determined and compared with that of control cells. As can be seen from the table, the respective deficiencies, in stationary cells, stimulate an increase in the content of phosphatidyl choline and a decrease in the content of phosphatidyl ethanolamine. The changes registered in the content of phosphatidyl inositol and phosphatidyl serine in stationary cells are dependent on the nature of the deficiency. A uniform decrease in the content of phosphatidyl ethanolamine was also registered in deficient exponential cells. On the other hand, in these cells the content of phosphatidyl choline, phosphatidyl inositol and phosphatidyl serine is unchanged or only slightly increased.

Considering the role of sterols or phospholipids in the microarchitecture of biological membranes we can assume that both components play a role in membrane susceptibility to polyene antibiotics especially, if polyene sensitivity is dependent on the degree of membrane fluidity. In this connection, our results suffer from the shortcoming that the lipids of both classes are extracted from the whole, unfractionated cells and that changes in the composition of the cytoplasmic membrane only may be masked. Similarly, it should be pointed out that nystatin sensitivity may be determined in part by binding factors in the cell wall[13], and that our analyses would not reveal changes in cell wall organization. Nevertheless, our results suggest that growth factor deficiencies allow us to manipulate the content and composition of membrane components and nystatin sensitivity of cells. This approach could be used for further studies of the mode of action of polyene antibiotics at the molecular level.

1 J.M. Hamilton-Miller, Bact. Rev. *37*, 166 (1973).
2 A.W. Norman, A.M. Spielvogel and R.G. Wong, Adv. Lipid Res. *14*, 127 (1976).
3 M. Fryberg, A.C. Oehlschlager and A.M. Unrau, Archs Biochem. Biophys. *173*, 171 (1976).
4 A.M. Pierce, H.D. Pierce, Jr, A.M. Unrau and A.C. Oehlschlager, Can. J. Biochem. *56*, 135 (1978).
5 B.H. Olson and M.J. Johnson, J. Bact. *57*, 235 (1949).
6 V. Bryson and W. Szybalski, Science *116*, 45 (1952).
7 R.P. Longley, A.H. Rose and B.A. Knights, Biochem. J. *108*, 401 (1968).
8 R.A. Gonzales and L.W. Parks, Biochim. biophys. Acta *489*, 507 (1977).
9 A. Čejková and V. Jirků, Folia microbiol. *23*, 372 (1978).
10 C.W.F. McClare, Analyt. Biochem. *39*, 527 (1970).
11 C.C. HsuChen and D.S. Feingold, Nature, Lond. *251*, 656 (1974).
12 C.C. HsuChen and D.S. Feingold, Biochem. biophys. Res. Commun. *51*, 972 (1973).
13 S.M. Hammond and B.N. Kliger, Antimicrob. Agents Chemother. *9*, 561 (1976).

Encapsulation of *Psorospermium haeckeli* by the haemocytes of *Astacus leptodactylus*

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Summary. Psorospermium haeckeli, a parasite of freshwater crayfish, has sometimes been observed encapsulated within the tissues of *Astacus leptodactylus*. This observation does not agree with earlier reports. The lack of tolerance is discussed in relation to variations affecting either the parasite or its host.

In arthropod species, penetration into the internal medium by either living or inert extraneous bodies generally induces a defense reaction of the host with an encapsulation of these foreign 'organisms'. A progressive accumulation of blood cells tightly grouped around the organism is involved; this is a common defence reaction in invertebrates[1,2]. Such encapsulation effectively prevents the development of parasites such as trematodes, cestodes, nematodes, Hymenoptera and tachinids, as well as pathogenic fungi[3,4]. However, a parasite which is sometimes present in large numbers within the tissues of different crayfish species has always been found unencapsulated, and seems to be tolerated by its host. It was first described by Haeckel[5], and then defined as *Psorospermium haeckeli* by Hilgendorf[6]. This parasite has been observed in several *Astacus* species[5,8-11] and in *Cambarus affinis*[12-14]. It is poorly known and its taxonomic status has not been clearly determined, although it is in fact considered to be a sporozoan protozoon. It invades various tissues[10,11] but, until now, no evident cellular reaction of the crayfish has been reported and the direct damage to the crayfish tissues is unknown[15]. Since 1976 we have periodically found an analogous parasite within the tissues of some groups of *Astacus leptodactylus*, but it sometimes appears to be encapsulated. It has been identified, with the assistance of M. Vey, as belonging to the species *Psorospermium haeckeli*. However it is able to induce an encapsulation reaction by the host. Thus, it seems that the reaction of the crayfish may show different modalities after aggression by this pathogen.

