14 Hows, J.M., Palmer, S., and Gordon-Smith, E.C., in: Cyclosporin A, p.511. Ed. D.J.G. White. Elsevier Biomedical Press, Oxford 1982.
15 Donatsch, P., Abisch, E., Homberger, M., Traber, R., Trapp, M., and Voges, R., J. Immunoass. *2* (1981) 19.
16 Lemaire, M., Niederberger, W., and Nussbaumer, K., personal communication.
17 Kahan, B.D., Van Buren, C.T., Boileau, M., Ried, M., and Payne, W.D., Transplantation *35* (1983) 96.
18 Hows, J.M., Chipping, P.M., Fairhead, S., Smith, J., Baughan, A., and Gordon-Smith, E.C., Br. J. Haemat. *54* (1983) 69.
19 Bunjes, D., Hardt, C., Solbach, W., Deusch, K., Röllinghoff, M., and Wagner, H., in: Cyclosporin A, p.261. Ed. D.J.G. White. Elsevier Biomedical Press, Oxford 1982.

0014-4754/84/060559-03$1.50 + 0.20/0

Passage of hemolysins through the midgut epithelium of female *Ixodes ricinus* L. fed on rabbits infested or reinfested with ticks[1]

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Summary. Antibodies considered in this study are hemolysins synthesized by rabbits against sheep red blood cells. Ingested with the blood meal, they cross the tick midgut epithelium and retain their immunological properties in the hemolymph. During a reinfestation of rabbits, more ticks present these antibodies, and titres are generally higher than during a first infestation. Hemolysins are only found in ticks weighing 180 mg or more.

Rabbits infested with 10 female *Ixodes ricinus* L. acquire a resistance which disturbs the Ixodid biology[2,3]. During reinfestations, the ectoparasites often ingest less blood and lay fewer eggs. Tissue at tick fixation sites is infiltrated with mononuclear and polymorphonuclear cells, in which eosinophils and degranulating basophils are recognized, especially during reinfestation[4]. Some degranulating mast cells are also present. Animals develop immediate and delayed hypersensitivity reactions against tick saliva[3,5]. Specific antibodies against ♀ *I. ricinus* salivary glands have been detected in infested rabbits. They undoubtedly take part in establishment of immunity, since passive transfer of immune serum produce partial resistance against ticks[6,7].

Our work demonstrates that hemolysins, detected with a sensitive hemolysis reaction, cross the midgut epithelium of ♀ *I. ricinus*. They were identified in ticks fed on rabbits immunized against sheep red blood cells. The influence of anti-tick immunity on this phenomenon was assessed.

Materials and methods. Infestation of rabbits. 13 Himalayan male rabbits, weighing approximately 2 kg, were used in the experiment. Each rabbit was exposed to 14 ♀ *I. ricinus* fed under capsules on their backs.

Immunization of rabbits. Rabbits were immunized with i.v. injections of 1 ml of 10% sheep erythrocyte suspension per kg of rabbit weight[8]. 2 injections and infestation schemes were used. In 1 scheme, 5 rabbits were given injections on days 1, 2, 3, 4, 8, 10, 13 and 17 of the experiment and were infested with ticks on day 9. In another scheme, 5 other rabbits were first infested with ticks. The immunization process with sheep blood cells began on the day when the last tick dropped from the rabbit and then followed the same procedure as the first scheme. Reinfestation of the second group of rabbits took place on the 13th day of the immunization with sheep red blood cells. 3 control rabbits were not injected with erythrocytes suspensions but were infested and reinfested with ticks.

Collection of hemolymph. The process for collecting hemolymph was similar to that described by Burgdorfer[9]. Generally 2–6 µl (extreme values: 1–30 µl) of hemolymph were collected in capillary tubes. Hemolymph was stored at −20 °C until used.

