A NOTE ON THE PROBLEM OF ACQUIRED IMMUNITY TO ARGASID TICKS

Journal of Parasitology 26(1): 71-74 1940

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It has been shown (Trager, 1939a) that guinea pigs once infested with the ixodid tick, *Dermacentor variabilis* Say, develop an acquired immunity which effectively prevents subsequent groups of larvae from engorging. The immunity is a generalized one and lasts about 3 months. A similar acquired immunity is developed by rabbits and deer mice against the larvae of *D. variabilis*, by guinea pigs against the larvae of *D. andersoni*, and by rabbits against the larvae of *Haemaphysalis leporis-palustris*. *D. variabilis*, *D. andersoni* and *H. leporis-palustris* are all members of the family IXODIDAE. The ticks of this family have 3 stages in their life cycle and take one large blood meal in each stage, remaining attached to the host at each meal for a time varying from several days to about 2 weeks.

The majority of blood sucking arthropods, unlike the ixodid ticks, engorge in a relatively short time, and it was of interest to see whether an animal could develop any immunity to parasites of such a type. Ticks of the family ARGASIDAE, which in most of their stages engorge in a few minutes to a few hours, were selected for the work.

MATERIALS AND METHODS

Large numbers of *Argas persicus* Oken. were collected from chicken coops in the public market of Panamá. In order to obtain eggs and larvae engorged adults were kept in pairs of 1 ♂ and 1 ♀ in small vials held over moist sand. Groups of larvae which were to be compared in any one experiment were always counted out from the same vial and were hence of identical parentage. Unfed nymphs and adults were kept in large groups in vials over moist sand and counted numbers were removed when needed for experimental purposes. White Leghorn chickens from 6 weeks to 3 months old, obtained from a battery brooder and not previously exposed to ticks, were used for all the experiments with *A. persicus*. In a few experiments with nymphs and adults, the chicken was placed in a large covered battery jar equipped with paper toweling on the

Received for publication, September 18, 1939.

* It is a pleasure to thank Dr. H. C. Clark, director of the Gorgas Memorial Laboratory, and the members of his staff, notably Dr. D. M. Jobbins, senior medical entomologist, for the many courtesies extended to me during my two months' stay in Panamá.

Journal of Parasitology 26(1): 71-74, 1940

bottom and a raised wooden platform. This simulated natural conditions, since the ticks could hide under the platform during the day and feed on the chicken at night. In most of the work, the pill box method previously described was used, one 1¼" pill box being attached to either side of a chicken on the region underneath the wing, a portion of skin which in young chickens is almost free from feathers. In such a covered box the majority of unfed nymphs and adults would engorge within 10 minutes, but they were usually left overnight to give as many as possible an opportunity to feed. The larvae, as is normal for the species, engorged within 5 to 8 days.

Ornithodoros venezuelensis Brumpt was collected from native houses in several villages in the interior of the Republic of Panamá. Eggs and larvae were secured by keeping several males and females together in small vials. In this way, one vial would contain enough larvae for a single experiment. Guinea pigs equipped with a 1½" pill box on each side were used. The larvae, as well as the nymphs and adults of *O. venezuelensis*, engorged within an hour or less after attaching, but they were usually left on the host several hours or overnight so that as many as possible might feed.

TABLE 1.—*Acquired immunity to the larvae of* Argas persicus

Exper. No.	Chick No.	Previous treatment	Ticks put on			No. engorged
			No.	Date	Side	
				1939		
1	702	Fed upon by 118 nymphs and adults from 6/22 to 6/30, 39 not restricted to 1 region of body	50	7/12	Left	21
	703	Fed upon by 7 larvae 7/6 to 7/10 on right side	50	7/12	"	38
	704	Fed upon by 100 nymphs and adults from 6/23 to 6/30 not restricted to 1 region of body and by 24 on right side only on 7/6	50	7/12	"	26
2a	720	Fed upon by 203 nymphs and adults on right side only from 7/8 to 7/17	100	7/22	"	43
	722	Fed upon by 202 nymphs and adults on right side only from 7/6 to 7/17	100	7/22	"	33
	724	Worc pill box on right side 7/8 to 7/17	100	7/22	"	72
2b	721	Fed upon by 113 nymphs and adults on right side only from 7/8 to 7/12	100	7/20	"	49
	723	Fed upon by 103 nymphs and adults on right side only from 7/8 to 7/12	100	7/20	"	29
	725	Worc pill box on right side 7/8 to 7/12	100	7/20	"	64
3	701	See Exper. 1	100	7/31	"	14
	726	42 larvae engorged on 7/22 to 7/29	100	7/31	"	36
4	716	Fed upon by 92 nymphs and adults from 7/6 to 7/13 not restricted to 1 region of body	100	7/20	Right	45
	717	Fed upon by 187 nymphs and adults from 7/6 to 7/13 not restricted to 1 region of body	100	7/20	"	56
	718	Fed upon by 11 nymphs from 7/11 to 7/13	100	7/20	"	4*
	719	Not exposed to ticks	100	7/20	"	50
5	717	See Exper. 4	100	7/31	"	2
	718	See Exper. 4	100	7/31	"	45
	719	See Exper. 4	100	7/31	"	33

* Chicken 718 scratched its box loose soon after the larvae were put in, so that most of them escaped.

