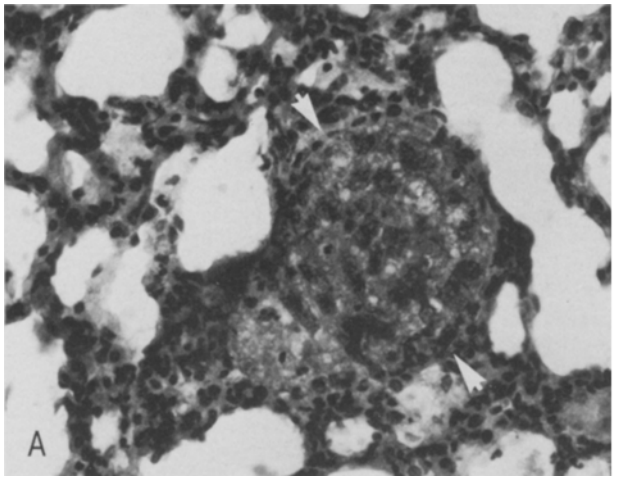
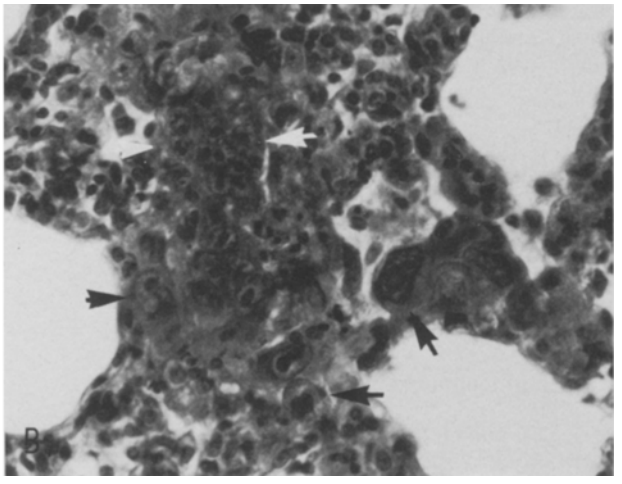


A Trophoblast nuclei in the lung from a mouse killed 4 days after injecting blastocyst into circulation. All the trophoblast cell nuclei, lying within the area indicated by arrows, show marked lysis. ×210.



B Small and giant trophoblast cells in the lung of a mouse killed 8 days after injecting blastocysts into the circulation. The giant trophoblast nuclei are indicated by black arrows and the small nuclei by white ones. ×270.

that the mucopolysaccharide of the zona acted as a focal point of concentration of the virus and that breakdown products affected the growing trophoblast adversely. The initial failure of blastocysts to produce trophoblast giant cells in the original experiments appears, therefore, to have been due to or associated with an endemic infection in the mice.

Thus, the lungs can be added to brain, spleen, kidney, liver, testis, peritoneum and eyes, as organs in which trophoblast grows from blastocysts or ectoplacental cones when they are experimentally implanted[5–7,9]. The lungs do not apparently contain a specific enzyme which protects them from trophoblast growth as has been suggested[10], at least not in mice.

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Rabbits infested with adult *Ixodes ricinus* L.: effects of mepyramine on acquired resistance[1]

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Summary. The results of this work demonstrate that histamine seems to be involved in the expression of the resistance acquired by rabbits to ♀♀ *I. ricinus.* Daily treatment of animals with the H_1-antihistaminic mepyramine inhibited the effects of immunity. This observation applies to the effect of resistance on the weight of engorged ticks, the duration of the blood meal and the success of egg laying and hatching.

In our preceding work, we demonstrated that rabbits infested by ♀♀ *I. ricinus* progressively acquire resistance during repeated infestations[2]. This immunity is manifested by an increase in the average duration of the blood meal, by inadequate feeding and by poor oviposition. The duration of pre-oviposition and embryogenesis can also be prolonged[3]. Experimentally, resistance can be partially transferred by serum from immune animals[4,5]. As described previously, the skin of the animals is rendered sensitive to antigens of tick saliva, resulting in an immediate hypersensitivity reaction[5]. Infested animals show a marked infiltration of cells at the tick attachment site, notably of mast cells and basophils[6]. We have observed that some cells degranulate during a reinfestation. This phenomenon is due, undoubtedly, to a type I hypersensitivity reaction of mast cells, and also to a reaction similar to cutaneous basophil hypersensitivity[3]. Using a degranulation test, we have shown a progressive sensitization of basophils to tick salivary antigens.

