sues, and they were not significantly different from zero. However, at the 15th postoperative day, the PVI count was increased and differed significantly from zero, but was of the same extent whichever lymphoid tissue was implanted.

The analysis of the different pretreatment of the donors demonstrated that ALS as well as adult thymectomy depressed considerably the PVI-forming capacity of the 4 lymphoid tissues tested in the experimental groups. At the 8th and 30th postoperative days, no significant difference between the two kinds of pretreatment was observed for each tissue under study. However, it must be noted that at the 15th day the Peyer's patches as well as the spleen or lymph nodes from ATx donors induced an hepatic paravascular infiltration of lesser importance than in control animals but significantly higher than after ALS treatment ($p < 0.05$). Thus, based on this criterion, it is suggested that T-lymphocytes present in the Peyer's patches have a reponse to ALS treatment and adult thymectomy closely related to that of other peripheral T-lymphocytes.

Discussion. T-lymphocytes subpopulations have been recently reviewed by Cantor and Weissman[18]. One class of them (T1) is sensitive to adult thymectomy, relatively insensitive to ALS and homes preferentially to the spleen. A second subpopulation (T2) is relatively insensitive to adult thymectomy, sensitive to ALS treatment and is found in highest concentration in lymph nodes, thoracic duct and blood. In the GVH reaction, the T1 lymphocytes are responsible for cytotoxicity whereas T2-lymphocytes act as 'amplifier' cells responsible for the cell proliferation[12]. The results reported above confirm the sensitivity of the T-cell subpopulations of the peripheral lymphoid tissues to ALS treatment and adult thymectomy. However, the relative proportion of these 2 lymphocytes subsets cannot be evaluated adequately with the experimental model used in this study. In fact, the GVH reaction observed after intrahepatic lymphoid tissue implantation is modulated by the interaction of a suppressor factor, probably the T-suppressor cell, to which the high values of PVI in non-treated F1 Hybrid recipients (group b) might be related[19,20]. The delayed increase (on the 15th day) of the PVI formation in the recipients of ATx lymph nodes spleen and Peyer's patches is in keeping with the previous finding that thymectomy in the adult life caused only a partial loss of capacity to develop suppressive activity[21]. The data reported in this study demonstrated that the T-lymphocytes of the Peyer's patches exhibited the same sensitivity to ALS or ATx treatment as those present in other peripheral lymphoid tissues. Thus it is concluded that all the known subpopulations of T-lymphocytes acting in the GVH reaction are present in Peyer's patches.

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Effects and duration of resistance acquired by rabbits on feeding and egg laying in Ixodes ricinus L.[1]

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Summary. Rabbits gradually developed a resistance against Ixodes ricinus ticks as a result of sequential experimental infestations. The resistance was characterized by an increased duration of feeding, by ticks engorging to a smaller extent and by reduced egg production. Once established, the immunity persisted for at least 9 months. Changes in the titre of circulating anti-I. ricinus antibodies was measured by indirect immunofluorescence.

Although the ecology, behaviour and epidemiology of I. ricinus have been extensively studied, the immunological reactions of the host to the bite of this tick are very poorly understood. In this work, the development, effects and duration of resistance acquired by the rabbit on feeding, egg production and hence fecundity are described. The occurrence of specific anti-I. ricinus saliva antibodies was also examined.

Materials and methods. To study the development of acquired resistance, 5 male rabbits (Himalayan breed, genotype aac^Hc^H), weighing 2 kg, were infested 4 times, each with 10 ♀♀ I. ricinus on days 0, 21, 49 and 84 of the experiment. The duration of immunity was investigated by re-infesting 2 of these animals 56 days after the 4th infestation, 2 others 183 days and the last rabbit 269 days after. The ♀♀ ticks, captured in the field on a 'blanket', were held at high humidity (RH > 90%) and allowed to engorge along with ♂♂. It is known that fertilization is necessary for the final phase of feeding in ♀♀[2]. In order to avoid local cutaneous lesions impeding attachment, the ears were infested in turn, sometimes also the animals backs were used.

