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Rigidity and resistance of larval- and adult schistosomes-medium interface

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ABSTRACT

Schistosomiasis is second only to malaria in prevalence and severity, and is still a major health problem in many tropical countries worldwide with about 200–300 million cases and with more than 800 million people at risk of infection. Based on these data, the World Health Organization recommends fostering research efforts for understanding at any level the mechanisms of the infection and then decreasing the social and economical impact of schistosomiasis. A key role is played by the parasite apical lipid membrane, which is entirely impervious to the surrounding elements of the immune system. We have previously demonstrated that the interaction between schistosomes and surrounding medium is governed by a parasite surface membrane sphingomyelin-based hydrogen barrier. In the present article, the elastic contribution to the total motion as a function of the exchanged wave-vector Q and the mean square displacement values for *Schistosoma mansoni* larvae and worms and *Schistosoma haematobium* worms have been evaluated by quasi elastic neutron scattering (QENS). The results point out that *S. mansoni* larvae show a smaller mean square displacement in comparison to *S. mansoni* and *S. haematobium* worms. These values increased by repeating the measurements after one day. These differences, which are analogous to those observed for the diffusion coefficient we previously evaluated, are interpreted in terms of rigidity of the parasite-medium interaction. *S. mansoni* larvae are the most rigid systems, while *S. haematobium* worms are the most flexible. In addition, temperature and hypoxia induce a weakening of the schistosome-medium interaction. These evidences are related to the strength of the hydrogen-bonded interaction between parasites and environment that we previously determined. We have shown that *S. mansoni* worms are characterized by a weakened interaction in respect to the larvae, while the *S. haematobium* worms more weakly interact with the surrounding medium than *S. mansoni*. The present QENS analysis allowed us to characterize the rigidity of larval- and adult *S. mansoni* and *S. haematobium*-host interface and to relate it to the parasite resistance to the hostile elements of the surrounding medium and to the immune effectors attack.

1. Introduction

Schistosomiasis is a severe parasitic disease endemic in 78 countries of the developing world, with most serious impact on children residing in rural areas. It is caused by several species of the genus *Schistosoma*, namely *Schistosoma mansoni* and *Schistosoma haematobium* [1,2]. Cercariae shed by infected snails invade host skin, and remain in the epidermis for up to 72 h exchanging the classical trilaminate membrane for an outer double lipid bilayer. This heptalaminate membrane surrounds the parasites, which migrate, develop, mature, copulate, lay eggs and reside exclusively in the mammalian host blood capillaries [3,4]. The outer membrane protects the developing larvae and adult worms from the surrounding hostile immune elements, while allowing entry of nutrients (small molecules such as glucose, amino acids and cholesterol which do not exceed 400 Da) and exit of wastes [5–9]. Indeed, the apical membrane molecules of larvae older than 3–24 h, lung-stage schistosomula and adult worms do not bind antibodies (large molecules exceeding 150,000 Da), while are accessible to the different nutrients [5–8] which interact with their respective binding-proteins or protein transporters. We have hypothesized that sphingomyelin (SM) molecules in the outer lipid bilayer is the main factor responsible for these protective and sieving properties, via formation of a tight hydrogen bond barrier

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with the surrounding water [10–12]. The existence of a SM-based hydrogen barrier around lung-stage schistosomula and adult schistosomes was recently documented using quasi elastic neutron scattering (QENS) [13]. More specifically, we have previously gathered QENS data to determine the nature and the strength of the interaction of both larval and adult parasites with the surrounding medium. The evaluation of the quasi-elastic contribution to the total motion allowed characterization of the mobility features of schistosomes using the obtained diffusion coefficient values. Of note, the outer lipid bilayer and surrounding hydrogen bond network at the host parasite interface was shown to be easily disrupted via heating, or hypoxia-mediated activation of a parasite tegument-associated neutral sphingomyelinase (nSMase) [10–14].