Materials and methods. Adult crayfish *Astacus leptodactylus* originating from Central Europe were obtained from local fish merchants. 25 to 30 groups of crayfish, each with 20-30 animals, were all examined; a total of over 500 subjects. Pieces of several tissues, as well as hemolymph withdrawn on anticoagulant (10% aqueous sodium citrate = 1:9), were collected and examined through an optical microscope to look for parasites. Histochemical tests with PAS hematin were performed after fixing in formalin.

Results. Every year the parasites were found only in certain groups of crayfish, mainly during the spring and sometimes early in the autumn. The rate of infestation of each type and each animal was not precisely recorded since the presence of this parasite was a common feature. All these groups of crayfish were infected and in 2 of them, cellular reactions of encapsulation were observed. Both encapsulated or unencapsulated parasites invaded the same tissues: they were located at the base of the antenna and the antennula, within the ventral sinus, the sub-epidermal tissues, and in fact everywhere within the adipocyte rich organs. The vitality of the crayfish, as well as their outer appearance, seemed to be unchanged. Parasites with 2 different shapes were observed; the majority were ovoid but a few were round. The organisms had a very thick outer shell, which contained spherical bodies of different sizes

comparable to spores. Histochemical tests showed that the capsule substance consisted of polysaccharides, whatever the shape was. Only the ovoid parasites were sometimes found encapsulated (figure 1) but often they were merely inserted within the tissues (figure 2). The encapsulation rate does not seem to be related either to the number of parasites or to the delay of infestation. The encapsulated organisms were surrounded by a thin cap of hemocytes, which was built of one to several cellular layers. Although granulocytes are the most abundant cellular type in the crayfish hemolymph, mainly in premolt stages (without discussing their primitive circulating morphological aspect), it was noted that the accumulated amebocytes appeared to be semi-granular after they had encapsulated the parasite. On the other hand, no melanization was observed within the cellular mass.

Discussion. Psorospermium haeckeli was often present in the specimens of *Astacus leptodactylus* that we studied. However, in contrast to earlier reports, especially data of Unestam[15], who showed that this parasite does not induce a recognizable cellular reaction in *Astacus astacus* and is rarely melanized, it seems that this agent can be both free in the tissues or encapsulated by amebocytes. Thus, the common defence reaction of encapsulation, wide-spread among the invertebrates, appears in *Astacus leptodactylus* and the phenomenon of tolerance towards this parasite reported up to now appears to be abolished sometimes. These encapsulations have been found only within certain groups of the crayfish, mainly during spring.

The adipose tissue, which appears to be the preferential location for parasite accumulation, is mainly expanded in premolt stage animals. Nevertheless, it is not possible to ascertain whether this reaction is dependent on the molting cycle of the crayfish, since it also occurs in the autumn. Vey[11], working on strongly parasitized animals, has suggested that this agent might disturb the molt of crayfish and induce its death by damaging the integument. Moreover, external morphological diseases in the integument have

