races were reared and maintained routinely in our laboratory, as described in the previous paper[6]. Adults originating from nondiapausing pupae were used in each experiment in the case of the Japanese race (D). If parents were placed under a long day condition (15L:9D) at 20 °C, the offspring developed into only nondiapausing pupa regardless of the light regime in the following larval stages, in Japanese *peregrina*. Therefore, adults of both races were kept in a short day room (12L:12D, 20 °C) for the experiment. About 150 newly deposited larvae were transferred to a glass vessel (1.5 l in volume and 12 cm in diameter) in a room kept at 20 °C. Then each vessel was put into a 5-gallon can equipped with a 5-watt fluorescent lamp. Illumination of this lamp was controlled by an outside time switch. The inside temperature of each can was controlled at 20 °C by aerator. Photoperiods used were: continuous lighting, 16L:8D, 15L:9D, 14L:10D, 13L:11D, 12L:12D, 11L:13D, 10L:14D, 9L:15D and continuous darkness. The larvae reached their maximal sizes in 5 days at 20 °C. In order to synchronize pupation, fully grown larvae were left for an additional 4 days in the same vessels supplied daily with distilled water to keep the inside wet. The prepupal insects were allowed to pupate in sawdust in dry vessels (480 ml in volume and 8 cm in diameter) and kept for a further 2 weeks under the same conditions of photoperiod and temperature. At the end of this time, the number of diapause and nondiapause pupae were counted, using the method of judgement described by FRANKEL and HSIAO[7]. Nondiapause pupae at this stage of development were easily distinguished from those of diapause flies with their pigmented eyes.

When larvae of Japanese *peregrina* were reared at 20 °C under short day conditions 13L:11D to 10L:14D, pupal diapause approached 100%, as shown in the figure. Under a long day condition (15L:9D, 20 °C), they almost all did not enter diapause, as known from previous work[4]. None of the New Guinean *peregrina*, however, entered pupal diapause, irrespective of photoperiod, in any of the repeated experiments (figure, ND). The crossbreeding experiments (D♀ × ND♂; ND♀ × D♂) produced pupae of the offspring (F_1) which did not undergo diapause under long day conditions such as 15L:9D to 13L:11D. Under the light regime 12L:12D, 30% of the pupae entered diapause in the crossing D♀ × ND♂. The percentage of hybrid offspring diapausing increased up to 50% in D♀ × ND♂ and 66% in ND♀ × D♂ under the light regime 11L:13D. The figure shows that about 30–40% of hybrids did not enter diapause, even under the three short day lengths such as 11L:13D, 10L:14D and 9L:15D. In the reversed cross, ND♀ × D♂, the mean percentage of diapausing hybrids was apparently more than those of D♀ × ND♂ crossings, although there is no significant difference between them in the final analysis. Day length of less than 11 h seems to have been effective upon about 60% of the F_1. New Guinean and Japanese parents are considered to have anti-diapause and diapause factors respectively, which may be genetically transmitted to their progeny. Also, it is likely that there is another genetic factor which reflects the day length making the fly diapause. The Japanese race requires a critical day length of between 14 and 15 h for diapause. New Guinean flies may be considered to have a critical day length near to 11 h for entering diapause, as suggested by the fact that 2% of New Guinean flies diapaused under the 11L:13D light regime in the preliminary inbreeding experiment. The F_1 seems to require an intermediate day length (13L:11D) for entering diapause. The occurrence of anti-diapause allele(s) suppresses 30–40% of the latter allele(s) in F_1. Discovery of this kind of anti-diapause allele(s) may be applicable to the genetic control of Japanese *peregrina* which has to enter diapause under the severe climatic conditions of winter. VINOGRADOVA and ZINOVJEVA[8] reported that the diapause and nondiapause states of progeny are determined by a maternally operated photoperiod in *Calliphora vicina*, and suggested that the photoperiod regulates the physiological state of the larvae via the female, but does not influence the process of oögenesis directly in the larval diapause of the blow-fly. In the study on the embryonic diapause of field crickets, OHMACHI and MASAKI[9] found that the development of the hybrid egg is not determined by the yolk or cytoplasm provided by the mother, but the genetic constituents of the embryo or interaction between the two. Our experiment also suggests that the physiological state of the progeny is determined by the combination of allele(s) and environmental conditions.

[6] T. OHTAKI, Jap. J. med. Sci. Biol. *19*, 97–104 (1966).
[7] G. FRANKEL and C. HSIAO, J. Insect. Physiol. *14*, 707–718 (1968).
[8] E. B. VINOGRADOVA and K. B. ZINOVJEVA, J. Insect. Physiol. *18*, 2401–2409 (1972).
[9] F. OHMACHI and S. MASAKI, Evolution *18*, 405–416 (1964).

Oxygen consumption of the rat small intestine during infection with Nematospiroides dubius

A. M. SCOFIELD

Unit of Applied Zoology, Wye College (University of London), Wye, Ashford, Kent. TN25 5AH (England), 22 June 1976

Summary. Qo_2 of jejunal rings did not differ significantly between uninfected rats and rats infected for 7 days with *Nematospiroides dubius*. Qo_2 of isolated jejunal mucosal epithelial cells was significantly greater 7 days after infection than in uninfected controls or at 29–36 days after infection.

Although the absorptive capacity of the small intestine of a number of host species is altered during gastrointestinal nematode infections[1–3] relatively little else is known of intestinal metabolism in these infections, although work with rats infected with *Nematospiroides dubius* indicated an increase in glucose utilization during infection[2]. The present work measures the oxygen consumption by the small intestine of rats infected with *N. dubius*, a nematode which causes pronounced intestinal malabsorption of nutrients in the rat[2,3].

Methods. Female Wistar rats, aged 2.5–3 months at slaughter were used. A single dose of 4000 infective larvae in 0.5 ml tap water was given orally. Control animals were given 0.5 ml tap water 7 days before use. Animals were starved 16 h before use. The small intestine was

[1] L. E. A. SYMONS, Int. Rev. trop. Med. *3*, 49 (1969).
[2] A. M. SCOFIELD, Comp. Biochem. Physiol. *47A*, 219 (1974).
[3] A. M. SCOFIELD, Comp. Biochem. Physiol. *52A*, 685 (1975).

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

Vitor M. C. Madeira and M. C. Antunes-Madeira[1]

Department of Zoology, University of Coimbra, Coimbra (Portugal), 13 August 1976

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^{++}.