Continuing our previous work, we herein aimed to examine the different characteristics of the formidable interfacial barrier between the host medium and the parasite, via focusing on the correlation between the flexibility and the resistance of the parasites under different external conditions. Since we needed to define these fundamental properties on a molecular level, the larval and adult *S. mansoni* and adult *S. haematobium* are investigated in the same experimental conditions as previously. QENS data were generated in order to use the evaluation of the elastically scattered intensity and the mean square displacement as a function of exchanged vector and temperature for assessing the rigidity of the apical lipid bilayer SM-based hydrogen bond network surrounding larval and adult *S. mansoni* and adult *S. haematobium*. Incoherent neutron scattering was employed to investigate such living systems, since it has already been successfully used on other complex biological samples, such as *Escherichia coli*, *Artemia* cysts, mammalian cells, extremophile bacteria [15–18], where the very large presence of hydrogen atoms distributed nearly homogeneously within these structures predominantly contributes to the scattered signal and gives access to detailed information about atomic motions on the picosecond-nanosecond time domain. This represents an important advantage since it is possible to study the dynamics of hydrogenated molecules also in samples which are not crystalline neither monodisperse, such as living cells. In addition, neutron scattering studies have unambiguously demonstrated that biomolecular motions are affected by environmental conditions where biological systems are immersed [15–23].

The methodological approach used in the data analysis is based on the determination of the elastically scattered intensity on the exchanged vector Q and then on a model proposed by Zaccai and co-workers [16,17,19] to evaluate the "pseudo-force constant", which is related to the "resilience" of biological systems, i.e. the capability to maintain the stability as a response to a thermal stress, by the mean square displacement behavior. Zaccai and co-workers [16,17,19] used this model to associate the biomolecular dynamics to the thermo-adaptation in many systems and in particular on extremophile biomolecules, demonstrating that proteins extracted from thermophile organisms are characterized by a higher rigidity and a lower flexibility than those extracted from psichrophile organisms that possess a quite high flexibility. These features allow these organisms to maintain a perfect balance between the flexibility fundamental to exert their functions and activities and the structural resistance to the thermal stress [15,17,19].

In line with these studies, here we compare the rigidity of larval and adult *S. mansoni* and adult *S. haematobium* in order to relate it to their adaptive strategies activated as a response to different external stress, i.e. hypoxia and temperature. We emphasise that the different flexibility degree of the parasites is directly related to their different mobility as characterized in our previous work [13]. In other terms, the previous determination of the nature of the parasite-medium barrier, i.e. the role played by the hydrogen bond network in this interaction, provides a clear explanation to the present findings dealing with the rigidity and then with resistance of parasites against immune system attack and harsh external conditions, such as thermal stress and hypoxia.

2. Materials and methods

2.1. Parasites

Male Syrian hamsters and cercariae of *S. mansoni* and *S. haematobium* were obtained from the Schistosome Biological Materials Supply Program, Theodore Bilharz Research Institute, Giza, Egypt. Hamster Infection and perfusion were performed under anaesthesia, following the recommendations of the current edition of the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, U.S.A. Lung-stage schistosomula were recovered six days after hamster percutaneous infection with 2000 cercariae of *S. mansoni* as described [13], and then suspended in RPMI medium containing 20% fetal calf serum (FCS), placed in sterile polypropylene tubes (Costar), and transported to Grenoble, France, within 18 h. Adult worms were obtained from hamsters 6 and 13 weeks after exposure to 200 cercariae of *S. mansoni* and *S. haematobium*, respectively. Worms were washed in RPMI medium, resuspended in RPMI medium/20% FCS, incubated for 6 h at 37 °C/3% CO_2, placed in sterile, vented, 25 ml tissue culture vessels (Costar) filled to the rim with medium/20% FCS, and reached Grenoble, France, within 18 h. Intact and hypoxia-exposed larvae were examined by indirect membrane immunofluorescence using radiation vaccine serum as described [10,11].

2.2. Experimental

The IN4 spectrometer at the Institute Laue Langevin (ILL, Grenoble, France) was used to carry out QENS measurements as a function of energy, exchanged wave-vector Q and temperature. The instrumental configuration was: an incident wavelength of 2.96 Å, an energy resolution of 450 μeV, a sample holder orientation of 135° relative to the incident beam. Measurements have been performed on *S. mansoni* larvae and adult worms and *S. haematobium* worms at a temperature value of 300 K, by repeating after 24 h the measurements on *S. mansoni* larvae and adult worms. In addition *S. mansoni* larvae have been also investigated at a temperature value of 320 K.

The usual corrections, such as empty cell subtraction and vanadium normalization as well as the multiple scattering and multiphonon contribution evaluations, were performed before the data analysis by using the Lamp software.