RESULTS

I. *Argas persicus.* The results with nymphs and adults need not be given in detail. As many nymphs or adults would engorge upon a chicken previously fed upon by 100 to 150 nymphs over a period of 1 to 2 weeks as on a chicken not previously exposed. This was true even if successive batches of 30 to 40 ticks were placed repeatedly over the same region of the body. Usually 80 to 100 per cent of the ticks became engorged.

One infestation with larvae (about 50 engorged out of 100 applied to each chicken) did not confer any immunity to a second infestation with larvae. However, repeated infestations with a large number of nymphs and adults appeared to confer a slight immunity to the larvae as shown in Table 1, Exper. 1, 2, and 3. In another experiment (4), somewhat similar to these, no immunity was developed, but when chickens 717, 718, and 719 were again exposed to larvae much fewer engorged on 717, previously fed upon by 187 nymphs and 56 larvae, than on 718, previously fed upon by 11 nymphs and only 4 larvae, or 719, previously fed upon by 50 larvae only (Exper. 5). As might have been expected from these results, the intracutaneous or intraperitoneal inoculation of extracts of *Argas persicus* did not produce any immunity to larvae, nymphs or adults.

Attempts to demonstrate complement fixation (see Trager, 1939b) with serum from the partially immune chickens as antibody and extract of *Argas persicus* as antigen failed because of the very high anti-complementary activity of the two antigens tried. One, a saline extract of nymphal and adult ticks, was anti-complementary even at a dilution of 1 : 25,000 on the basis of the weight of ticks used. The other, an extract of larval ticks, lost its anti-complementary effect at a dilution of 1 : 20,000 on a weight basis. At this dilution it did not fix complement in the presence of immune chicken serum.

II. *Ornithodoros venezuelensis.* In a small series of suitably controlled experiments, there was no evidence of any fewer larvae feeding on guinea pigs previously exposed to even as many as 5 successive infestations, each with about 50 ticks, over a period of 2 to 3 weeks than on animals not previously infested. Definite red marks were noted in the skin at the site of attachment of larvae as well as nymphs and adults, but these did not appear any different after a fifth or sixth infestation than after a first.

DISCUSSION

It is evident that the exposure, over a relatively short time, of chickens to infestation with *A. persicus* or of guinea pigs to infestation with *O. venezuelensis* does not result in any immunity which is capable of acting within the 10 minutes to an hour required for engorgement by the nymphs and adults of the former tick and by all stages of the latter.

That the nymphs and adults of *A. persicus* do, however, inject their host with antigenic substance is demonstrated by the partial immunity which repeatedly infested chickens exhibit against the larvae of the fowl tick. One may assume that, during the several days required for the larvae to engorge, a cellular reaction, of the type described in the studies with *Dermacentor variabilis*, occurs in the immune animals.

In spite of the negative results of the present study, the possibility remains that a very prolonged exposure of an animal to repeated infestations with a rapidly feeding blood-sucking arthropod might eventually result in an immune response which could occur within the short time of attachment of the parasite. This possibility is strengthened by the indications that the rapidly feeding nymphs and adults of *A. persicus* do apparently inject effective amounts of an antigenic substance.

Cherney, Wheeler and Reed (1939) have recently reported a partial "immunization" of people to flea bites. From their work it is impossible to decide whether the "immune" people were immune to the fleas themselves or to the effects of the flea bite. This is an important and necessary distinction and has been previously emphasized (Trager, 1939a). A person who did not react to flea bites could be repeatedly bitten without his being aware of it. One can cite again the observation of Rozeboom (1936) that the donor of blood to a colony of *Anopheles albimanus* soon became immune to the effects of the bites, although the mosquitoes continued to feed on him as well as ever. An experimental demonstration of a true immunity against rapidly feeding blood-sucking arthropods themselves, that is, an immune mechanism which would actually prevent them from feeding, would be most interesting and important.

SUMMARY

Chickens which had been repeatedly infested with nymphs and adults of *Argas persicus* did not show any immunity to these stages of the tick, which engorge in 5 to 10 minutes. The chickens did, however, exhibit a partial immunity to the larvae of *Argas persicus*, which require at least 4 days for engorgement.

Guinea pigs exposed to repeated infestation with *Ornithodoros venezuelensis*, all stages of which engorge rapidly, showed no immunity to any of the stages of this tick.

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