Titration of hemolysins. Hemolysin titres of rabbit serum or of tick hemolymph were measured[10]. Briefly, 0.5 ml of a serum or hemolymph dilution, prepared in veronal buffer pH 7.4, was mixed with 0.3 ml guinea-pig complement (1/20) and sheep red blood cells 2%. To each tube, 1.7 ml veronal buffer was added. The mixture was incubated for 1 h at 37 °C under gentle stirring. After centrifugation (3000 t/min during 5 min), hemolysin titres in the supernatant were defined spectrophotometrically (524 nm). Specificity threshold of the reaction was fixed at 1/10 for tick hemolymph. Rabbits sera were tested from 1/100.

Results. Hemolysins cross the midgut epithelium of ticks. At the end of the blood-meal during the first infestation (day 0, first scheme), 16.0% of ♀ *I. ricinus* (4/25) show the presence of hemolysins in their hemolymph (table 1). 7 days after feeding, this percentage increases to 50.0% (8/16). During reinfestation

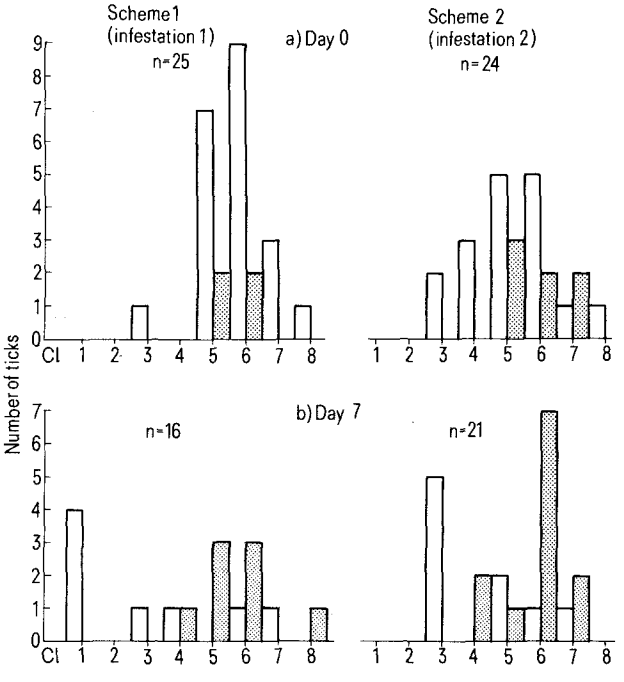

Figure 1. Relation between tick weight and hemolysins crossing. For some ticks, hemolymph was not prepared; these cases are not represented in the figure. □, ticks without hemolysin; ▨, ticks with hemolysins; Cl. 1–7: weight class of 60 mg; Cl. 8: ⩾ 420 mg.

of other animals (second scheme), more ticks present these antibodies. Immediately or 7 days after the blood-meal, 29,2% (7/24) and 57.1% (12/21) of the ticks, respectively, possess hemolysins. As a whole, during a first infestation, the hemolymph of 29.3% (12/41) of the ticks contains them. During a reinfestation, this percentage increases to 42.2% (19/45). The test used is highly specific. Hemolymph of ticks fed on control rabbits never produces hemolysis of sheep red blood cells.

Relation between the presence of hemolysins in tick hemolymph and tick weight. At the end of the blood-meal taken during the first or second infestations, hemolysins are only detected in ectoparasites weighing more than 240 mg (fig. 1a). However 7 days later, antibodies are found in the majority of the ticks weighing 180 mg or more (inf. 1: 8/11; inf. 2: 12/16).

Relation between rabbit hemolysins titres and the presence of these antibodies in tick hemolymph. The hemolysin titres of immunized rabbits vary between 1/10,000 and 1/50,000 (fig. 2). The higher number of ticks containing these antibodies during reinfestation could be due to higher titres often observed in the reinfested animals. In fact, all positive ectoparasites are fed on rabbits with titres of 1/40,000 and 1/50,000 during that infestation. Therefore, these antibodies are detected only in 1/8 ticks gorged on a rabbit with a titre of 1/50,000 during a first infestation (table 2). During reinfestation, this proportion increases significantly (15/27 ticks fed on 3 animals; $p < 0.05$).