3. Results and discussion

3.1. Analysis of the elastically scattered intensity and the mean square displacement

The behavior of the elastic contribution to the total motion of lung-stage *S. mansoni* larvae and *S. mansoni* and *S. haematobium* worms has been characterized as a function of Q in the 0.6–3.4 $Å^{-1}$ range at the temperature value of $T = 300$ K, as shown in Fig. 1. By observing Fig. 1, a Gaussian contribution that is related to the vibrational motions, and a non-Gaussian contribution, which is linked to the diffusive motions, can be considered [24–26]. This latter contribution provides a measure of the dynamic heterogeneity degree of the investigated systems and furnishes information about their rigidity and flexibility. In order to point out these spectral features, in the insert of Fig. 1 we consider the logarithm of the elastic contribution to the total motion as a function of Q^2.

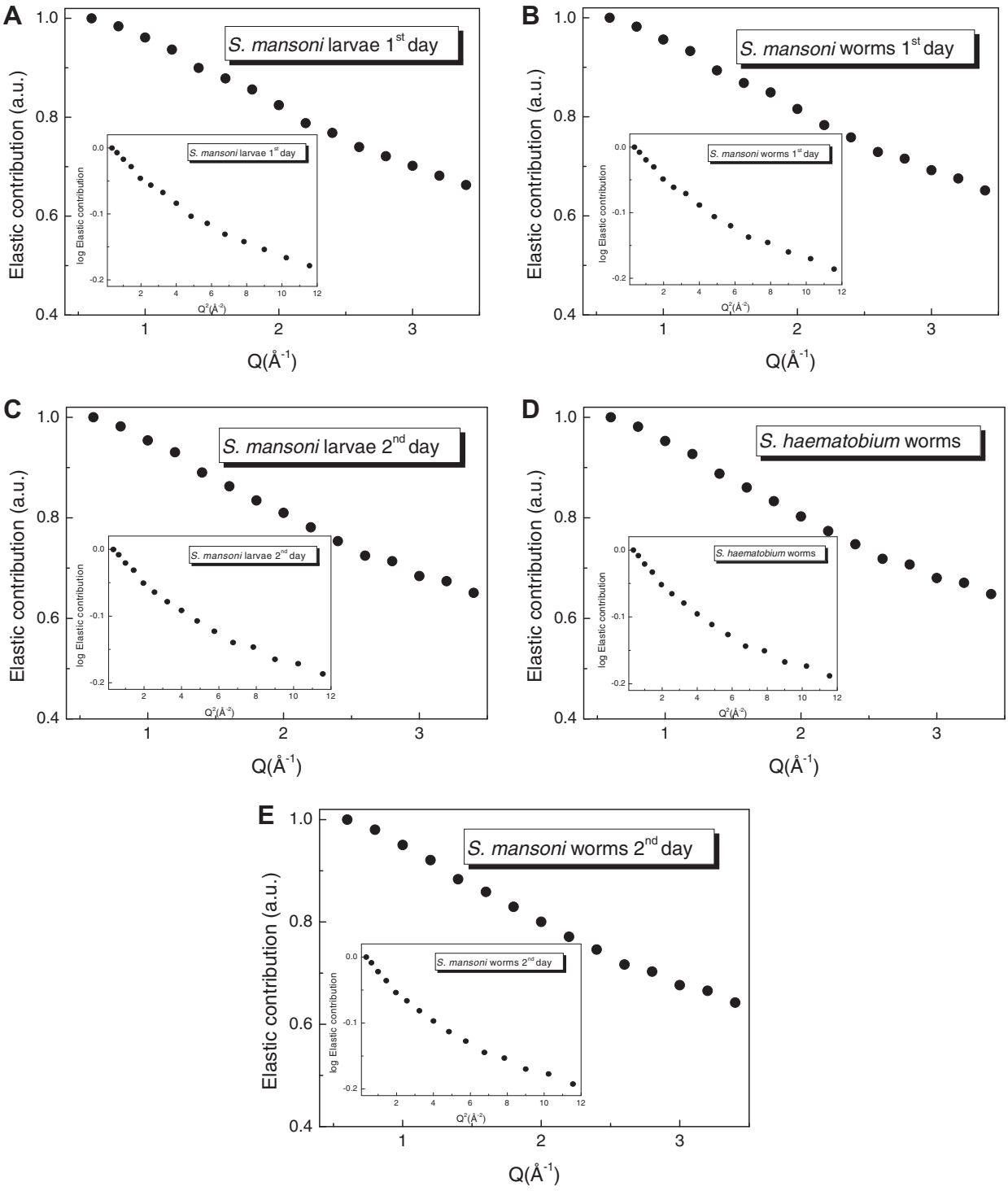

Fig. 1. Elastic contribution to the total motion of (A) *S. mansoni* larvae on the first day of measurements, (B) *S. mansoni* worms on the first day of measurements, (C) *S. mansoni* larvae on the second day of measurements, (D) *S. haematobium* worms, (E) *S. mansoni* worms on the second day of measurements as a function of Q in the 0.6–3.4 Å^{-1} range at T = 300 K. In the insert, the corresponding logarithm of the elastic contribution to the total motion as a function of Q^2 at T = 300 K is shown.

