HOST FACTORS AFFECTING INFESTATION BY

RHIPICEPHALUS SANGUINEUS WITH

PARTICULAR REFERENCE TO AGE,

SEX AND HORMONES

Sami R. Ali

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RHIPICEPHALUS SANGUINEUS

ABSTRACT

Immature and mature albino mice of both sexes were exposed to R. sangui-
neus larvae. The former were significantly more susceptible than mature
mice. Male mice in both age groups, and immature females became infested
with significantly more R. sanguineus larvae when treated with cortisone
than did control mice. The naturally more resistant mature female became
even more resistant upon cortisone treatment. The greater susceptibility
of untreated mice attibutable to immaturity was nullified by cortisone
administration. Oestrogen-treated male mice became more resistant than
controls in both age groups. It seems clear that female mice are more
resistant to infestation because of some factor associated with the female
hormone level.

Observations were made on the behaviour and factors concerned with
the imbibition of adult R. sanguineus. Equal and unequal numbers of
male and female R. sanguineus were applied to one hedgehog and 3 dogs.
There was evidence of an increase in the weight of the ticks with a longer
period of imbibition on the hedgehog. In dogs there was no clear relation-
ship between the weight of the ticks and their period of imbibition. The
presence of males partly affected the period of imbibition and the number
of females imbibing. On the hedgehog, there was no detectable site of
predilection, while on dogs most ticks moved from their backs to the
underside of the ears to complete their imbibition.

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Immature and mature albino mice of both sexes were exposed to R. sanguineus larvae. The former were significantly more susceptible than mature mice. Male mice in both age groups, and immature females became infested with significantly more R. sanguineus larvae when treated with cortisone than did control mice. The naturally more resistant mature female became even more resistant upon cortisone treatment. The greater susceptibility of untreated mice attibutable to immaturity was nullified by cortisone administration. Oestrogen-treated male mice became more resistant than controls in both age groups. It seems clear that female mice are more resistant to infestation because of some factor associated with the female hormone level.

Observations were made on the behaviour and factors concerned with the imbibition of adult R. sanguineus. Equal and unequal numbers of male and female R. sanguineus were applied to one hedgehog and 3 dogs. There was evidence of an increase in the weight of the ticks with a longer period of imbibition on the hedgehog. In dogs there was no clear relationship between the weight of the ticks and their period of imbibition. The presence of males partly affected the period of imbibition and the number of females imbibing. On the hedgehog, there was no detectable site of predilection, while on dogs most ticks moved from their backs to the underside of the ears to complete their imbibition.

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INTRODUCTION

Host resistance to parasitic infection is intricately related to many factors including age, sex and hormone balance. For certain parasites, it has been demonstrated that immature animals are more susceptible than adult animals. In secondary echinococcosis when white mice were inoculated intraperitoneally with Echinococcus granulosus scolices, young animals (48 days of age or under) developed a greater number of cysts than older mice (Schwabe et al., 1959). Hamsters experimentally infected with Nippostrongylus muris showed that one-month-old animals harboured more worms than 2-to 6-month old hamsters (Haley, 1958a). On the other hand, Dudzinski and Mykytowycz (1963) noted a much lower level of infection with Graphidium strigosum in young than in adult experimental rabbits (Oryctolagus cuniculus). In comparable rabbits observed by the same authors, however, age did not seem to affect the number of Trichostrongylus retortaeformis, but larger worms producing more eggs were seen in 1-to 4-month-old rabbits compared with 50-month-old rabbits. In a study of the helminths in a natural population of the European trout, Salmo trutta, Thomas (1964) noted that trout aged 3 years and over had more worms than younger animals.

Among ectoparasites, the significance of the age of the host appears to have been noted only in few cases. Buxton (1948) observed that Xenopsylla cheopis that fed on very young mice laid fewer eggs than those fed on adult mice. Rothschild and Ford (1964a) in their work on the rabbit flea Spilopsyllus cuniculi noted that 2-to 6-week old rabbits were unattractive to fleas, while very young rabbits (5 or 6 days old) and pregnant does during the last 10 days of pregnancy were the most attractive. Cook and Beer (1958) noted that old

male meadow voles, Microtus pennsylvanicus, harboured more lice (Hoplopleura acanthopus) than young male animals. It seems clear that susceptibility to certain parasites by an animal is related to its age, but in different ways in different host-parasite relationships.

The sex of the host and its effect on different parasitic infections has received some attention. In most experimental studies, male hosts harboured more parasites than females: for example, hamsters infected with Nippostrongylus muris (Haley, 1954; 1958a; 1958b); guinea pigs and mice infected with Nippostrongylus brasiliensis (Parker, 1961; Neafie and Haley, 1962); mice and rats, the latter an abnormal host, infected with Nematospiroides dubius (Dobson, 1961a; 1961b; 1962); and mice infected with the pinworm Syphacia obvelata (Mathies, 1954). Dobson (1964) noted that male lambs experimentally infected with Oesophagostomum columbianum were not only more susceptible than females, but also harboured larger worms. Gonadectomy of lambs removed these differences. With Haemonchus contortus, however, the same author noted that the sex of the host had no effect on the infectability to this parasite. In contrast with the above, Dudzinski and Mykytowycz (1963) observed that experimentally infected male rabbits (O. cuniculus) harboured fewer Graphidium strigosum than female hosts, and that the numbers of Trichostrongylus retortaeformis were not influenced by the sex of the host. In a survey of wild rabbits (O. cuniculus) in New Zealand, Bull (1959) noted that seasonal factors were also involved in the infection rate and intensity of T. retortaeformis. Male rabbits were more resistant in summer but less resistant in winter than females.

Examination of the helminths in a large number of Rana temporaria over a period of 4 years by Lees and Bass (1960) revealed that the level of parasitization in male frogs during and immediately before the breeding

season was considerably higher than in females, while after the breeding season
this difference became less marked. When male frogs were infected with oestra-
diol benzoate, there was some evidence of decrease in the level of parasitiza-
tion in the treated frogs.

Thomas (1964) in his study of the helminths of male and female Salmo
trutta from a natural population found that when female trout were spawning
or recovering from the effects of spawning they were infected with more worms
than males. This trend was less marked or reversed at other times of the year.

The sex of the host has been shown to influence the sex ratio of the
parasite in at least one parasitic infection. Two groups of male mice,
castrated and uncastrated, when subsequently infected with Schistosoma mansoni
showed a significant difference in the total number of worms recovered from
both groups. The castrated group harboured fewer parasites than the normal
uncastrated group. The reduction in the number of adult schistosomes was
attributed to a reduction in the number of male parasites. When testosterone
was administered to both castrated and uncastrated male mice, however, there
was a reduction in the number of both sexes of parasites (Berg, 1953; 1957).
Since castration decreased the number of parasites and the effect of injected
testosterone was questionable, the author suggested that variation in testo-
sterone concentration might be responsible for the change in response of the
host to this infection.