In the present study, we have tried to demonstrate the importance of liberated histamine in the biological expression of resistance. After treatment of rabbits, reinfested with ♀♀ *I. ricinus,* with the H_1-antihistaminic drug mepyramine, we compared the duration of the blood meal, weights of engorged ticks, and the success of egg laying and hatching with the results obtained from both primary infestation and reinfestation of untreated, immune animals.

Materials and methods. 12 male rabbits of a Russian race (Himalayan breed, genotype aac^Hc^H) weighing approximately 2 kg were used. They were infested twice at an interval of 3 weeks, with 10 ♀♀ and 10 ♂♂ *I. ricinus,* raised in our laboratory. 4 animals served as controls and these

Table 1. Contingency tables

	1st infestation Success	Failure		2nd infestation Success	Failure	
a) Egg laying			n_1			n_1
On control rabbits	33	4	37*	16	13	29
On treated rabbits	47	10	57	39	17	56
b) Hatching			n_2			n_2
On control rabbits	32	1	33*	10	6	16
On treated rabbits	41	6	47	31	8	39

n_1, Total No. of fed ♀♀ *I. ricinus;* n_2, total No. of fed ♀♀, which laid eggs; * $p<0.05$. Significance of results was tested with a χ^2-test.

Table 2. Effects of resistance on feeding of ♀♀ *I. ricinus*

	1st infestation	n	2nd infestation	n
a) Mean weight of fed ♀♀ (mg)				
On control rabbits	234.6± 85.9	37	97.9±86.3	29
On treated rabbits	197.7±103.1	57	152.4±89.5	56
b) Mean duration of blood meal (h)				
On control rabbits	163.9± 16.1	37	195.1±39.5	29
On treated rabbits	166.8± 26.2	57	169.0±34.9	56

n, No. of fed ♀♀ *I. ricinus.*

received a s.c. injection of 0.1 ml of water on the 1st day and all subsequent days of the 2nd infestation. The other 8 animals were injected similarly with 0.1 ml of a solution of mepyramine (Neoantergan®, Specia, 0.5 mg/kg b.wt). This dose has been shown to produce nearly maximal antihistaminic effects in rabbits (Julou, personal communication).

The effects of the treatment on the resistance of rabbits to ♀♀ *I. ricinus* were analyzed statistically. Using a χ^2-test for contingency tables, we compared:

a) the distribution of weights of ticks engorged during the 1st and/or 2nd infestation on control and/or treated rabbits. Class intervals were generally 60 mg, occasionally 120 mg; b) under the same conditions, the distribution of the duration of feeding. Class intervals were generally 1 day except for the last interval (4 days); c) the proportion of ticks laying eggs; d) the proportion of successful hatching from deposited eggs. Contingency tables about weights of ticks and duration of the blood meal can be reconstituted from figures 1 and 2.

Results. The treatment with the antihistamine drug decreased the effects of resistance on the biology of the tick. In fact, the distributions of weights of ticks engorged during a primary infestation and a reinfestation were not different (fig. 1, $\chi^2=6.08$, ndf=4). It was also true for the duration of feeding (fig. 2, $\chi^2=1.63$, ndf=4). The majority of ♀♀ *I. ricinus* laid eggs after a primary infestation (table 1a, 47/57 or 82.5%). After reinfestation of the same rabbits, the yields and percentage were modified slightly (39/56 or 69.6%) but the observed difference was not significant ($\chi^2=1.89$, ndf=1). The results with hatching were similar (table 1b). On a primary infestation, 41/47 or 87.2% of the egg batches hatched while on reinfestation the results were 31/39 or 79.5%. The differences again were non-significant ($\chi^2=0.46$, ndf=1).

In control animals the results were markedly different. The tick populations from primary infestation and reinfestation differed significantly for all the parameters considered: a) weight of engorged ticks (fig. 1, $\chi^2=23.93$, ndf=3); b) duration of blood meal (fig. 2, $\chi^2=25.26$, ndf=3); c) egg laying (table 1a, $\chi^2=8.14$, ndf=1); d) hatching (table 1b, $\chi^2=7.83$, ndf=1).