Hemolysin titres of ticks (1/20 to 1/500) are at least 20 times smaller than the titres of rabbits (1/10,000 to 1/50,000, table 3). Numerous antibodies are probably digested by the ectoparasites. Titres detected in ixodids during a reinfestation are generally higher. In fact, during a first infestation, not more than 6/12 ticks showed a titre higher than 1/100. This proportion increased greatly during a reinfestation (17/19, $p < 0.05$).

Discussion. In this work, we report that hemolysins cross the midgut epithelium of ♀ *I. ricinus* better in ticks fed on reinfested rabbits. Passage of host serum components, including antibodies, through the digestive tract of *Dermacentor variabilis*[11] also occurs. In other arthropods e.g. *Ephestia kuniella*[12], meal proteins have been detected in midgut cells. These proteins enter the hemolymph of *Rhodnius prolixus*[13] and are also found in fat bodies and ovaries of *Hypoderma bovis*[14]. Antibodies produced artificially against brain, muscles or hemolymph of *Sarcophaga falculata* cross the intestinal epithelium of these flies and react specifically with antigens[15]. In *Glossina morsitans morsitans,* the crossing of proteins of high molecular weight can be limited by the peritrophic membrane[16], which would select molecules of about mol. wt 45,000. In fact IgG fragments (Fab fragments excluding intact immunoglobulins) have been detected in hemolymph of this insect. Therefore, specific antibodies ingested with the blood-meal are still detrimental to this arthropod[17,18]. Formation of antigen-antibody complexes (intervention of Fab fragments) and fixation of complement on the Fc fragment represent a prerequisite for the hemolysis test. Thus the *I. ricinus* hemolymph contains antibodies with classical immunological properties. The absence of extracellular digestion in ticks would facilitate the crossing of intact immunoglobulins.

Antibodies naturally synthesized against tick saliva[2,3] could also cross the midgut epithelium of ectoparasites and thus interfere with other tick tissues – e.g. ovaries[11] – disturbing physiological mechanisms. However, some immunoglobulins are fixed on the intestinal epithelium of ♀ *I. ricinus* fed on reinfested rabbits. The immunity developed against ticks also disturbs the physiology of this tissue, altering hemoglobin digestion[19].

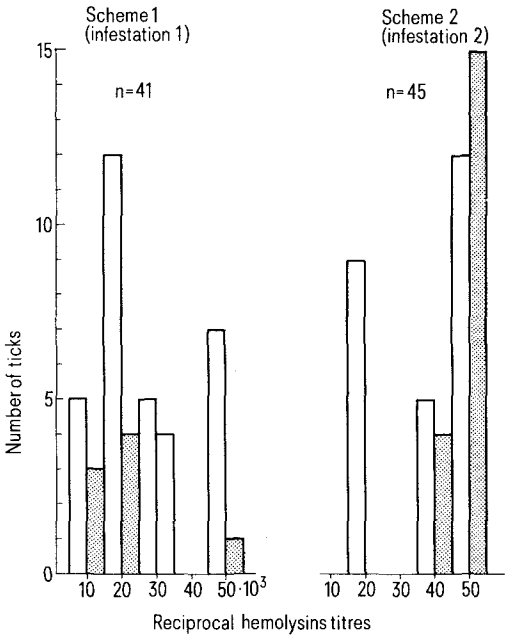

Figure 2. Relation between rabbit hemolysin titres and presence of these antibodies in tick hemolymph (day 0 and day 7); □, ticks without hemolysin; ▨, ticks with hemolysins.

Table 1. Percentages and ratios of ticks in which hemolysins crossed the midgut epithelium (No. of positive ticks/total No. of ticks)

	Scheme 1 (infestation 1)	Scheme 2 (infestation 2)	Controls
Day 0	16.0 (4/25)	29.2 (7/24)	0 (0/28)
Day 7	50.0 (8/16)	57.1 (12/21)	0 (0/18)
Total	29.3 (12/41)	42.2 (19/45)	0 (0/46)

Table 2. Ratios of ticks fed on rabbits with hemolysin titres of 1/50,000 in which hemolysins crossed the midgut epithelium (no. of positive ticks/total no. of ticks fed on rabbits with titres of 1/50,000)

	Scheme 1 (infestation 1)	Scheme 2 (infestation 2)
Day 0	0/4	6/15
Day 7	1/4	9/12
Total	1/8*	15/27

* $p < 0.05$; significance of total results only, tested with the Fisher exact probability.