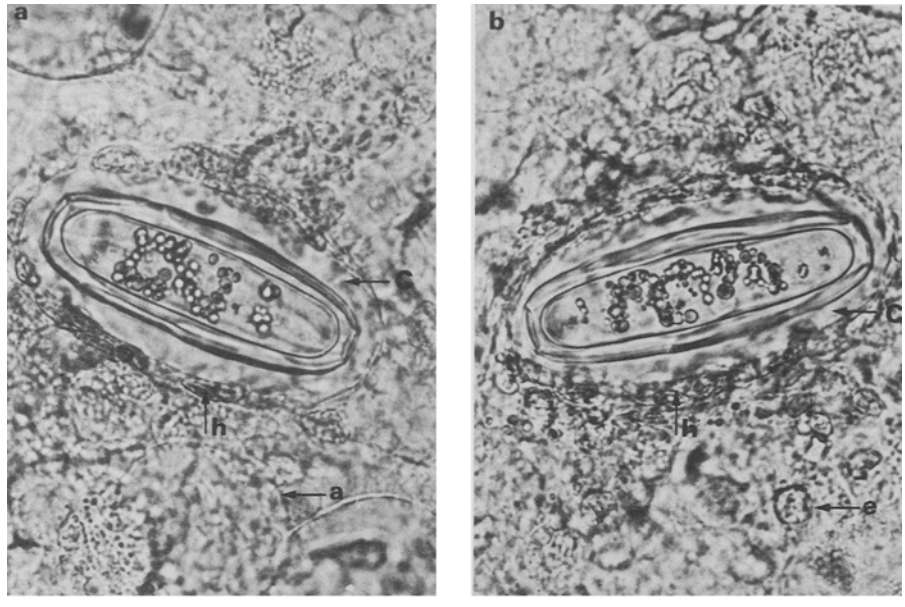

Fig. 1. *Psorospermium haeckeli* encapsulated by the hemocytes within the tissues of *Astacus leptodactylus*. *a* early encapsulation of parasite; *b* encapsulated parasite. C, glucidic shell; e, epithelial cell; a, adipocyte; h, hemocyte.

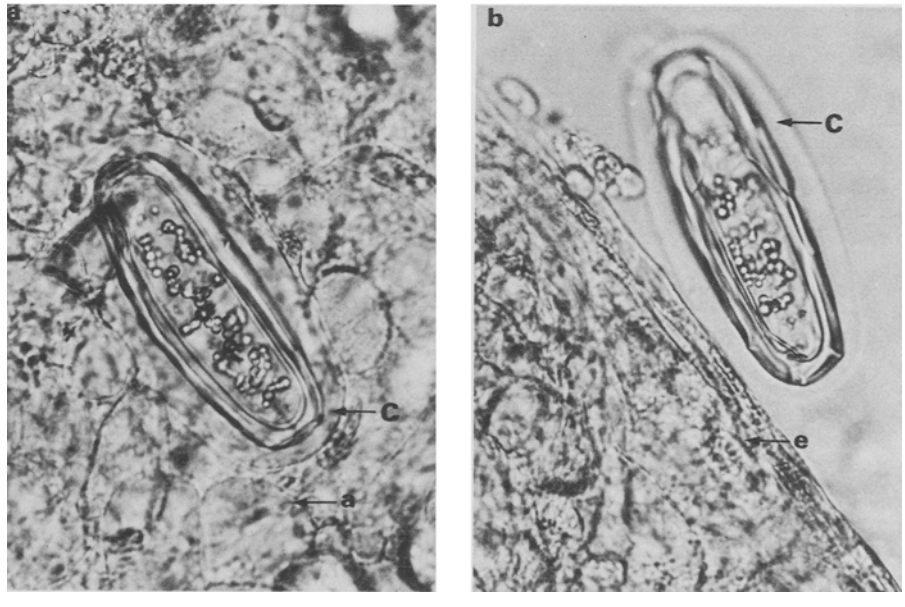

Fig. 2. Free *Psorospermium haeckeli* within the tissues of the same group of crayfish. *a* parasite within the adipocyte-type tissues; *b* free parasite outside the tissues. Symbols are the same as in figure 1.

been noted by him in contrast to the observations of the authors and Nylund and Westman[10]. These different data might be explained by the more or less advanced stage of infestation which can induce the death of the crayfish. Nevertheless, the encapsulating reaction does not seem to be linked to the stage of infestation since in the same group of crayfish, and for a given number of parasites, we have always observed both free organisms and others surrounded by amebocytes, while no encapsulation has been noted when a group of animals was more abundantly parasitized. Thus, these crayfish would have an activating capacity, to identify the shell of *Psorospermium* as "not self", and would be able to perform encapsulating reactions. Only some groups of *Astacus* showed these cellular reactions. It seems that the encapsulation could depend either on genetic variations between populations of different geographical origin, or on changes at the parasite level. These different reactions in *Astacus leptodactylus* are indeed very noticeable since the identification of parasites as "self" or "not self" by the cells of their hosts is probably connected with the biochemical structure of the shell and is probably concerned with a change in polysaccharides which take part in cellular immunological reactions. This might also explain the reaction of "not self" against the extraneous bodies by the hemocytes of invertebrates. Indeed, *Psorospermium haeckeli* could exist under 3 forms: the 1st free and round, the 2nd, which is the most common one, free and ovoid, and 3rd, an encapsulated one which might derive from the 2nd as a result of variations in the chemical components of the shell.