The ticks were protected by a nylon sheath, fixed at its extremities with an adhesive band; a collar prevented the rabbit from scratching. At the end of feeding, the ♀♀ I ricinus. (the male does not feed) detached from the host and were collected by opening the sheath. They were weighed and placed in a humid environment (RH > 90%) for egg laying and hatching. The presence of circulating, specific anti-I. ricinus saliva antibodies were examined by indirect immunofluorescence, according to the techniques described elsewhere[3]. Salivary glands of ♀♀ ixodids, taken on the 4th day of feeding, were used as antigen. At this stage of engorgement of the ticks, the salivary glands contain a large number of antigenic acini. The threshold of specificity of the reaction was fixed experimentally at $^1/_{20}$.

Results. 1. Effects of acquired resistance in rabbits on the nutrition of ♀♀ I. ricinus. Preliminary note. During the 4 experimental infestations, all the ♀♀ I. ricinus attached; also most fed. However, on the 4th infestation 36.0% died on the host without engorging. In the text, the weight of fasting ♀♀ I. ricinus (which the mean weight is

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Table 1. Effect of resistance on feeding in ♀♀ I. ricinus

	Infestation number 1	2	3	4
Mean weight of fed ♀♀ (mg)	254.8 ± 75.8	175.7* ± 114.1	183.0* ± 104.7	81.1* ± 68.8
Coefficient of dispersion (%)	29.7	65.0	57.2	78.7
Percent ♀♀ normally engorged (⩾ 120 mg)	100.0	60.5	68.2	25.0
Mean feeding time (h)	172 ± 36	182 ± 30	194** ± 50	220* ± 57

*$p < 0.001$; **$0.01 < p < 0.05$. Significance of results was tested with Student's t-test.

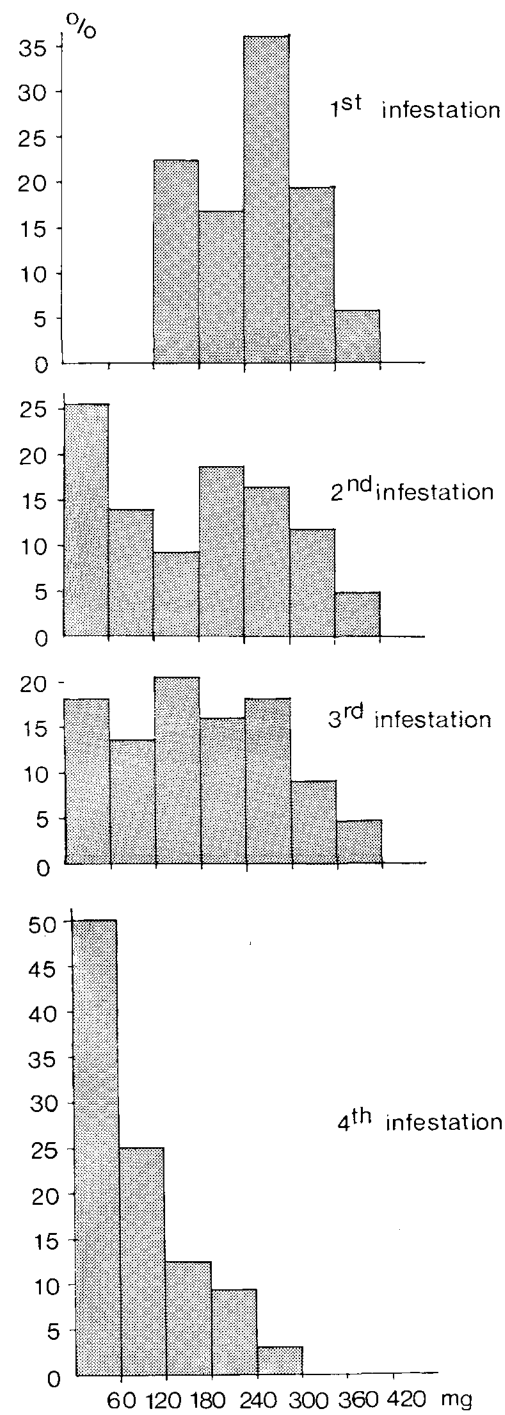

Fig. 1. Frequency distributions of weights of ♀♀ I. ricinus from 4 successive infestations on 5 rabbits. Class intervals are 60 mg.

