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THE EFFECT OF SODIUM IONS ON THE EXCYSTMENT OF *HYMENOLEPIS DIMINUTA* CYSTICERCOIDS

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The cysticercoids of *Hymenolepis diminuta* can be excysted *in vitro* by mimicking the conditions found in the mammalian gut. Excystment is usually carried out in physiological saline and the presence of specific bile salts and a temperature of approximately 37° C are required. Proteolytic enzymes, such as pepsin and trypsin, speed up excystment but are not essential. Although the bile salt requirements for excystment have been studied in some detail, the role of the inorganic ions in the medium has not been investigated. (Sawada I. 1990: Exp. Parasitol. 8: 325-335; Rothman A.H. 1959: Exp. Parasitol. 8: 336-364; Campbell W.C., Richardson T. 1960: J. Parasitol. 46: 490; Lackie A. M. 1975: Biol. Rev. 50: 285-323).

Cysticercoids were recovered from *Tribolium confusum* 8-12 weeks after infection and excysted at 37° C in Krebs Ringer (140 mM Na Cl, 6 mM KCl, 3 mM $CaCl_2$, 1 mM KH_2PO_4) containing 0.5% (w/v) trypsin and 0.3% (w/v)glycotaurocholate. Using isotonic stock solutions of the different salts, the ionic composition of the Ringer was varied without altering the overall ionic strength (Laser H. 1961: in C. Long (Ed.), Biochemist's Handbook, E.&F.N. Spon, London, pp. 58-60). Omission of K^+, Mg^{2+} or Ca^{2+} from the Ringer solution had no significant effect on either the rate or percentage excystment. The NaCl in the medium could be replaced by Na_2SO_4, Na_2NO_3, or Na_2HPO_4/NaH_2PO_4, again with little effect on excystment. However, sodium ions appear to be essential for excystment and NaCl in the medium could not be replaced by LiCl, KCl, RbCl, CsCl, NH_4Cl, choline chloride, N-methylD-glucamine, mannitol or sucrose. A dose response curve for sodium (Fig.1) shows that cysticercoids require at least 70 mM sodium to excyst. This relatively high level suggests that sodium is required either for the operation of a specific transport mechanism or for the maintenance of an electrogenic potential. To further characterize the role of sodium ions in excystment, a range of inhibitors of sodium dependent mechanisms was tested. No significant effect on excystment was found with 1mM *p*-chloromercuriphenylsulphate, 1mM orthovanadate, 1mM sodium tungstate (all non-specific ATPase inhibitors), 1 µM amiloride (sodium transport inhibitor), 5 mM strontium chloride (sodium /calcium exchange inhibitor), 0.5 µM ruthenium red (calcium uniport inhibitor), 1mM oubain (sodium/potassium exchange inhibitor) or 1µM monensin (calcium ionophore). However, an 80% inhibition of excystment was found with 0.1 µM acetazolamide, a sodium/proton exchange inhibitor. Excystment was unaffected by 1mM procaine, but was totally inhibited by 2% (v/v) ethanol and by 0.5 mM praziquantel. Ethanol acts as a general membrane disrupting agent and praziquantel causes paralysis of cestode musculature by opening calcium channels. The failure of some of the inhibitors tested to show any appreciable effects could be due to their inability to penetrate the glycocalyx.

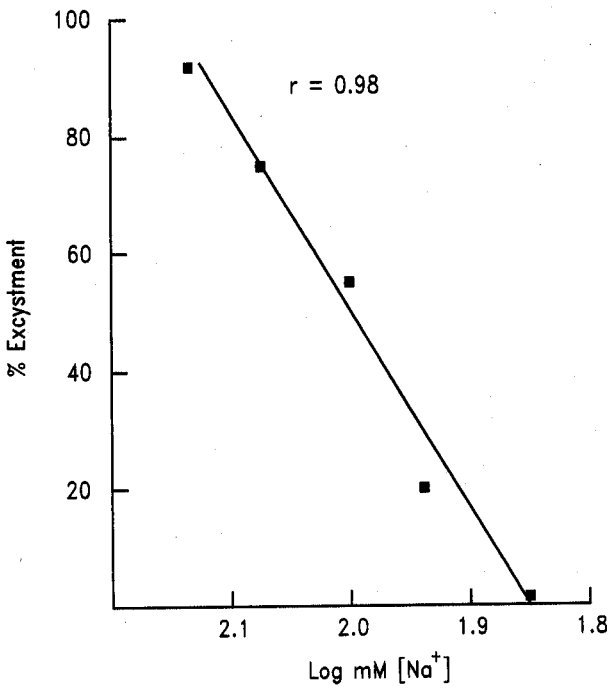

Fig.1. The effect of sodium ion concentration on the % excystment of *Hymenolepis diminuta* cysticercoids.

The results suggest that excystment of the cysticercoids may involve the activation of a tegumental proton pump, with a subsequent change in internal pH. Whether this activation is due to a direct effect by bile salts, or whether it is mediated through second messengers is unknown.

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