Table 3. Ratios of positive ticks with titres higher than 1/100 (No. of ticks with titres greater than 1/100 to the total No. of positive ticks)

	Scheme 1 (infestation 1)	Scheme 2 (infestation 2)
Titre of hemolysins	> 1/100	> 1/100
Day 0	0/4	6/7
Day 7	6/8	11/12
Total	6/12*	17/19

Maximal hemolysin titres detected in tick hemolymph: 1/500; * $p < 0.05$; significance of total results only, tested with the Fisher exact probability test.

1 The present work was supported by the Swiss National Foundation for Scientific Research, grant No. 3070.81.

2 Bowessidjaou, J., Brossard, M., and Aeschlimann, A., Experientia *33* (1977) 528.
3 Brossard, M., Monneron, J.P., and Papatheodorou, V., Parasite Immun. *4* (1982) 355.
4 Brossard, M., and Fivaz, V., Parasitology *85* (1982) 583.
5 Brossard, M., Experientia *38* (1982) 702.
6 Brossard, M., Bull. Soc. Path. exot. *70* (1977) 289.

7 Brossard, M., and Girardin, P., Experientia *35* (1979) 1395.
8 Gervey, J.S., Cremer, N.E., and Sussdorf, D.H., in: Methods in Immunology, p.207. W.A. Benjamin Inc. London, Amsterdam, Dom Mills, Sydney, Tokyo 1977.
9 Burgdorfer, W., J. trop. Med. Hyg. *15* (1970) 1010.
10 Paris-Homelin, A., and Devillechabrolle, A., in: Travaux pratiques du C.E.S. d'Immunologie générale, p.64. Librairie Le François, Paris 1977.
11 Ackermann, S., Brian Clare, F., McGill, T.W., and Sonenshine, D.E., J. Parasit. *67* (1981) 737.
12 Smith, D.S., Compher, K., Jenners, M., Lipton, C., and Witte, L., J. Morph. *127* (1969) 41.
13 Wigglesworth, V.B., Rec. R. Soc. Ser. B *131* (1943) 313.
14 Nogge, G., Experientia *27* (1979) 524.
15 Schlein, A., Spira, D.T., and Jacobson, R.L., Ann. trop. Med. Parasit. *70* (1976) 227.
16 Nogge, G., and Giannetti, M., J. med. Ent. *16* (1979) 263.
17 Nogge, G., J. Insect Physiol. *24* (1978) 299.
18 Nogge, G., and Giannetti, M., Science *203* (1980) 1028.
19 Brossard, M., in preparation.

0014-4754/84/060561-03$1.50 + 0.20/0

Chloroplast nucleoids in a unicellular hot spring alga *Cyanidium caldarium* and related algae

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Summary. Algal chloroplast nucleoids were compared by epifluorescent microscopy. *Cyanidium caldarium* strain RK-1 or 001 has a rod-shaped chloroplast nucleoid while *Cyanidium caldarium* (*Chroococcidiopsis* sp.) strain M-8 or 002 has a circular chloroplast nucleoid along the periphery of a multilobed chloroplast.

A unicellular eukaryotic hot-spring alga *Cyanidium caldarium* has various features relating it to several phyla such as blue-green algae, green algae, red algae and so on[1]. In addition, some strains such as Allen's strain[2] and strain M-8 from a hot spring of Japan[3] are clearly different from *Cyanidium caldarium* (Tilden) Geitler[4] in cell size, endospore number, fine structure and biochemical properties. Nagashima and Fukuda[3] have proposed that strain M-8 must belong to different genus, *Chroococcidiopsis* Geitler, and also that it may be closely related to primitive Rhodophyta. However, few studies of the organelle DNA of these algae have been made. Kuroiwa et al[5] recently developed an improved method for visualization of chloroplast nucleus (nucleoid) in situ with a DNA-specific dye DAPI (4'6-diamidino-2-phenylindole) by epifluorescent microscopy. In this paper, chloroplast nucleoids of several hot-spring algae are compared by this method.