1.695 mg[2]) is neglected. – During the first feeding, ticks took 120.0 to 399.7 mg of blood, with a mean of 254.8 mg. Thus in reinfestations, ticks weighing 120.0 mg or more were considered fully engorged (table 1). In the 2nd, 3rd and 4th infestations, the mean weight of fed ticks fell respectively by 79.1, 71.8 and 173.7 mg ($p < 0.001$). Only 60.5, 68.2 and 25.8% of the ticks were fully engorged. Thus, from the weight of blood ingested, the populations of ticks in the 2nd and 3rd infestation were divided into 2 groups on either side of the limit, earlier set at 120.0 mg (figure 1). This particular division was characterized by an extention of the bases of the 2 histograms towards the left (i.e. lower weights). For the 2nd infestation particularly, there were 2 peaks; for the 4th infestation, the only peak of the histogram was situated below 120.0 mg. For the 3 reinfestations, the coefficients of dispersion were above 30.0%, being 65.0, 57.2 and 78.7 respectively (table 2). This undoubtedly indicated an alimentary disturbance due to the immune response of the rabbits. On resistant animals, attachment time was prolonged by 48 h ($p < 0.001$); from 172 h in the first infestation, it rose to 220 h in the last.

2. Effect of acquired resistance in rabbits to egg laying in I. ricinus. All the ♀♀ I. ricinus from the first infestation of the rabbits laid eggs normally, the number of eggs laid being proportional to the weight attained by the females during feeding[2]. In the 3 following infestations, 23.3, 29.5 and 84.4% respectively of the females did not lay eggs. In the main, these females were not fully engorged, i.e. 90.0, 69.2 and 77.8% respectively. Thus acquired resistance in the rabbits impeded normal nutrition in the ticks and possibly prematurely blocked the onset of vitellogenesis.

3. Acquired resistance and fecundity of ♀♀ I. ricinus. From the first infestation, the eggs laid by 22.2% of the females did not hatch; they all came from ticks fed on a single rabbit (table 2). The failure percentages from the following 2 infestations were similar, 21.2 and 29.0%, but from the 4th infestation, eggs laid by 80.0% of the ticks did not develop. However, because of the results of the

Table 2. Effect of resistance on egg laying and hatching

	Infestation number 1	2	3	4
Failure to lay eggs (as percent of ♀♀ which fed)	0	23.3	29.5	84.4
Failures due to abnormal feeding (< 120 mg; %)	0	90.0	69.2	77.8
Failure of egg hatching (percent of ♀♀ laying eggs)	22.2	21.2	29.0	80.0
Fecund ♀♀ (%)	77.8	60.5	50.0	3.1

Table 3. Duration of acquired resistance

	Rabbit number 1	2	3	4	5
Time elapsed since 4th infestation (days)	56	56	183	183	269
Mean weight of fed ♀♀ (mg)	139.6 ± 79.2	93.9 ± 86.7	83.8 ± 38.7	80.2 ± 56.6	57.3 ± 34.3
♀♀ normally engorged (⩾ 120 mg; %)	66.7	30.0	22.2	20.0	0
Mean feeding time (h)	219	215	200	217	209
Failure to lay eggs (as percent of ♀♀ which fed)	55.5	70.0	55.5	30.0	50.0
Failure of egg hatching (percent of ♀♀ laying eggs)	75.0	66.7	100.0	85.7	100.0
Fecund ♀♀ (%)	11.1	10.0	0	10.0	0

first infestation, we cannot conclude that one of the influences of acquired resistance is to affect embryogenesis in the egg.

Fed on rabbits which had had no previous contact with the ticks, 77.8% of the ♀♀ I. ricinus were fertile (table 2). From the 2nd and 3rd infestations, 60.5 and 50.0% respectively laid eggs which hatched. From the 4th infestation, the percentage became very low, 3.1%. In this case, the cycle of I. ricinus was nearly completely stopped by immunity in the rabbits.

4. Acquired resistance in rabbits and circulating anti-I. ricinus antibodies. Circulating anti-I. ricinus saliva antibodies were detected 7 days after the first infestation. The titre rose rapidly and 18 days after the beginning of the experiment was $^1/_{320}$ in all but one case (figure 2). Several days before each of the other 3 infestations, the titre of antibodies was high (geometric mean: $^1/_{242}$, $^1/_{367}$, $^1/_{367}$ respectively) and generally varied little between animals. Thus, the extremely strong immunity observed at the 4th infestation was not due to a higher level of circulating antibodies (see discussion).