It is evident from Fig. 1 that the general trend of the elastic profiles is quite similar for all the investigated systems. More specifically, from the insert of Fig. 1 it is clear that the elastic intensity depends linearly on Q^2 up to the Q value of about 2.00 Å^{-1}, corresponding to a distance of 3.14 Å, where a deviation from the linear behavior is evident. This transition can be related to an increased flexibility of the system.

However, some small differences can be highlighted among larvae and worms and both qualitatively and quantitatively analysed. On this purpose, we derive the mean square displacement values by the slope of the linear trend observed in the small Q region: by a formal point of view, in the Gaussian approximation, the orientational average of the elastic contribution can be performed for small Q values in order to eliminate angular coordinates yielding [16,23]:

$$I(Q,t) \cong \sum_{\alpha=1}^{N} x_\alpha \exp\left[-\frac{Q^2}{6}\langle[r_\alpha(t) - r_\alpha(0)]^2\rangle\right] \quad (1)$$

This allows calculating the mean square displacement $\langle u^2 \rangle$ by the relation:

$$\langle u^2 \rangle = -6 \frac{d\left\{ \ln \left[S^{el}_{Inc.}(Q) \right] \right\}}{dQ^2} |_{Q=0} \quad (2)$$

The mean square displacement values of lung-stage *S. mansoni* larvae and *S. mansoni* and *S. haematobium* worms at $T = 300$ K are reported in Fig. 2. Since systems with larger values are characterized by a high level of fluctuations [23], by the comparison of the values of the mean square displacement we can conclude that the flexibility of these systems is increased. Of note, the *S. mansoni* larvae show the smallest mean square displacement whose value is increased after one day; analogously, the distance characterising the *S. mansoni* worms increased with time; finally, *S. haematobium* worms present the largest mean square displacement value.

3.2. Molecular mechanisms of the parasite-medium interaction

Since all the systems are investigated at the same temperature value, in the same surrounding medium and in the same experimental conditions, these differences can be explained just referring to the peculiar features of the specific larva-medium and worm-medium system interaction. In other terms, a small mean square displacement value implies a high rigidity of the larva-medium and worm-medium system since it is raised by an intense interaction between the larva and the medium as well as between the worm and the medium. A self-consistent explanation to these rigidity properties is provided by our previous findings [13] concerning the nature and the strength of these interactions; as a confirmation we can observe that an analogous trend is observed in the diffusion coefficient values obtained in our previous work [13] and in the mean square displacement values obtained in the present one for the different parasites. More in detail, the *S. mansoni* larvae present the smallest values of diffusion coefficient and mean square displacement; whereas for the *S. mansoni* worms in the second day of measurements the largest values are found. In addition, all the investigated systems under different conditions maintain the same trend of both these quantities. We can conclude that the hydrogen-bonding governed interaction between parasites and surrounding environment as characterized in our previous [13] and present works is strictly related to the global behavior of schistosomes as a response of different external stimuli.

Specifically, highly rigid interaction between larvae and surrounding medium, likely mediated by SM-hydrogen bond network [13], is necessary for the parasite survival as lung-stage schistosomula are extremely vulnerable to host immune attack while migrating in the convoluted and thin-walled capillaries of the lung [27]. The low rigidity of *S. haematobium*-medium interaction may explain their higher *in vitro* susceptibility than *S. mansoni* to unsaturated fatty acids-mediated nSMase activation, that leads to SM hydrolysis and collapse of the SM-based protective hydrogen bond network [10,11], and provide a clue for why these worms escape the unsaturated fatty acids-rich portal and mesenteric capillaries to the venous plexus around the urinary bladder [28].

