Environmental temperature and the growth of *Taenia crassiceps* cysticerci in mice

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Summary. Mice kept at low (5±1 °C) and high (35±1 °C) temperature harboured significantly less *Taenia crassiceps* cysticerci than controls kept at 21±1 °C. This effect was more pronounced in heat-stressed than in cold-stressed animals and more in males than in females.

Webster mice of both sexes, 4-month-old, were divided into 3 groups of 15 mice each and acclimated to their respective environmental temperatures 7 days prior to infection. One group was acclimated to 5±1 °C, and the other to 35±1 °C. The control group was kept at 21±1 °C. With the aid of electric timers, each group was placed on the same photoperiod of 15 h daylight and 9 h darkness. All mice were infected with 20 nonbudding cysticerci each and killed 20 days later. At autopsy the number of nonbudding individuals, budding individuals, and the total numbers of larvae were counted.

2 experiments were performed; in both the cold-stressed an heat-stressed mice of both sexes harboured significantly less larvae than the controls. Low temperature decreased the total number of larvae from 42.06±4.24 to 20.93±1.26 and 32.40±2.45 to 15.60±0.66 in females, and from 28.60±4.53 to 19.86±1.49 and 26.71±2.04 to 16.36±1.53 in males. High temperature depressed the number of larvae from 42.06±4.24 to 24.13±1.78 and 32.40±2.45 to 16.00±0.92 in females and from 28.60±4.53 to 14.40±1.04 and 26.71±2.04 to 8.14±1.04 in males. The mortality of larvae (with subsequent disintegration) was highest in heat-stressed males. In both experiments heat-stressed males had significantly less larvae than cold-stressed males. But there was no significant difference in the total number of larvae recovered from respective female groups.

All temperature-stressed mice had significantly lower numbers of nonbudding larvae than controls. In cold-stressed mice their number decreased from 28.93±3.14 to 9.33±0.81 and from 12.93±2.24 to 2.20±0.57 in females and from 23.07±3.85 to 7.60±0.88 and from 14.86±1.86 to 2.00±0.58 in males. In heat-stressed mice the mean number of nonbudding cysticerci decreased from 28.93±3.14 to 11.73±1.21 and from 12.93±2.24 to 3.13±0.41 in females and from 23.07±3.85 to 11.07±0.90 and from 14.86±1.86 to 4.36±0.50 in males. However the cold-stressed males harboured in both experiments significantly less nonbudding larvae than heat-stressed males.

In cold-stressed males of the 1st experiment the number of budders increased significantly from 5.53±0.82 in controls to 12.33±1.47, but remained more or less constant in the 2nd experiment (11.86±0.78 and 14.36±1.18 respectively). There was no significant difference between the number of budders in cold-stressed and control females in the 1st experiment (13.13±1.42 and 11.53±1.46 respectively), but there was a significant decrease in this number in the 2nd experiment (19.47±1.23 and 13.40±0.65 respectively). High temperature decreased significantly the number of budders in males in both experiments, from 5.53±0.82 to 3.33±0.48 and from 11.86±0.78 to 3.79±1.06 respectively. Also in both experiments heat-stressed males had significantly less budding larvae than cold-stressed males. The number of budders in heat-stressed females was close to that in cold-stressed females. It remained almost constant in the 1st experiment (13.13±1.42 in controls and 12.40±0.83 in heat-stressed mice) but was significantly lower in the 2nd experiment 19.47±1.23 and 12.87±0.74 respectively. The present study, which seems to be the first dealing with the effect of environmental temperature on proliferating cestode larvae in homeothermic hosts, showed that this temperature affects the growth of *T. crassiceps* cysticerci in mice. Similar studies with other proliferating cestode larvae are currently in progress.

Demonstration of the presence of M-creatine kinase in mammalian myogenic cell lines

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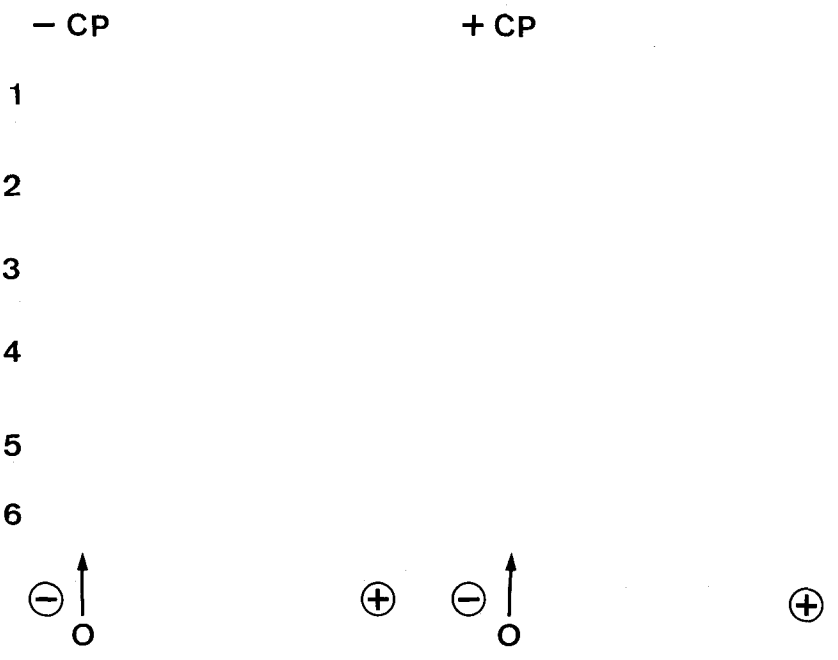
Summary. Unequivocal identification of M-CK in cell extracts from fused cells of myogenic cell lines is difficult due to almost identical behaviour of the M-CK and a contaminating enzyme activity in electrophoresis. If CK dimers present in cell extracts were subjected to dissociation and reassociation in the presence of exogenous B-CK subunits, the formation of easily identifiable MB-CK was demonstrated, indicating the presence of M-CK in the myogenic rat cell lines.