In a study of the effect of sex hormones on the normal resistance
of rats to strobilocerci of Taenia taeniaeformis, it has been found that
males harboured a larger number of cysts than females (Campbell, 1939).
When theelin (=estrone) was administered it did not affect the resistance
of females, but males that received theelin showed fewer cysts than untreated

controls.. Females that received testosterone propionate harboured more
strobilocerci than untreated females while males that received this hormone
showed no change in resistance (Campbell, 1939). Campbell and Melcher (1940)
showed that castration enhanced the degree of infection with the strobilocerci
of T. taeniaeformis in female rats but diminished it in males. Administration
of androgen to ovariectomized rats lowered their resistance still further.
Estrogen administration to orchiectomized males increased their resistance
to this infection to a greater extent. Addis (1946) studied the effect of
sex hormones on the growth of Hymenolepis diminuta in rats and found that
there was normal growth in uncastrated rats while in castrated animals the
growth of the worms was stunted. When castrated male rats were treated with
testosterone, worm development returned to normal. These observations
suggested the necessity of male sex hormones for the normal growth of the
worms. When the female hormones theelin and progesterone were injected into
castrated male rats, the former failed to prevent the stunting of the worms
while progesterone appeared to be as effective as testosterone. In spayed
females the growth of worms was normal in both testosterone treated and
untreated groups. Immature male rats treated with testosterone showed normal
worm growth by contrast with untreated immature males where there was stunting
of the worms. H. diminuta in immature females, on the other hand, was
indifferent to female sex hormone treatment. Egg production of H. diminuta
in relation to sex hormones showed that castration of male rats caused
reduction in the egg output of this parasite. When testosterone or proges-
terone were administered to male rats, each acted similarly by raising the
egg output. This was lowered by castration of the host (Beck, 1952.)..
These data on helminths infections suggest that they are often times more
successful in male than in female hosts.

With regard to ectoparasites and sex of the host, little information
is available. Cook and Beer (1958) observed that male meadow voles (Microtus
pennsylvanicus) harboured more lice (Hoplopleura acanthopus) than females.
Nutting (unpub. data) noted that more male dogs were infested with Demodex
canis than bitches. Rothschild (1964) recorded that pregnant rabbits during
the last 10 days of pregnancy became particularly attractive to fleas,
especially female fleas. Bat fleas of the genus Ischnopsyllus crowd onto female
bats during migration to their summer colonies. With the human flea Pulex
irritans, there is a marked predilection for women (in Rothschild, 1964).
On the other hand, bird fleas of the family Ceratophyllidae and various fleas
on small mammals are generally more numerous on male than on female hosts.
Here as in internal parasites, the sex of the host appears to be significant
in its susceptibility to at least some ectoparasites.

Cortisone is an anti-inflammatoy substance which some workers in
parasitology have assessed in the resistance mechanism of various hosts to
parasitic infections. Thomas (1955) stated that "Cortisone and ACTH cause
enhancement of infection by a great variety of different infectious agents,
and they appear to do so by lowering the host's resistance rather than by
affecting the agents themselves". Ashcroft (1959) studied the effect of
cortisone on Trypanosoma rhodesiense infections in albino rats and noted
that the daily administration of 25 to 50 mg. cortisone acetate/kg. body
weight on the day before or on the same day of inoculation caused an initial
enhancement of the infection followed by its inhibition. When a dose of
12.5 mg/kg was administered to 2 groups, one group for 14 days starting 7
days before infection and the other group for 6 days from the 15th day to the
20th day of infection, the effect of cortisone was entirely inhibitory.

When a 5 mg/kg dose was used, no inhibition was observed and enhancement occurred later. It was suggested that cortisone has several actions, some of which, such as the inhibition of the defense mechanisms of the rat may enhance the trypanosomal infection, while an altered blood composition may inhibit the infection. Changes in the infection will depend on which effects are predominant, which will in turn depend on the dose of cortisone, the time when it is given, the response of the rat and the susceptibility and adaptability of the trypanosome. Seneca and Ides (1955) recorded that cortisone treatment of mice infected with Trypanosoma cruzi markedly lowered their resistance to this infection as shown by a heavy peripheral parasitaemia. Activation by cortisone of latent trypanosomal infections in rhesus monkeys have been reported by Wolf et al. (1951). Cantrell (1955) reported that cortisone when administered to rats experimentally infected with Trypanosoma equiperdum had no effect, but when administered with a low dose of oxophenarsine there was a delay in the disappearance of peripheral trypanosomes. With higher doses in the presence of cortisone there was earlier relapse and higher death rate among the rats than in those treated with oxophenarsine alone. This phenomenon was related to a possible impaired antibody formation in cortisone treated animals in response to trypanosomal infection. In the case of Plasmodium berghei Findlay and Howard (1951) observed that cortisone treated mice were infected with more peripheral parasites than untreated controls.

The effect of cortisone on Trichinella spiralis infections have been studied in mice (Coker, 1955; 1956a & b; Stoner and Godwin, 1954), rats (Markell and Lewis, 1957; Merkell, 1958; Lord, 1958), and Chinese hamsters (Cricetulus barabensis), a host normally highly refractory to this parasite

(Ritterson, 1959). In all of these studies, cortisone-treated animals harboured greater numbers of adult worms in the intestine and more larvae in the musculature than in controls. In some hosts immunized against this infection cortisone treatment broke down the acquired immunity to the challenge infection.

White rats are known to be highly resistant to the mouse nematodes Nippostrongylus muris and Nematospiroides dubius and to the cotton rat nematode Litomosoides carinii. Weinstein (1953; 1955) noted that there was a suppression of cellular response in the skin and a greater number of worms in the intestine of treated white rats infected with N. muris when compared with non-treated rats which showed an intense inflammatory skin reaction with many larvae trapped in nodules. Cortisone did not, however, prevent the stunting of adult worms or suppress the formation of antibody. When Weinstein administered cortisone to rats immunized against N. muris, the inflammatory skin reaction was suppressed and larval penetration was successful. Many larvae were trapped in the internal organs, but larger numbers matured to adults and were larger in size than in the non-treated immune controls. Cross (1960) demonstrated that when white rats were infected with N. dubius and treated with cortisone, the parasites behaved as if in its normal host. Briggs (1959) noted that cortisone made white rats less resistant to L. carinii. A similar observation was made by Parker (1961) in guinea pigs, a host normally highly resistant to Nippostrongylus brasiliensis. Naturally resistant strains of mice to Taenia taeniaeformis strobilocerci became highly susceptible when treated with cortisone (Oliver, 1962). In contrast with the above observations is that on Schistosoma mansoni in mice by Coker (1957) and Weinmann and Hunter (1960). These workers noted that mice infected with this trematode and treated with cortisone harboured smaller

numbers of parasites than untreated controls. It seems clear, however, that almost all tested protozoa and helminth infections became enhanced in the presence of cortisone.

Work on ectoparasites appears to be confined to the sheep ked, Melophagus ovinus, and rabbit flea, Spilopsyllus cuniculi. Nelson (1962) showed that cortisone breaks down the acquired resistance of lambs to M. ovinus with consequent establishment of a greater number of keds than on untreated control lambs. Moreover, the parasites were able to engorge in a shorter period of time on cortisone treated animals.

Rothschild and Ford (1964b) noted that the daily injection of non-pregnant adult rabbits with 1.3 mg of cortisone or more induced partial to complete maturation of the fleas (S. cuniculi), but 0.6 mg or less did not. The same authors observed that during the last 10 days of the host's pregnancy, female fleas become firmly fixed to the skin of the rabbits' ears. This behaviour could be induced on non-pregnant rabbits by injecting small doses of hydrocortisone into the host. Larger doses of the same hormone showed the opposite effect and the fleas all detached themselves from the ears and moved onto the rabbits' head. The importance of dosage level is apparent. These few observations suggest that modification of the host with cortisone has a similar effect on ecto-parasite numbers and infectability as in the case of internal parasites.

The parasite used in the current study was the Kennel Tick, Rhipicephalus sanguineus (Latr.). This is a three host tick with an artificial worldwide distribution presumably attributable to its most important host, the dog. A variety of other animals, however, have been infested including domestic livestock and man, wild carnivores, insectivores

and ruminants, ground-feeding birds, and reptiles (Leeson, 1951; Hoogstraal, 1956). Significantly, the experimental animals used in the present study (the white mouse Mus musculus; Eurasian hedgehog Erinaceus europaeus; and dog Canis familiaris) are all known natural hosts.

