-ATPase of SR is an oligomeric complex. Furthermore, the present techniques open the possibility of collecting the individual monomers for further biochemical characterization, namely molecular reconstitution of the Ca^{++}-transport sites, thus permitting clarification of the vaster problem of ion transport across biomembranes. Studies are in progress to isolate preparatively the ATPase peptides by isotachophoresis.

7 A. P. Carvalho and A. Mota, Archs Biochem. Biophys. *142*, 201 (1971).
8 G. B. Warren, P. A. Toon, N. J. M. Birdsall, A. G. Lee and J. C. Metcalfe, Proc. Nat. Acad. Sci. USA *71*, 622 (1974).
9 A. Griffith and N. Catsimpoolas, in: Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel. Ed. R. C. Allen and H. R. Maurer. Walter de Gruyter, Berlin 1974.
10 A. J. Murphy, Biochem. biophys. Res. Commun. *70*, 160 (1976).
11 M. le Maire, J. V. Møller and C. Tanford, Biochemistry *15*, 2336 (1976).