The transition of B-creatine kinase (B-CK) to muscle-specific M-creatine kinase (M-CK) is a well documented event during terminal differentiation of myogenic cells in the embryo[2,3] or in culture[3,4]. Terminally differentiated myotubes, derived from presumptive skeletal muscle contain, among other typical muscle proteins, also M-CK, which has proved to be a useful marker to evaluate the differentiated state of myogenic cells[4-6]. In mammalian myogenic cell lines, the determination of the creatine kinase isoenzymes has been hampered by the fact that creatine kinase activity and some contaminating enzyme, probably myokinase, giving rise to high background staining, cannot be separated easily from each other by electrophoresis.

So far no B-CK has been found in these cell lines, and thus even if the M-CK gene were active one could not hope to find any hybrid enzyme MB-CK, an activity that clearly can be separated from the unspecific staining by electrophoresis. The presence of MB-CK would indicate both the M-CK and B-CK genes to be active within the same cytoplasm and give some further information on the state of differentiation of such cells. As there is no BB-CK



Electrophoresis of creatine kinases on cellulose acetate supports and stained for creatine kinase in the presence of the substrate creatine phosphate (+CP) and in its absence for unspecific blank activity (−CP).
1°Purified M-CK from rat muscle;
2°purified B-CK from rat brain;
3°20 µl of L-8 after dissociation reassociation procedure in the presence of exogenous B-CK;
4°same preparation as in *3* but with twice the amount;
5°cell extract of fused L-8 cells;
6°desalted ammonium sulfate precipitate (40–70% saturation) of the cell extract.

or MB-CK detectable in extracts of these rat cell lines[7,8], one would expect to be able to use spectrophotometric analysis of the CK activity, since any change in CK activity could be attributed to M-CK present in these cells. This proved, however, not to be easy, because again the blank values, regardless of whether creatine or creatine phosphate was used as a substrate for the assay, were very high and variable (D. Turner, personal communication).

In this communication, we want to clarify the situation and describe a way to demonstrate unequivocally the presence of M-CK in fused cultures of rat myogenic cell lines by the 'in vitro' formation of enzymatically active MB-CK formed between exogenous, added B-CK subunits and the CK present in the cell extract.

Materials and methods. Extracts of confluent, well fused cultures of L-8 cells were prepared according to routine methods[4]. The extract was precipitated with ammonium sulfate and the fraction of proteins precipitating between 40 and 70% saturated ammonium sulfate was dissolved in Tris-HCl buffer pH 8.0, 1 mM 2-mercapto-ethanol and desalted over a Sephadex G-25 column equilibrated with the same buffer. A sample of the desalted fraction was kept for further analysis, the rest was mixed with a 5fold excess of purified B-CK from rat brain, calculated on the basis of apparent CK activity determined spectrophotometrically[9]. This mixture was made 8 M in urea by the addition of cristalline urea. After 1 h at 4 °C, mixture was then transferred to a dialysis bag and dialyzed overnight against low ionic strength buffer (50 mM Tris-HCl pH 8.0, 10% glycerol, 10 mM 2-mercaptoethanol, 0.1 mM EDTA) to allow renaturation of enzymatically active dimers[10,11]. Samples of the dialyzed fraction were analyzed by electrophoresis on Gelman Sepraphore III cellulose acetate supports and CK enzymatic activity was revealed as described[4].

L-8 myogenic cells (gift of D. Yaffe) and L-5 and L-6 cells (gift of A. Cohen) were cultured on gelatinized falcon tissue culture dishes in the medium described by Yaffe[12]. The purified rat enzymes were from stocks purified in this laboratory by R. Gmür from rat muscle (M-CK) and from rat brain (B-CK) by M. Specker.