Materials and methods. Cyanidium caldarium strain RK-1 and *C. caldarium* strain M-8 (named *Chroococcidiopsis* sp. M-8 in the text) were originally isolated from Yumoto-spa, Noboribetsu-spa, Japan, respectively[3]. *C. caldarium* strain 001 and *C. caldarium* strain 002 (named *Chroococcidiopsis* sp. 002 in the text) isolated in Campi Flegrei, Italy, were kindly provided by Prof. R. Taddei and Prof. G. Pinto, Università di Napoli, Italy. RK-1 and 001 strains were cultured autotrophically by

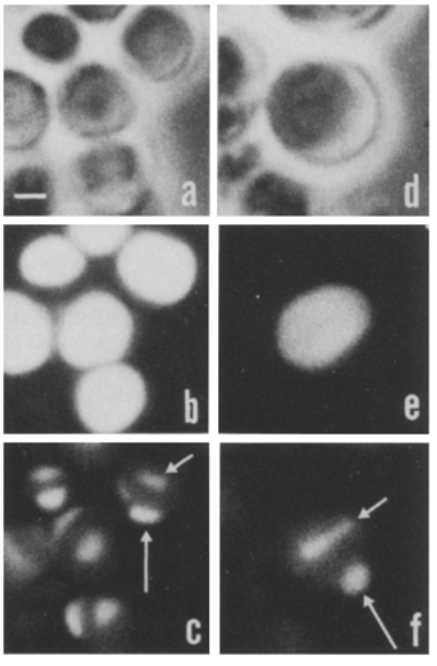

Figure 1. Phase contrast (a, d) and fluorescent micrographs (b, c, e, f) of *Cyanidium caldarium* strain RK-1 (a–c) and strain 001 (d–f) after DAPI staining. Phase contrast (a, d) and fluorescent micrographs with green (b, e) and UV (c, f) lights are taken in the same field in each strain. The ovule chloroplast can be seen in a cell excited with green light (b, e). A spherical cell nucleus (long arrows in c, f) and a rod-shaped chloroplast nucleoid in the chloroplast appears in the cell excited with UV instead of green light (short arrows in c, f). Scale bar, 1 μm.

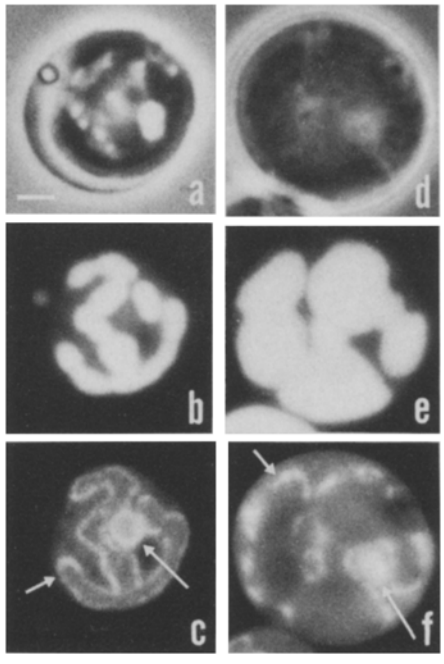

Figure 2. Phase contrast (a, d) and fluorescent micrographs (b, c, e, f) of *Chroococcidiopsis* sp. *(Cyanidium caldarium)* strain M-8 (a–c) and strain 002 (d–f) after DAPI staining. Phase contrast (a, d) and fluorescent micrographs with green (b, e) and UV (c, f) lights are taken in the same field in each strain. The multilobed chloroplast is shown with green light (b, e). A spherical cell nucleus (long arrows in c, f) and a circular chloroplast nucleoid along the periphery of the chloroplast appears with UV instead of green light (short arrows in c, f). Scale bar, 2 μm.