Wilson (1950) in his work on Ixodoidea in Nyasaland stated that R. sanguineus was not closely related to any other species in the genus. In 1952, however, Feldman-Muhsam (in Cwilich and Hadani, 1964) divided R. sanguineus into two species, R. sanguineus sens. str. and R. secundus. The criteria used for differentiation were (a) the shape of the genital aperture in the adult female; (b) the angle of the lateral border of the basis capituli, shape of palps, and the presence or absence of a spur on coxa I in the nymphal stage; and (c) the contour of the upper external line of the palp in the larval stage. Adult males were indistinguishable. The same author in 1957 observed the infested hosts of these two species in Palestine and found that 81% of the ticks from dogs were R. sanguineus sens. str. while 19% were R. secundus. On cattle, sheep, goat, horse, donkey, jackals and hedgehogs the respective percentages of R. secundus were 97, 94, 91, 89, 79, 69 and 73, with the remainder being R. sanguineus sens. str. Cwilick and Hadani (1964) doubted this classification from a survey of ticks they carried out in the same country following which they were able to cross-breed R. sanguineus sens str. females with R. secundus males to the F3 generation. Attempts to cross-breed F3 male hybrids with R. secundus females, were, however, unsuccessful. The genital aperture of hybrid females showed a number of intermediate forms between R. sanguineus sens. str. and R. secundus described by Feldman-Muhsam. Our observations on larvae and nymphs reared from a single female R. sanguineus from a dog in Lebanon also showed intermediary morphological characters.

Therefore, for the purpose of the present study, R. secundus is considered
to be conspecific with R. sanguineus.

The purpose of the present study is to assess the significance of
experimental observations on the effect of cortisone, age, sex and sex hormones
in the laboratory mouse on R. sanguineus larval infestations. Additional
information is presented on adult tick behaviour on the dog and hedgehog.

I. Experimental Hosts

Four dogs and one hedgehog were used for observations on the adult tick. During exposure, these animals were kept in a covered box sealed with adhesive tape. Two holes covered with silk boltingcloth at each side of the box provided ventilation. Since most ticks attached to the ear of dogs a 10 x 10 x 1/10 inch fiberboard collar was fitted around the neck to prevent loss of ticks from scratching. The collar did not interfere with the dogs' normal feeding.

The progeny of 2 male and 2 female albino mice provided all the mice used in the current study. Worden (1947) noted that the age of sexual maturity of the laboratory mouse was 42 to 56 days. In the present study, all mature mice were 2 to 3 months old, while immature mice were less than 40 days old at the completion of the experiments. In order to prevent loss of ticks by the grooming activities of the mice, a round plastic collar (1 to $1\frac{1}{2}$ mm. thick and 3 to 5 cm. wide) was fixed around the neck while another was fitted in front of the pelvis (Fig. 1) before exposure to the larvae. Immediately

Fig. 1. Collars fixed around the neck
and in front of the pelvis of a
mouse

after exposure, each mouse was put in a separate one-gallon jar with a 5 cm.

diameter opening in the lid covered with silk bolting cloth (74 mesh/inch)

cemented in place with "Duco Cement". Adequate ventilation was provided by

this method. The edge of the lid after closure was sealed with adhesive tape

to prevent any loss of larvae. Mice were transferred daily to clean jars, and

food in the form of pellets moistened with water was provided.

II. The Parasite

Adult female ticks were permitted to drop from naturally infected

dogs and hedgehogs taken in Lebanon. The ticks were placed in separate

vials covered with silk bolting cloth held in a desiccator at 21 to 25°C

and a relative humidity of about 93% maintained by a saturated solution of

$(NH_4) H_2PO_4$. A series of egg masses were obtained in this way.

Larvae used on experiment were of the same approximate age since all

of them were held 30 to 45 days after the 3 week hatching period of the egg

mass. In the cortisone experiment, each mouse was exposed to 500 larvae,

while in the sex hormone experiments 400 larvae were used. Larval counts

were made under a dissecting microscope in a petri dish containing water.

The larvae floated on the water which also impeded their mobility and

permitted ready transfer of the larvae to mice with a camel's hair brush.

III. Hormones

An aquous suspension of cortisone acetate (Merck "Cortone") was

provided in vials of 20 cc. at a strength of 25 mg/ml. Sterile physiological

saline solution was used as a diluent to provide a dose of 25 mg/kg body

weight of the mouse, which was administered subcutaneously. Injections were

given daily starting 3 days before exposure of the mice and were continued

for 3 days after the final larval drop. Control mice were injected with

a comparable volume of physiological saline.

Oestradiol benzoate (The British Drug House L.T.D. "Oestroform") in oil was administered intramuscularly in doses of 50 ugm/kg body weight into mature and immature male mice. Injections were given every third day beginning 3 days before exposure. Control mice received corn oil injections.

Testosterone (Charles E. Frosst & Co. "Orchisterone-P") in corn oil was administered intramuscularly in doses of 0.43 mg/kg body weight into mature and immature female mice. Injections were given every third day starting 3 days before exposure. Control mice received corn oil injections.

IV. Leucocyte Counts

Blood for total white cell and differential counts was taken as free-flow blood from the tip of the tail of the mice a day before and four days after exposure. The blood smears were stained with Giemsa or Wright's Stain.

RESULTS

I. Experiments with Larvae.

A. Age of Host

The significance of the age of the host in the number of R. sanguineus larvae infesting mice was determined by exposing 5 immature and 5 mature mice of each sex to 500 larvae in one experiment and 400 larvae in another. These mice were the same animals that served as controls in the cortisone and oestrogen experiments to be discussed later. Regardless of the sex of the host, all immature mice dropped greater numbers of larvae than mature mice. Differences were highly significant as shown by Student's "t" test. In one group, almost twice as many larvae dropped from the immature mice as from the mature mice (Fig. 2). The highest number that dropped from a single immature mouse on the day of peak drop was 229 while from a mature mouse the comparable number was 197 (Fig. 3). It seems clear that immature mice are distinctly more susceptible to R. sanguineus larvae than mature mice.

B. Sex of Host.

The total numbers of larvae that dropped from the control mice in the cortisone experiment were analyzed to determine whether the sex of the host had any influence on the number of parasites that were able to imbibe and drop. Fig. 2 shows that male mice dropped more larvae than females. The largest number of larvae that dropped from a single male mouse on one day was 180 for immature mice and 72 for mature mice. The comparable numbers for females were 79 and 66. The difference was highly significant between the sexes of the immature mice but did not reach a statistically significant level in the case of mature mice. These data suggest that the positive effect

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P VALUES OF TOTAL NUMBERS

AGE	SEX	CORTISONE
EI♀>EM♀ P<0.001	EI♀<EI♂ P<0.08	EI♀>CI♀ P<0.001
EI♂>EM♂ P<0.08	EM♀<EM♂ P<0.001	EM♀<CM♀ P<0.05
CI♀>CM♀ P<0.001	CI♀<CI♂ P<0.001	EI♂>CI♂ P<0.001
CI♂>CM♂ P<0.001	CM♀<CM♂ P<0.27	EM♂>CM♂ P<0.001