Finally, it is important to note that tick populations from primary infestations of control and treated animals did

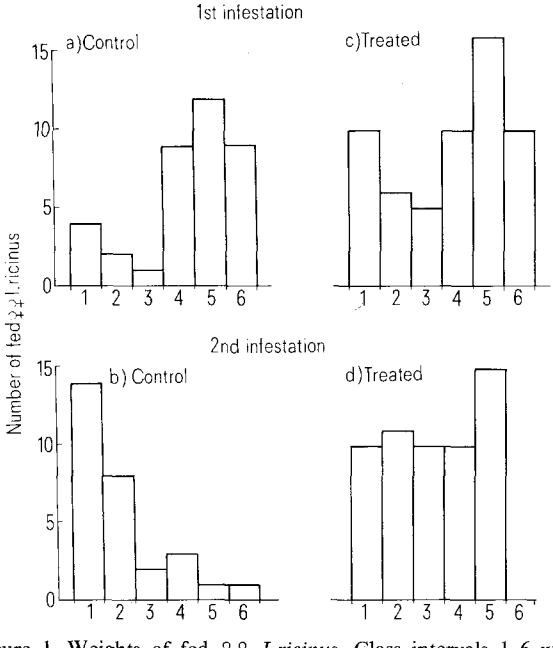

Figure 1. Weights of fed ♀♀ *I. ricinus*. Class intervals 1–6 were 60 mg. Significance of results was tested with a χ^2-test for contingency tables. a vs b and b vs d: $p<0.05$.

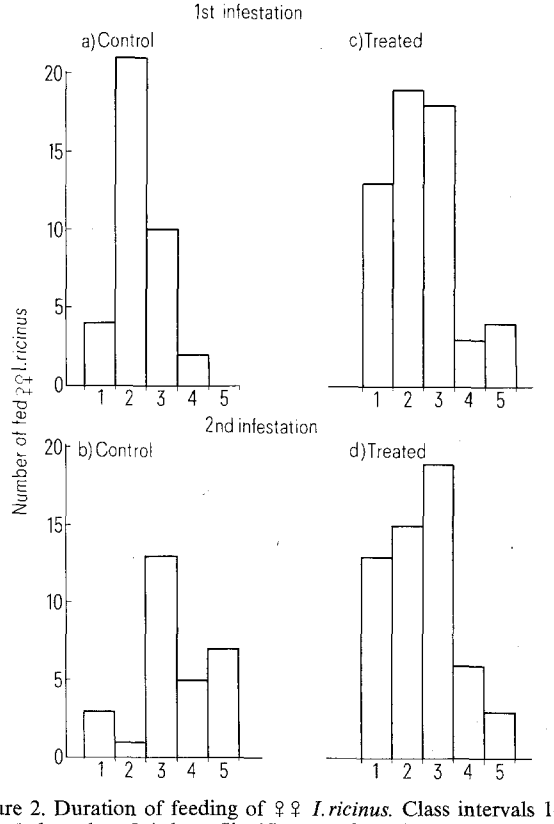

Figure 2. Duration of feeding of ♀♀ *I. ricinus*. Class intervals 1–4 were 1 day, class 5:4 days. Significance of results was tested with a χ^2-test for contingency tables. a vs b and b vs d: $p<0.05$.

not differ significantly with respect to the weight (fig. 1, $\chi^2 = 3.83$, ndf = 5) and the duration of feeding (fig. 2, $\chi^2 = 5.94$, ndf = 3). On the other hand, on reinfestation, we observed significant differences (weight of ticks: $\chi^2 = 12.91$, ndf = 4; duration of feeding, $\chi^2 = 14.17$, ndf = 4). This last result confirms the inhibition of resistance by the antihistamine treatment.

The effect of the antihistaminic is also seen on the means of the weights of engorged ticks and the duration of the blood meal (table 2). The assertion made above that the antihistaminic decreased the effects of resistance is also valid in this case. As seen in table 2 the weights of ticks engorged on rabbits in the treated group during a primary and a subsequent infestation differed less than those of ticks engorged on the control rabbits. The mean duration of feeding of ticks on the treated groups did not differ on primary infestation or reinfestation, whereas the figures were distinctly different on control animals.