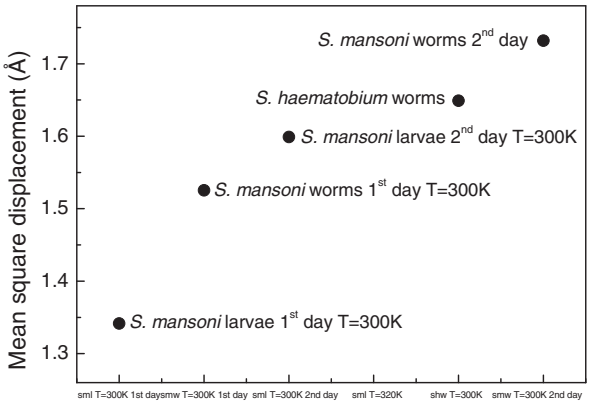

Fig. 2. Comparison among the mean square displacement values of lung-stage *S. mansoni* larvae and *S. mansoni* and *S. haematobium* worms at $T = 300$ K.

3.3. Effect of temperature on S. mansoni larvae

In order to complete the analysis of the elastic contribution to the total motion of the *S. mansoni* larvae, measurements were also performed at two different temperature values, i.e. $T = 300$ K and $T = 320$ K. In Fig. 3A the comparison between the elastic intensity as a function of Q of *S. mansoni* larvae at $T = 300$ K and $T = 320$ K is reported, while in the insert of Fig. 3A the logarithm of the elastic contribution to the total motion as a function of Q^2 is shown. Heating disrupts the hydrogen bond network around schistosomes, and, as expected, the decrease of the intensity as well as the deviation by the Gaussian shape are more marked for the *S. mansoni* larvae at higher temperature.

It is evident that the mean square displacement (Fig. 3b) has a higher value for the *S. mansoni* larvae at $T = 320$ K than at 300 K since temperature increases the fluidity and then the fluctuations

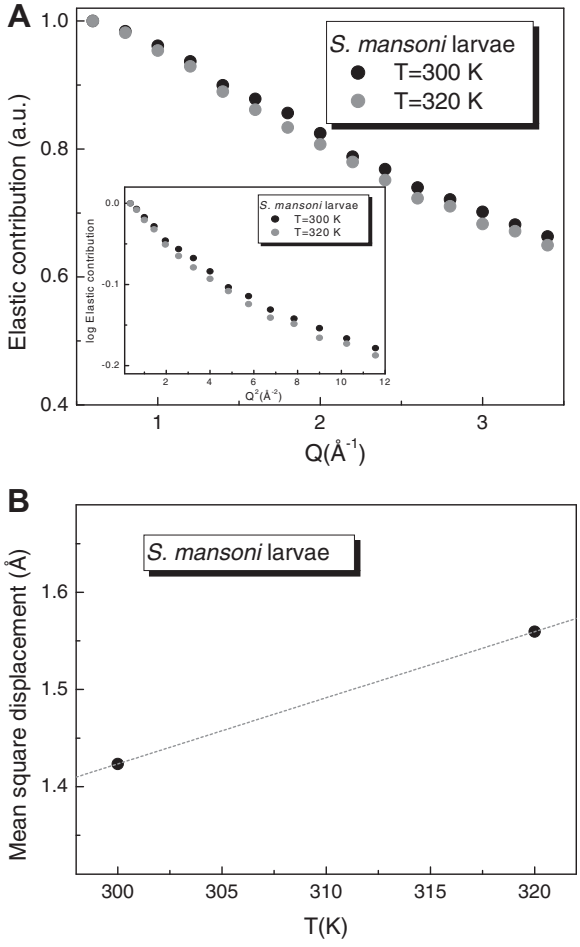

Fig. 3. (A) Comparison between the elastic intensity as a function of Q of *S. mansoni* larvae at $T = 300$ K and $T = 320$ K. In the insert the corresponding logarithm of the elastic contribution to the total motion as a function of Q^2 is shown. (B) Temperature behavior of the mean square displacement of *S. mansoni* larvae.

of the system. However, the dependence of the mean square displacement on temperature provides useful information about the rigidity of larvae in terms of the pseudo-force constant <*k*>, which is calculated according to $< k >= 0.00138/d\langle u^2\rangle/dT$ [17]. This parameter allows to quantitatively evaluate the "mean resilience", which is related to the stiffness degree of the larvae, for which we get the value of <*k*>=0.2 N/m. This value is comparable to those obtained for other biological systems investigated *in vivo* and analysed by using the present model [17] and reveals a relative flexibility which allows to *S. mansoni* larvae to better adapt themselves to the environment changes induced by the temperature increase.