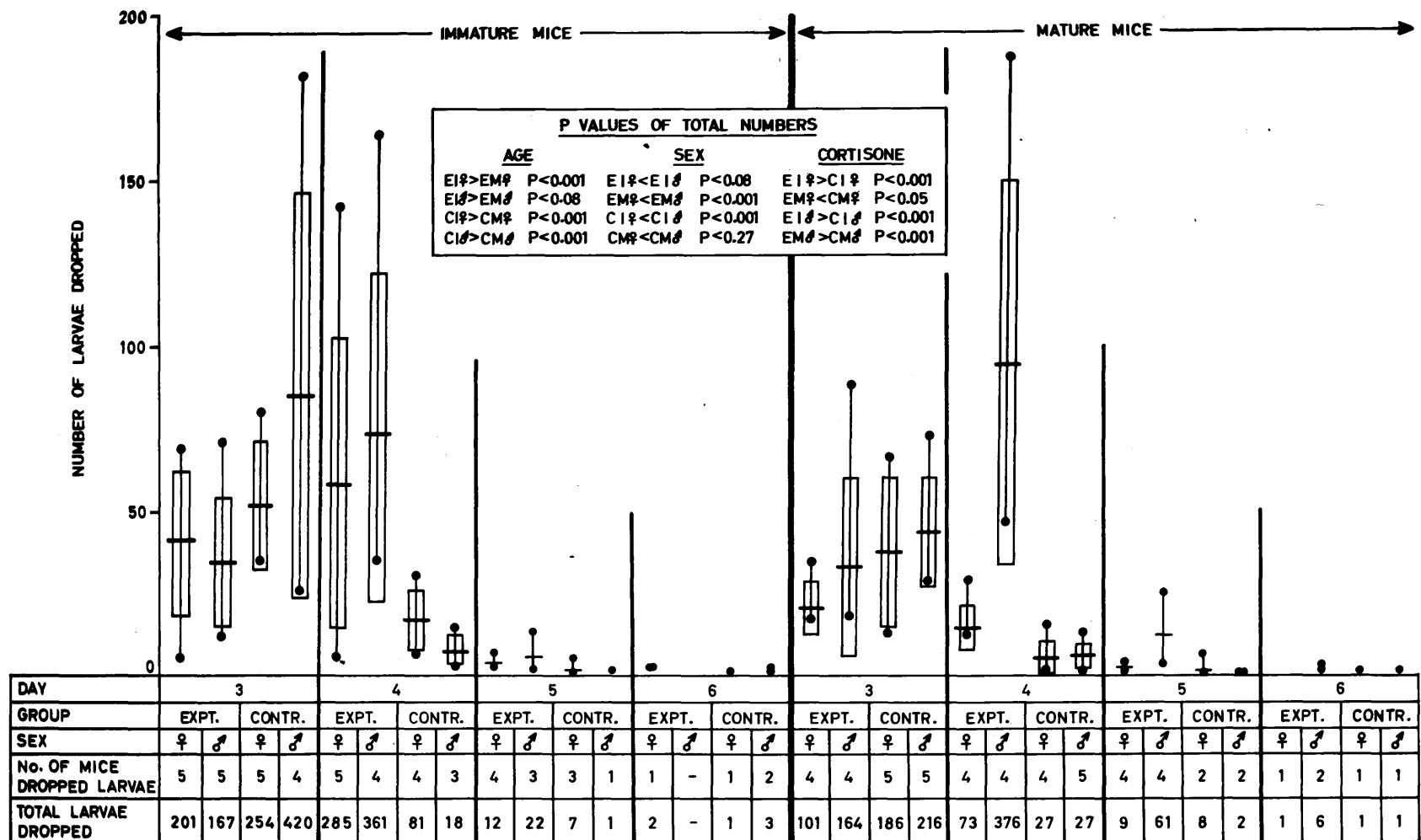| DAY	3				4				5				6				3				4				5				6			
GROUP	EXPT.		CONTR.		EXPT.		CONTR.		EXPT.		CONTR.		EXPT.		CONTR.		EXPT.		CONTR.		EXPT.		CONTR.		EXPT.		CONTR.		EXPT.		CONTR.	
SEX	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
No. OF MICE DROPPED LARVAE	5	5	5	4	5	4	4	3	4	3	3	1	1	-	1	2	4	4	5	5	4	4	4	5	4	4	2	2	1	2	1	1
TOTAL LARVAE DROPPED	201	167	254	420	285	361	81	18	12	22	7	1	2	-	1	3	101	164	186	216	73	376	27	27	9	61	8	2	1	6	1	1

Fig. 2. The effect of age, sex and cortisone administration on the susceptibility of immature and mature mice of both sexes to R. sanguineus larvae. The horizontal bar denotes the mean, and the box on either side of the mean represents one standard deviation.

E = Experimental   C = Control   I = Immature   M = Mature

NUMBER OF LARVAE DROPPED

P values of total
numbers
EM < CM   P < 0.001
E I < C I   P < 0.001
EM > E I   P < 0.24
CM < C I   P < 0.001

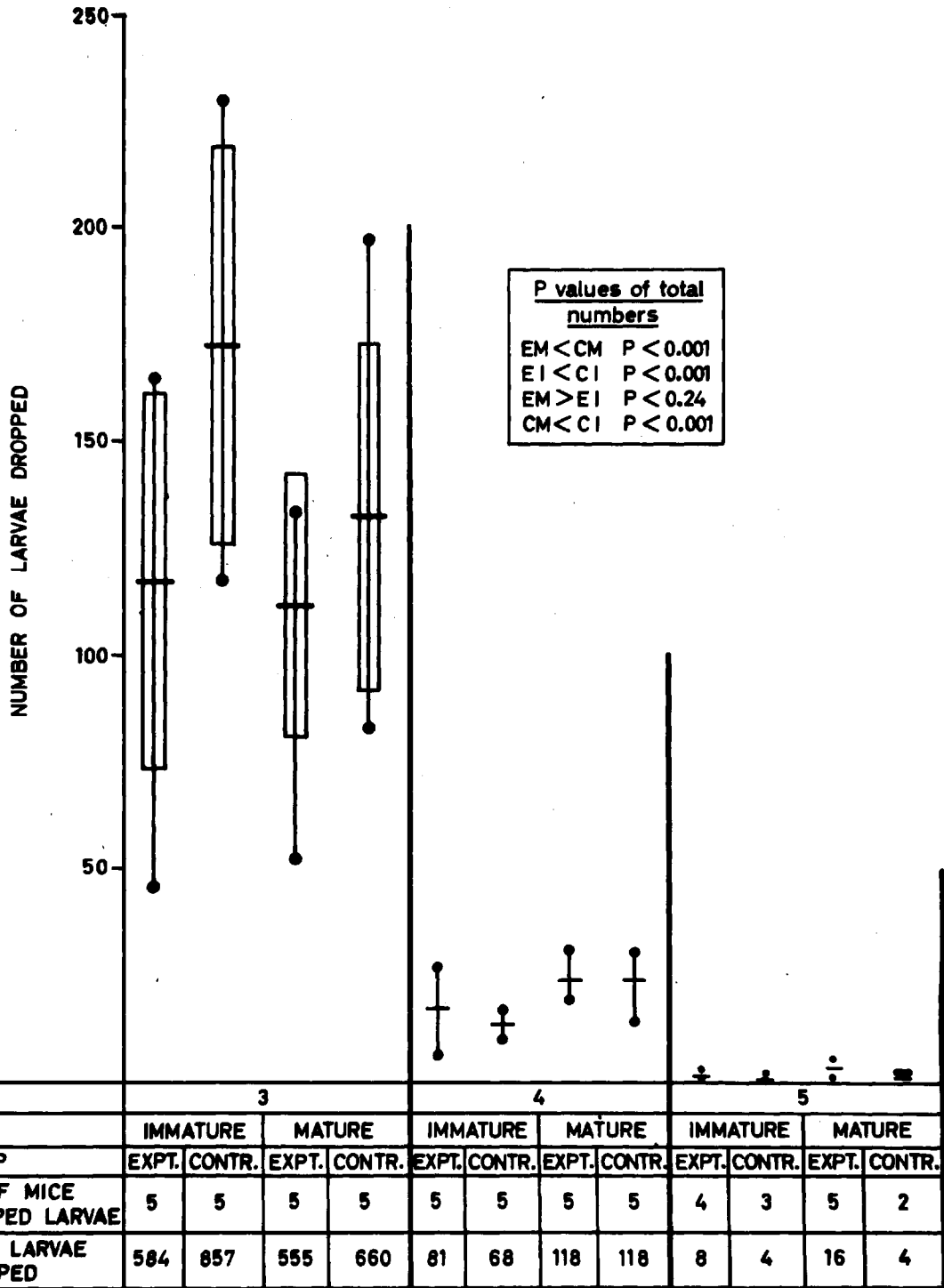| DAY	3				4				5			
AGE	IMMATURE		MATURE		IMMATURE		MATURE		IMMATURE		MATURE	
GROUP	EXPT.	CONTR.	EXPT.	CONTR.	EXPT.	CONTR.	EXPT.	CONTR.	EXPT.	CONTR.	EXPT.	CONTR.
No. OF MICE DROPPED LARVAE	5	5	5	5	5	5	5	5	4	3	5	2
TOTAL LARVAE DROPPED	584	857	555	660	81	68	118	118	8	4	16	4