Discussion and conclusions. The effects of mepyramine described in this study indicate that histamine may play an important role in the expression of the resistance acquired by rabbits to *I. ricinus.*

Histological examination of the skin of infested animals has shown degranulation of mast cells and basophils, particularly during a reinfestation[6]. Further, using a degranulation test[3], we have demonstrated progressive sensitization of circulating basophils to tick salivary antigens. Thus, during a reinfestation of rabbits by ♀♀ *I. ricinus,* one observes 2 types of local anaphylaxis; a type I hypersensitivity and a cutaneous basophil hypersensitivity. The antihistaminic blocks the action of histamine liberated as a result of these 2 reactions by binding to histamine receptors and therefore inhibiting the H_1 effects of histamine in a specific manner[7]. Consequently, mepyramine does not have an antagonistic action on all the effects of histamine. In the present study the drug would have inhibited the increase in vascular permeability resulting from the local anaphylactic reaction. Thus, the assumption can be made that both the humoral factors such as antibody and complement which are important for the expression of resistance (demonstrated in a preliminary way by the transfer of immune serum[4,5]) as well as cellular factors (eosinophils, etc.) do not appear with the same intensity as protective agents in the feeding lesion of the tick.

The importance of type I hypersensitivity in the establishment of resistance has been shown in other parasitic systems, notably in cattle parasitized by *Boophilus microplus*[8]. Histamine liberated naturally under these conditions could induce an unstable attachment of larvae, which then became more vulnerable to grooming by the host[9]. Detachment of larvae of this species has been reported to occur after the injection of histamine under the site of tick attachment[10].

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Different methotrexate effects in cultured normal and leukaemic human leukocytes[1]

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Summary. Evidence is presented that the use of 2 different culture media, with or without methionine, can help to distinguish between normal and leukaemic methotrexate (MTX)-sensitive cells derived from chronic myeloid leukaemia patients. During the blast crisis MTX assays may help directly in the diagnostics of lymphoid involvement.

First experiments performed by Gunz[3] 30 years ago showed the decrease in proliferative activity by acute leukaemic cells grown in vitro after the addition of aminopterin to the culture. As yet selective toxicity of antifolates has not been demonstrated for leukocytes from patients with chronic myelocytic leukaemia (CML). This phenomenon might have its basis in subnormal rates of methionine synthetase activity (EC 2.1.1.13) in CML cells (Bloss and Sauer[4] and Peytreman et al.[5]). Under conditions in vitro some protection against methotrexate (MTX) may be offered in the form of 5-methyl-tetrahydrofolic acid, which under normal conditions balances the intracellular tetrahydrofolate deficiency due to dihydrofolate reductase inhibition by MTX.

Material and methods. Leukaemic leukocytes obtained from the bone marrow and peripheral blood of CML patients were investigated in short-term suspension culture. Normal proliferating bone marrow cells were used as a control. The material was enriched in proliferating granulocyte precursors by the buffy coat technique and the dextran procedure[6]. Methotrexate (Lederle) was made up in physiological saline and added to 10-ml leukocyte cultures (cell density 1×10^6 per ml) to the final concentration 10^{-5} M for 48 h. The uptake of this high dose of MTX by normal and leukaemic leukocytes was expected to be similar i.e. by diffusion as shown by Kessel and al.[7] and by Hoffbrand et al.[8]. The effect of MTX was studied in 2 culture media: the 1st medium was Eagle's MEM 59 containing methionine 15 mg per l and folic acid 2.3×10 M^{-6}; the 2nd medium was Eagle's MEM 59 deprived of methionine and supplemented with homocystine (Fluka) 15 mg/l, 5-methyltetrahydrofolate 1×10 M^{-5} and vitamin B_{12} 4 mg/l. Inactivated calf serum was added to final 20% of culture medium volume. The serum was previously dialyzed against 30 vol. of normal saline with 3 changes at 4 °C for 48 h. Mitotic activity was studied by morphological examination of stained air-dried cell preparations after previous incubation with Colcemide (Ciba) 0.2 µg/ml for 2 h. Tritiated thymidine uptake into cell nuclei was measured over 2-h periods of exposure in separate cultures and examined autoradiographically. The final external concentration of ^{3}H-thymidine was 1.0 µCi in 1 ml incubation fluid.

Results and discussion. Incubation with medium 1 and medium 2 caused a markedly increased number of cells incorporating tritiated thymidine, i.e. entering the S phase,