3.4. Biological implications

In conclusion, the analysis of the elastic contribution to the total motion provides new findings about the rigidity degree and the extent of the fluctuations at the *S. mansoni* larvae and *S. mansoni* and *S. haematobium* worms-medium interface. It should be observed that the present results are in good agreement with those obtained by the analysis of the quasi-elastic contribution [13] to the total motion performed on the same systems. In respect with our previous work where we characterized the mobility of schistosomes, here the different strength of the interaction between the larvae and the medium and between the worms and the medium is evaluated in terms of rigidity, typical distances and peculiar fluctuations. In this frame, the mean square displacement reveals a weakened interaction by the *S. mansoni* worms in comparison to the larvae and by the *S. haematobium* worms in comparison to the *S. mansoni* worms, in line with the strength of the hydrogen barrier formed with the medium as characterized by the analysis of the quasi-elastic contribution to the total motion [13]. Analogously, larvae and worms interact less strongly with the medium after one day since hypoxia activates the parasite tegument-associated nSMase inducing SM hydrolysis and collapse of the hydrogen barrier [11,29]. As a confirmation, by comparing seven day-old and intact *S. mansoni* larvae examined by indirect membrane immunofluorescence using radiation cercariae vaccine serum (Fig. 4), it is evident that larvae exposed overnight to hypoxic conditions allowed access of antibodies to, and hence visualization of, the surface membrane antigens, likely due to collapse of the surrounding sphingomyelin-based hydrogen bond network. This effect is similar to that observed as a consequence of a temperature increase in the *S. mansoni* larvae.

Since both mobility and flexibility are fundamental interplaying aspects in the global behavior of a living system, which is linked to the biological functions, our previous [13] and present works provide complementary information on the defence mechanisms that parasites activate as a response to the immune system attack and on hostile external conditions, such as hypoxia and thermal stress.

3.5. Future prospects

We are planning to apply our novel methodology to examine the interaction of the surface membrane of normal, cancer, and metastatic cancer cells with the surrounding medium, and investigate whether SM in the apical lipid bilayer of mammalian cells forms with the neighboring lipid molecules and surrounding water a hydrogen bond network, similarly to schistosomes. Indeed, an increasingly tight SM-based hydrogen barrier could be responsible for lack of cancer cells contact inhibition [30,31], decrease and loss of MHC and other surface membrane antigens visualization [32–35], and evasion from immune effectors recognition and destruction during metastasis [36]. This study is prompted by the numerous reports documenting the key role of SM in cancer development, metastasis, and control [37–40].

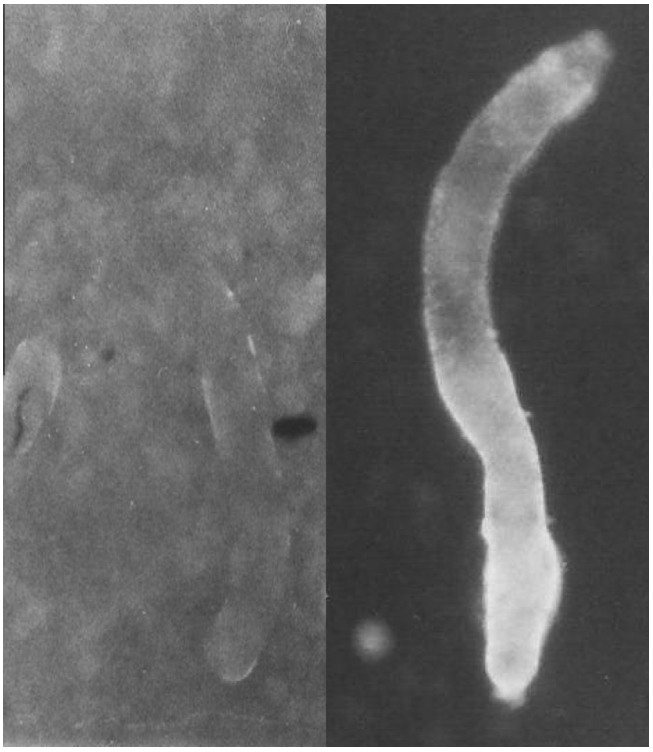

Fig. 4. Seven day-old *S. mansoni* larvae examined by indirect membrane immunofluorescence using radiation cercariae vaccine serum. On the left, intact larva is almost entirely negative, while on the right larva exposed overnight to hypoxic conditions allowed access of antibodies to, and hence visualization of, the surface membrane antigens, likely due to collapse of the surrounding sphingomyelin-based hydrogen bond network.

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