1 G. Salt, in: The cellular defence reactions of insects. Cambridge University Press, Cambridge 1970.
2 R.F. Whitcomb, M. Shapiro and R.R. Granados, in: The physiology of Insecta, vol. 5, p. 447. Ed. M. Rockstein. Academic Press, New York 1974.
3 A. Vey and C. Vago, Annls Inst. Pasteur, Paris *121*, 527 (1971).
4 T. Unestam and J.E. Nylund, J. invert. Path. *19*, 94 (1972).
5 E. Haeckel, Archs Anat. Physiol. Med. *24*, 561 (1857).
6 F. Hilgendorf, S.B. Ges. Naturf. Fr. Berlin *9*, 179 (1883).
7 O. Ljungberg and L. Monne, Bull. Off. int. Epizoot. *69*, 1231 (1968).
8 T. Unestam, in: First international symposium on freshwater crayfish 1973, p. 135. Ed. S. Abrahamsson.
9 V. Nylund and K. Westman, Fisk-Tidskr. Finl. *7*, 162 (1977).
10 V. Nylund and K. Westman, in: Fourth international symposium on freshwater crayfish 1978, p. 385. Ed. P.J. Laurent.
11 A. Vey, in: Fourth international symposium on freshwater crayfish 1978, p. 411. Ed. P.J. Laurent.
12 A. Wierzejski, Zool. Anz. *11*, 230 (1888).
13 E. Grabda, Mém. Acad. pol. Sci. Série B *6*, 123 (1934).
14 J. Krucinska and E. Simon, Przegl. zool. *12*, 288 (1968).
15 T. Unestam, in: Second international symposium on freshwater crayfish 1975, p. 327. Ed. J.W. Avault Jr.

Activity of the ciliary ampules through successive ages of the ciliate *Euplotes crassus*[1]

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Summary. In *Euplotes crassus*, activity of the ciliary ampules is complete only in sexually mature cells. During immaturity, no change of activity occurs in cells stimulated to mate, though variations can take place during the cell cycle. At maturity, ampules become active during both vegetative and sexual reproduction.

In *Euplotes*, ciliary ampules (As) are organelles, probably secretory in nature[2], associated with the dorsal bristles and the ventral ciliary organelles[3-8].

We investigated the proportion of full or 'ripe' As (RAs) in order to appraise the degree of activity of the As system at any given moment of the cell's life. On the basis of the finding that variations in the activity occur when cells of a given mating type interact with cells of a compatible mating type, we suggested that As can play an important role in *Euplotes* interaction[7].

To test this hypothesis further we have examined the As activity in cells at different stages of their life cycle, and the variations of activity in immature and in mature cells were compared.

Materials and methods. Singlets and morphological mutant doublets have been used in order to discriminate between mixed sexually compatible cells. The singlet and doublet strains chosen were known to be homozygous for different *mt* genes, those in the former being dominant over those correspondingly carried in the latter. To be aware of this *mt* locus constitution of the 2 strains allowed us to know in advance[10] that any offspring derived by crossing singlets with doublets would have inherited the mating type of the singlet parent, that is the mating type complementary to that of the doublets.

Cells were grown in Erd-Schreiber sea water medium inoculated with *Dunaliella salina* at 22-23 °C, on a cycle of 12 h of light and 12 h of darkness.

Cells were prepared for electron microscopic observation and the percentage of RAs counted on 5 cells randomly chosen from a group of 10 cells as previously reported[7].

Results. Experiment 1. In figure 1 are reported the percentages of RAs in cells of a clone which was analyzed at

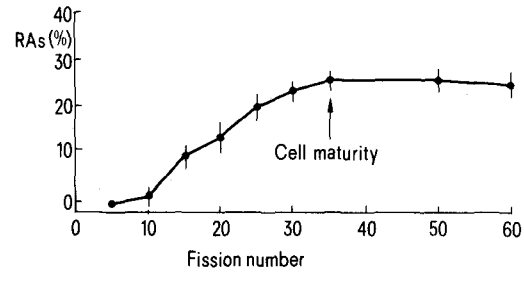

Fig. 1. Progressive increase of the proportion of RAs in cells passing from immaturity to maturity. Cell age is in number of fissions from conjugation.