5. Duration of resistance in rabbits. Each rabbit (No. 1–5), reinfested 56, 183 or 269 days after the 4th infestation with 10 ♀♀ I. ricinus showed resistance (table 3). The immunity varied from rabbit to rabbit, but always led, with the failure of egg laying or hatching, to a low percentage of fecund females (too 11.1%). In fact, the mean weights of blood ingested were between 57.3 and 139,6 mg; they were therefore less than at the first infestation (254,8 mg) and only 0 to 66.7% of the ticks were normally engorged. Mean feeding times were between 200 and 219 h, figures exceeding the attachment time observed in normal rabbits (172 h). Fed on resistant rabbits, 30.0 to 70.0% of ♀♀ I. ricinus did not lay eggs and 66.7 to 100% of egg batches laid did not hatch (see tables 1 and 2).

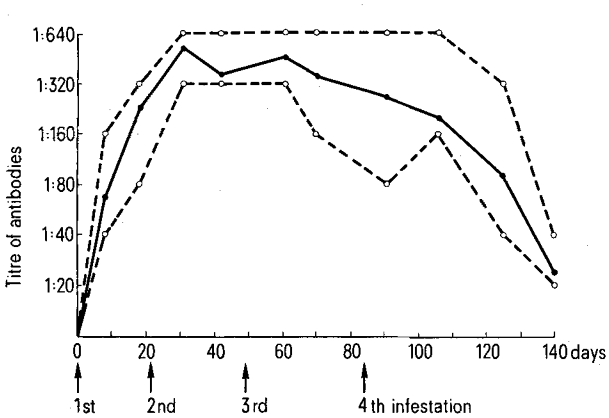

Fig. 2. Changes in titre of circulating anti-I. ricinus saliva antibodies. —.—.— Geometric mean of antibody titre. — ○ — — ○ — Extreme values (lowest and highest) on particular day.

Discussion and conclusions. Acquired resistance in rabbits disturbs the feeding of ♀♀ I. ricinus, by increasing the mean feeding time from 172 h on the first infestation to 220 h at the 4th. In fact, during the 2nd and 3rd infestations only about $^2/_3$ of the ticks fed normally; the others ingested less than 120 mg of blood, the lowest weight attained on rabbits previously unexposed to infestation (table 1)[2]. Adult *Rhipicephalus bursa*[4] or *R. appendiculatus*[5] engorge equally poorly on resistant rabbits. This is also the case of larval *Dermacentor andersoni* fed on immune guinea-pigs[6].

Using the technique of indirect immunofluorescence, circulating anti-I. ricinus saliva antibodies were detected from the end of the first infestation. They are probably involved in the development of resistance as immunity has been partly transferred by i.v. injection of immune serum into non-resistant animals[7]. The specific antibodies may neutralize the digestive or salivary enzymes of the ticks, an hypothesis already suggested to explain the resistance of cattle to Boophilus microplus[3,8]. However, another explanation is possible. In resistant animals, an intense inflammation of the skin develops at the attachment point of the ticks[9]. A normal flow of blood is thus probably impeded.

Resistance during the 4th infestation was much stronger and only $^1/_4$ of the ticks fed normally (table 1). However, the antibody titre was not elevated above that of the preceding infestation. A cell-mediated immunity, already demonstrated in the larval D. andersoni-guinea-pig system[10], could re-inforce the protective action of antibodies. On immune rabbits, an increase in the failure of egg laying by ♀♀ I. ricinus was noted from the first to the 4th infestation (0 to 84.4%, table 2). Ticks experimentally detached before the end of feeding on non-immune rabbits only fail to lay eggs if they weigh less than 22.3 mg[2]. In our case, most failures occurred in incompletely engorged females (<120 mg), but 86% of these weighed more than 22.3 mg. This suggests that the ingested blood, though sufficient in quantity to assure egg laying, even if somewhat reduced, contained factors detrimental to the formation of eggs and perhaps also to embryogenesis.

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