Fig. 3. The effect of oestrogen on the susceptibility of immature
and mature male mice to R. sanguineus larvae.

of masculinity in the immature mice became less important upon the maturation of the host.

C. Effect of Injected Oestrogen.

The conclusion above prompted the following experiment with oestrogen injections in male mice. Five mature and 5 immature male mice were treated with oestrogen and each was exposed to 400 larvae taken in equal numbers from 10 female ticks. The larvae were starved for 30 days beforehand. A similar number of mature and immature mice were held as controls. Fig. 3 shows that the total larval drop from both the immature and mature experimental mice was distinctly lower than that from the controls. The highest number of larvae that dropped on any one day from a single treated immature mouse was 164 while that from a comparable control was 229. The maximum numbers for mature mice were 133 and 197 for experimental and control animals. Indeed, the treated mature mice became infested with fewer larvae than the untreated mature mice. It appears that oestrogen not only reduced the susceptibility of mature male mice but also nullified the effect of immaturity noted previously.

D. Effect of Injected Testosterone.

In another experiment, 5 mature and 5 immature female mice were treated with testosterone and comparable groups were held as controls. Each mouse in these groups was exposed to a similar number of larvae held for the same period of starvation as in the oestrogen experiment, but larvae in this experiment were reared from 10 females from a different source. After 2 days of exposure to larvae, mice started dying from unknown cause and by the fourth day most were dead. This made it impossible to reach a satisfactory conclusion concerning the role of testosterone administration.

E. Effect of Injected Cortisone.

To assess the effect of cortisone on a resultant infestation by R. sanguineus larvae on mice, 500 larvae, taken in equal numbers from 20 female ticks 40 days after the egg masses had stopped hatching, were applied to each of 5 immature and 5 mature mice of both sexes that were under cortisone treatment. Equal numbers of comparable controls received saline injections.

All mice injected with cortisone, with the exception of the mature females, dropped larger numbers of larvae than their comparable controls with statistical differences that were highly significant (Fig. 2). The total numbers for the experimental and control groups were respectively, 550 and 442 for immature male mice, 500 and 343 for immature females, 607 and 246 for mature males, and 184 and 222 for mature females. It follows that cortisone reduced the resistance of the mature male mice and the immature mice of both sexes, but slightly increased the resistance of adult females. This observation is consistent with the effect of the oestrogen injections noted in the previous experiment.

Another observation was consistent in the case of adult male, immature male and female, but not in adult female mice. The day of peak larval drop occurred on the fourth day after exposure in the experimental adult male, immature male and immature female mice whereas most larvae dropped on the third day in comparable controls. In the adult female, however, the third day was the day most larvae dropped in both experimental and control groups. It follows that where cortisone had a significant effect on increasing the number of ticks imbibing, it also had a prolonging effect on the period of imbibition.

In this experiment the blood from a single mouse in each experimental and control group was examined for total and differential leukocyte count one day before and 4 days after exposure to larvae. Table I shows that in 3 of 4 mice in the experimental group there was an increase in the total leukocyte count following tick attachment. This was, however, also true for 2 of 4 of the controls. Following tick attachment, the percent neutrophils increased in the immature male and mature female mouse from the experimental groups together with a decrease in the percent lymphocytes. Control immature mice showed the reverse trend, while no trend appeared in the mature animals. These few observations suggest no positive effect on the white cell count following tick attachment.

II. Experiments with Adults.

Observations were made on the relationship between the period of imbibition and weight of fully imbibed female R. sanguineus. A single female that dropped from a naturally infected dog provided all the adults in the subsequent generation that were used in the experiments. The intermediary larval and nymphal stages fed on an immature dog following artificial exposure. After a 30 day starvation period, an equal number (30) of male and female ticks were applied to a hedgehog (Fig. 4), while 20 pairs were applied to an immature dog (dog Al, Fig. 5). A daily record of the positions of the ticks on the hedgehog was made. The ticks became attached amongst the spines of the back, neck and on the legs. None was observed on the belly. On the first day after exposure, 14 males and 1 female were clustered towards the anterior end of the hedgehog near a previous injury with their chelicerae imbedded in the skin. Four other males and 8 females were attached elsewhere. By the third day, the

TABLE I

WHITE BLOOD CELL COUNTS OF MICE BEFORE
AND AFTER EXPOSURE TO
R. SANGUINEUS LARVAE

Cell Type	Immature Mouse								Mature Mouse							
	Experimental				Control				Experimental				Contol			
	Male		Female		Male		Female		Male		Female		Male		Female	
	B. E.	A. E.	B. E.	A. E.	B. E.	A. E.	B. E.	A. E.	B. E.	A. E.	B. E.	A. E.	B. E.	A. E.	B. E.	A.E.
Total Leukocytes	6750	13250	6700	9000	4800	4150	4250	7900	5700	4100	5200	9800	5800	5300	5300	8100
Neutrophils %	76	88	65	84	64	32	56	23	91	87	74	23	44	46	67	53
Lymphocytes %	23	5	29	14	34	65	42	72	9	10	24	72	52	49	28	42
Monocytes %	1	5	5	2	2	2	2	2	-	2	2	2	2	3	2	3
Eosinophils %	-	1	1	-	-	1	-	2	-	1	~	2	2	2	3	2
Basophils %	-	1	-	-	-	-	-	1	-	-	-	1	-	-	-	-