Results. The different fractions were analyzed as described above and the results are given in the figure. Each sample was applied as a line across the cellulose acetate support, and after electrophoretic separation the strip was cut longitudinally in 2 equal strips. One half was incubated for blank activity (no creatine phosphate added), the other half was stained for creatine kinase (with creatine phosphate). On strip 5, the initial extract of the confluent, well fused L-8 cultures, and on strip 6, the 40–70% saturated ammonium sulfate precipitate were applied for electrophoresis. Both fractions contain a high blank staining, but enzymatic staining of the same band seems to be more intense in the presence of the substrate creatine phosphate, indicating the possible presence of M-CK. Since the electrophoretic separation of both fractions gave identical results, the ammonium sulfate fractionation did not seem to separate the contaminating activity form the CK. Further purification steps, like chromatography on a G-150 column previously calibrated with M-CK, resulted in a marked loss of apparent CK activity, while the unspecific enzymatic activity remained stable (data not shown).

If, however, the cell extract was subjected to the dissociation and reassociation procedure in the presence of excess purified rat B-CK subunits, the formation of MB-CK can clearly be demonstrated as seen in strips 3 and 4. The blank activity, however, still migrated to the same position as purified M-CK as seen in lane 1. Thus we can conclude that fused, confluent L-8 cells contain M-CK, although in very small quantities as compared to primary rat myogenic cultures.

The same experiment was carried out with cell extracts from other rat myogenic cell lines and traces of M-CK were found in confluent cultures of L-5 and L-6 rat cell lines (data not shown).

Discussion. It appears that well fused L-8 cells contain creatine kinase of the muscle-specific type M-CK, indicating once more their myogenic nature. On the basis of the present results, it could not be decided whether all cells accumulate M-CK at very low levels or whether only a few cells contain rather large amounts of the enzyme.

Unfused growing cells at lower density do not contain any B-CK, as one would expect for embryonic cells. We cannot decide, however, if the very low level of M-CK and the undetectable quantities of B-CK are due to cellular selection of a cell type that lacks the capabilities to express fully the creatine kinases, or whether generally the accumulation

of many enzymes are much lower in these cell lines. Using 2 dimensional gel electrophoresis, the β and γ forms of actin were found to be expressed in nonfused L-6 cells as well as in nonmuscle cells, but the muscle-specific form of α actin was only found in fused cells[13]. This result could indicate that the lack of detectable B-CK expression could be a quantitative effect and not necessarily a loss of B-CK, as the ubiquitous forms of actin, β-actin and γ-actin could be found in unfused cells of similar cell lines. The expression of the M-CK however clearly indicates that the fused cells express another feature of terminally differentiated muscle cells and corroborates some results of activity measurements in these cells[14].

While this manuscript was being prepared, Cohen et al.[8] found similar results, obtained with a method using an inhibitor of myokinase to demonstrate the presence of M-CK in rat myogenic cell lines.

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Seasonal fluctuations of population densities of the fish-mite *Seudacia medanensis* independent of atmospheric temperature and humidity

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Summary. Seudacia medanensis is a species of mite which reaches pest proportions on dried stored fishery products in the warm conditions of the tropics. Studies extending for a period of 2 years have shown that the seasonal variation in the population intensity of the mite is not influenced by atmospheric humidity or temperature.

Even though the fish-mites can cause considerable damage and qualitative deterioration of dried, stored fishery products, there is, except for few taxonomic descriptions of some species, hardly any work on the biology of these mites.

The present paper discusses the results of a 2-year study conducted to discover the influence of atmospheric temperature and humidity on the seasonal density of *Seudacia medanensis,* which reaches pest proportions on dried fishery products stored in the warm conditions of the tropics.

Material and method. Several healthy cultures of *S. medanensis* were maintained in the laboratory for routine observations. 3 of these cultures were maintained under identical conditions of feeding and atmospheric humidity and room temperature. For feeding, fresh specimens of dried anchovy (*Anchoviella commersonii*) were added to each culture at the middle of every month.

For assessing the seasonal density of the mites, approximately 10 g of the feeding material was withdrawn from each culture in the 1st week of every month, and the number of the adult mites present on the material counted, after immobilizing the mites by dipping the substrative material in killing fluid, viz. 10% formalin. Subsequent to the counting of the mites, the feeding material withdrawn from each culture was thoroughly washed and separately dried to constant weights. From the weights of the material thus obtained, and respective counts of mites that were present on them, the number of mites on a unit weight of 10 g in the 3 cultures was calculated. The figures given in the tables, in respect to the density of the mites for each month, is the average of the 3 counts obtained for a unit weight of 10 g for each culture. The experiment was started in January 1975 and terminated in December 1976. The average relative humidity and temperature for each month was computed from daily readings of the same obtained from the government meteorological department.

Results and discussion. The average counts of the adult mite from the 3 cultures (in relation to the unit weight) and the relative humidity and temperature of the atmosphere for

Table 1. Density of adult *S. madanensis* on unit weight of anchovy and atmospheric humidity and temperature during 1975

	Density y	Relative humidity x	Temperature z
January	1,040	65	26.6
February	1,000	72	27.45
March	925	74.5	28.10
April	3,322	80.0	28.6
May	0	79.0	27.7
June	0	87.0	25.75
July	0	88.0	26.0
August	1,250	86.5	25.75
September	5,050	86.5	26.10
October	5,148	85.5	25.9
November	15,450	83.5	25.9
December	8,005	73.0	26.25