* B.E. Before Exposure
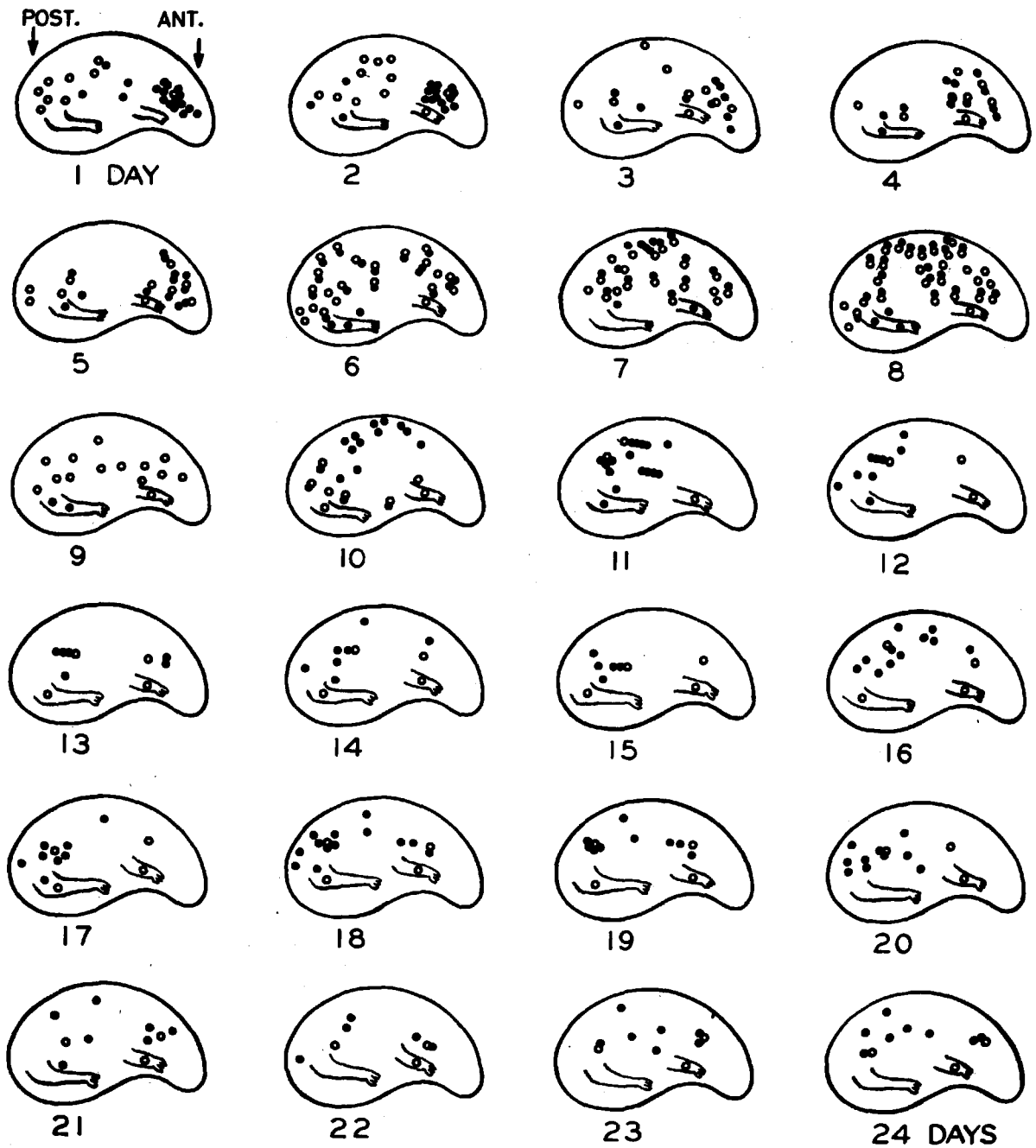** A.E. After Exposure

Fig. 4. The position of male (closed circle) and female (open circle) ticks on a hedgehog on different days following exposure. In each drawing, ticks on both sides of the animal appear on one face.
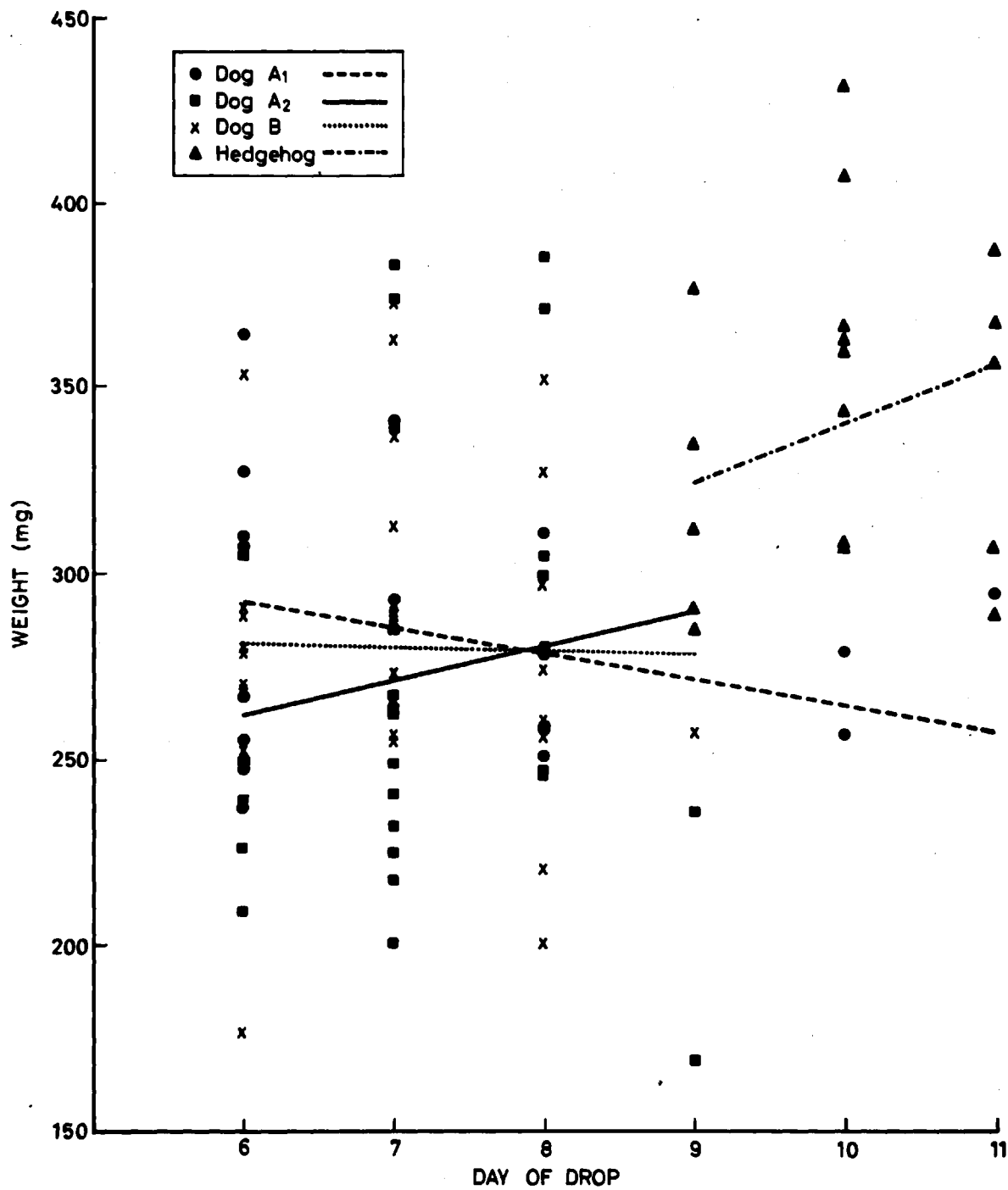
Fig. 5. The weight of female <u>R</u>. <u>sanguineus</u> at the day of drop from 4 hosts after different periods of exposure. The lines denote the change in weight with time.

males were more scattered and 2 were in apposition with 2 females. By the
fourth day, imbibition was apparent in females which were in apposition with
males. From 4 to 6 days, the number of paired ticks increased but there
were still individuals of both sexes not in apposition. By 9 days most females
had imbibed fully and no longer had males in apposition. It was also on this
day that the first female tick dropped. After 2 more days most of the ticks
dropped (Fig. 4). There were 4 females, however, that were sometimes seen in
apposition with males but which had not imbibed fully even by the eighteenth
day. On this day, all males were picked off and 9 previously unused males
were applied to the hedgehog to ascertain whether these males would induce the
4 remaining females to complete their imbibition. Subsequent daily observa-
tions showed no visible change, although they were seen in apposition with
the new males and persisted as such until the experiment was terminated at
24 days (Fig. 4).

The site of attachment of the ticks on the hedgehog suggested no
predilection to a particular body region. On the dog, on the other hand,
even though, the ticks were applied to the back and neck of the animal behind
the fiberboard collar, almost all the ticks became attached on the under side
of both ears. Two males and two females, however, attached to another part
of the body. This demonstrated a distinct movement by most of the ticks
from the dog's back to its ears.

The period of imbibition differed on the two hosts. In the case of
the hedgehog, 19 females dropped from 9 to 11 day following exposure, while
18 females dropped from 6 to 11 day from the dog. The absolute number of ticks
that dropped from the hedgehog was probably higher, when compared with the
original number applied, because of some loss attributable to the foraging

habits to the hedgehog.

The weight of the ticks, as determined on the day of drop, also differed between the hosts. Fig. 5 shows that ticks that dropped from the hedgehog weighed considerably more than those from the dog. In the former, there was an increase in weight with an increased period of imbibition $(y = 324.26 + 10.70 \ x)$. In dog A1, however, the longer the period of attachment the less the average weight of the ticks $(y = 297.66 - 5.55 \ x)$.

The possible effect on the period of imbibition of an unequal sex ratio among the ticks was assessed in two experiments by using twice as many males as females in one experiment and half as many in another experiment. In the first experiment, two immature dogs (dog A2 and dog B in Fig. 5) were used. Dog A2 was the same individual (dog A1) used in the previous observations. Each dog was exposed to 71 male and 35 female ticks. The ticks used were from the same source as in the hedgehog-and-dog experiment, but were 14 days older. In both dogs, as noted previously in the case of dog A1, ticks crawled from the back to the head and attached to the underside of the ears. Imbibed females started to drop 6 days after exposure and continued to do so for the following 4 days. This period of drop was 2 days shorter than was the case for dog A1. The total number of female ticks that dropped was 27 from dog A2 and 31 from dog B. There was an average weight increase $(y = 261.60 + 9.43 \ x)$ in the ticks during the period of drop for dog A2, while there was a slight decrease $(y = 282.35 - 1.13 \ x)$ in those that dropped from dog B (Fig. 5). The weight of the female tick does not, therefore, appear to be related to the period of attachment. It is possible that the presence of excess males in this experiment shortened the time required by the males to locate females and thus shortened the overall period of drop when compared with dog A1. There appeared

to be no relationship between the period of attachment and subsequent pre-oviposition period when the ticks were held at 21° to $25^{o}C$ and about 93% relative humidity. The periods were 8 to 11, 6 to 10, 5 to 7 and 5 to 10 days for dog A1, dog A2, dog B, and the hedgehog respectively.

In another experiment, half as many males (10) as females (20) were applied to an immature dog (dog C). The effect of this sex ratio on the period of imbibition of the females was noted. The ticks were applied as previously and each tick was marked with a different color or combination of colors for purposes of individual tick identification. Each tick was assigned a number (1-10 for males; 11-30 for females) according to its color code in the report to follow. Most ticks attached to the underside of the ears as in the case of the previous dogs. Fig. 6 shows that 8 females and 5 males reached the ears and became attached on the first day. There was one more female on the second day and 3 more on the third day. The number of males remained the same. On the third day, 2 of the females that were seen attached to the ears on the previous 2 days were now in apposition with males. This pairing persisted for 2 days in one instance (29 and 4) after which another male (8) which has been observed attached to the back of the dog for the four previous days, replaced male 4. This situation lasted for the following 2 days after which the female dropped. In the other instance (25 and 3) the pairing lasted for four days after which male 3 was replaced by male 8 and on the eighth day the the female (25) was not seen, while male 8 was seen in apposition with another female on the other ear. On the ninth day, female 25 was again seen on the ear and was again in apposition with male 3, a situation that lasted until the female dropped on the twelfth day. In 2 other instances, four days were
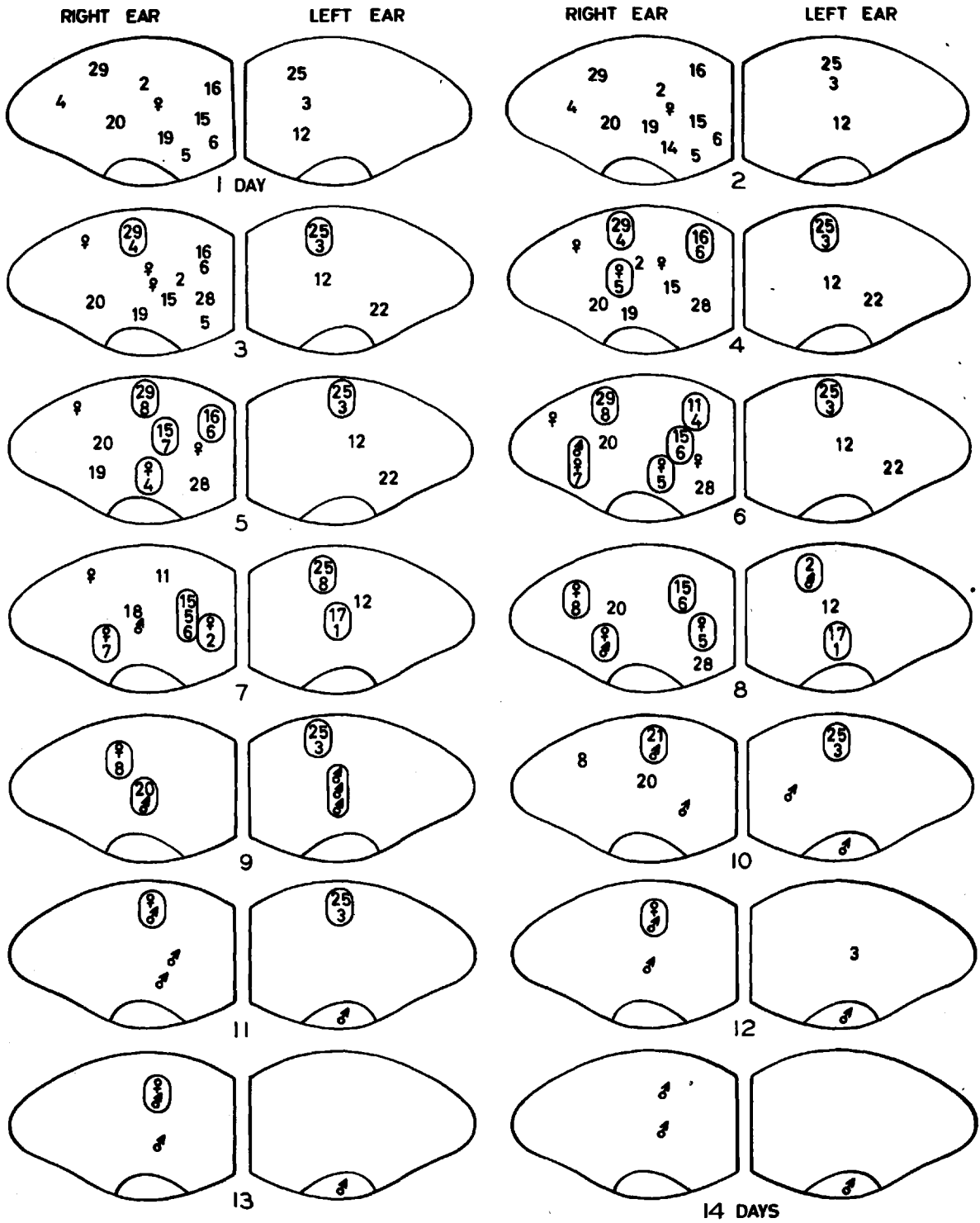
Fig. 6. The position of male (1-10) and female (11-30) ticks on the underside of the ears of a dog on different days following exposure. Ticks which could no longer be identified individually because of loss of their color code are indicated by the appropriate symbol for their sex.

required before males were seen in apposition (16 and 6; ♀ and 5). Two other
new combinations (15 and 7;♀ and 4) were seen at 5 days, and a new female with
2 males (♀, 7 and ♂) were observed at 6 days. When the females dropped, the
males showed some tendency to cluster together.

The overall period of drop was from 6 to 14 days. This is longer than
the 6 to 11 days observed for dog Al when the sex ratio was equal, and longer
still than the 6 to 9 days for dogs A2 and B where the males were twice as
numerous as the females. Moreover, the absolute number of the imbibed females
that dropped in this case was half of that recorded from dog Al with an equal
sex ratio even though the absolute number of femaleswas the same initially.
It seems possible that the period of imbibition, and perhaps also the number,
of female R. sanguineus that fully imbibe are controlled, at least in part,
by the availability of males.

DISCUSSION

Distinct trends have been reported in the literature concerning the effect of age, sex and cortisone treatment of hosts infected with different parasites, especially helminths and protozoa. Apparently no work has been attempted previously to assess the significance of the above factors on tick infestation on a host. In the present study, mature mice were less suscepti- ble than immature mice to R. sanguineus larvae, demonstrating the importance of the age of the host in this infestation. Little work in this respect has apparently been done with other ectoparasites. Rothschild and Ford (1964a) reported that young rabbits were more attractive to fleas than old rabbits. Cook and Beer (1958), on the other hand, noted that old male meadow voles (Microtus pennsylvanicus) harboured more lice (Hoplopleura acanthopus) than young males.

The sex of the host was found to be significant in the number of R. sanguineus larvae infesting mice. Immature male mice were more susceptible than immature females, while mature males were slightly more susceptible than mature female mice. When male mice were injected with oestrogen there was a pronounced effect of this treatment in rendering both immature and mature mice less susceptible to R. sanguineus larvae. The number of larvae that dropped from these mice was even fewer than that from the untreated mature mice. This showed that the positive effect of a difference in age noted previously disappeared upon treatment with oestrogen. Cook and Beer (1958) reported that male meadow voles harboured more lice (H. acanthopus) than females. Rothschild (1964) noted the same observation with bird fleas in the family Ceratophyllidae as well as with various fleas on small mammals.

Bat fleas of the genus Ischnopsyllus, however, as reported by the same author showed that more fleas crowd onto female bats than male bats during the migration of the host.

Cortisone is known to be an anti-inflammatoy substance and to break down the host's resistance to parasitism. Nelson (1962) in his work on the sheep ked (Melophagus ovinus) noted that cortisone treatment of lambs broke down the acquired resistance. This resistance was later suggested by Nelson and Beimborough (1963) to be primarily attributable to the vasospasm that cut the blood supply to the skin. Keds were therefore unable to obtain sufficient blood and died from starvation.. The breakdown in resistance by cortisone was manifested by the establishment of a greater number of keds on the cortisone-treated lambs than on the controls, and by shortening the period of imbibition of this parasite.

In the current study cortisone increased the susceptibility of immature male, immature female, and mature male, but not mature female mice to R. sanguineus larvae. The mature female showed the reverse trend with the controls dropping more larve than the cortisone-treated mice. Cortisone treatment of mice of both sexes reduced the significance of the sex difference noted above. The age difference that was encountered with immature males was also rendered less significant. If cortisone increases the output of oestrogen in females, and testosterone in males, then the increased resistance of females associated with both cortisone and oestrogen in contrast with the decreased resistance of males injected with cortisone which are already more susceptible than females constitute consistent observations.

The period of imbibition of the larvae of the cortisone-treated mice, except for the adult females, was one day longer than in controls. It is tempting to suggest that the reduced inflammatory reaction presumably associated with the larval attachment in the cortisone-treated adult male, immature male, and immature female mice affected the host in such a way that it was less able to reject ticks from the skin as it would a foreign body.

Adult R. sanguineus occur far more commonly on dogs in Lebanon and elsewhere in spring than at other times of the year, suggesting a "spring-rise" in this parasite. We can only assume that this may also be true for the less obvious immature stages. Cortisone administered experimentally increased the susceptibility of adult male mice. During the rodent mating season in early spring, fighting and increased "stress" with increased cortisone output among male rodents might, therefore, make them more susceptible to R. sanguineus larvae. The laboratory observation that immature mice are more susceptible than mature animals may also be significant in this regard, since it is springtime that immature rodents are most prevalant in nature. It follows that the physiological state of the host may be more significant in the "spring rise" phenomenon amongst ticks than has been realized heretofore.

In natural infestations, some species of adult ticks are found attached to particular regions of the host's body. A distinct movement by the tick from the point of contact to the site of attachment has been conjectured, but there appears to be no experimental evidence to confirm this. In the present study, adult R. sanguineus showed no obvious predilection to a particular site on the body of a hedgehog, while on 3 dogs most ticks moved from their backs to the ears. Throughout the experiments, ticks of both

sexes were also seen attached between the toes of the dogs, but the females did not fully imbibe. The fully imbibed female ticks that dropped from the hedgehog showed an increase in weight with the period of imbibition, while in the dogs there was no clear relationship between these two factors. Besides this hostal difference, it was also apparent that the number and period of imbibition of female R. sanguineus were dependant in part on the presence of males. Imbibition by adult R. sanguineus is, therefore, controlled by different factors than those noted previously for larval R. sanguineus.

CONCLUSIONS

1. Age: young mice were more susceptible to R. sanguineus larval infestation than adult mice.

2. Sex: immature male mice dropped more larvae than immature female mice. This difference was not significant in mature animals.

3. Cortisone treatment: the treatment of mice with cortisone rendered the immature female, and mature and immature male more susceptible to infestation by R. sanguineus larvae. Mature females showed the reverse trend, but the difference was not significant. This hormone increased the susceptibility of both mature and immature male mice. The susceptibility of the former was elevated to a height to nullify the difference noted above that was attributable to age. The highly significant difference between the sexes in immature mice under no treatment was also nullified upon cortisone administration, while in mature mice cortisone treatment induced a sex difference favouring male susceptibility.

4. Oestrogen treatment: both immature and mature male mice treated with oestrogen became more resistant to R. sanguineus larvae. The susceptibility of immature male mice was lowered to an even lower level than that of the control mature mice.

5. In dogs, adult R. sanguineus moved from their back to the underside of the ears to complete their imbibition. A comparable predilection was not observed on a hedgehog.

6. The period of imbibition of adult female R. sanguineus appeared to be partly influenced by the species of the host infested.

7. A dearth of male ticks led to a smaller number of female ticks imbibing, and prolonged their period of imbibition.

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