LABORATORY INVESTIGATIONS ON THE EFFECT OF INSECTICIDES AND HOST IMMUNISATION FOR CONTROL OF TICK INFESTATION

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THIS THESIS IS LOVINGLY DEDICATED

TO MY MOTHER

ABSTRACT

The thesis is in two parts. In the first, three species of economically important African ixodid ticks, Rhipicephalus appendiculatus, Amblyomma hebraeum and Amblyomma variegatum were investigated for the first time for their susceptibility to three newly developed photostable synthetic pyrethroids with long residual activity, permethrin, cypermethrin and decamethrin. Results of tests on unfed larvae, unfed nymphs and unfed adult ticks using different methods of testing such as the immersion technique, dipping, impregnated packet technique, "tea bag" technique and topical application are presented, and the relative susceptibility of the different species and of different developmental stages to the three pyrethroids is compared. A limited number of experiments were also done on the effect of the compounds on engorged nymphal ticks. The potential of the synthetic pyrethroids in the control of multi-host tick ectoparasites of animals in Africa is discussed.

The second part of the thesis is concerned with investigations on acquired immunity or resistance in host animals to tick infestation and its role in tick infestation of animals. This was investigated by using small animal models, rabbits and guinea pigs, and four species of ixodid ticks, R. appendiculatus, A. hebraeum and A. variegatum and an European species, Dermacentor marginatus. The development of immunity in the animals following a primary infestation with one species of tick, to a second infestation with the same species, and of cross-immunity to a second species, were investigated. Sera from immune animals were tested by serological methods for antibodies to tick salivary gland antigens. In a limited series of experiments, infection of animals with blood pathogens and the injection of tick

cell extracts and larval tick homogenates into animals were investigated for their effect on development of immunity to tick infestation.

The role of chemical acaricides and of acquired host immunity as an alternative or additional method to chemical control in the overall strategy of tick management on animals is discussed in the light of results obtained from the investigations.

LIST OF CONTENTS

List of Contents (Continued) Page No.

List of Contents (Continued) Page No.

List of Contents (Continued) Page No.

List of Contents (Continued) Page No.

List of Contents (Continued) Page No.

List of Contents (Continued) Page No.

LIST OF TABLES

LIST OF FIGURES

Fig. 36 Results of immersion tests with permethrin against 5 to 8 week old unfed larvae of R. appendiculatus, A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills

Fig. 37 Results of immersion tests with cypermethrin against 2 to 4 week old unfed larvae of R. appendiculatus, A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills

Fig. 38 Results of immersion tests with cypermethrin against 5 to 8 week old unfed larvae of R. appendiculatus, A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills

Fig. 39 Results of immersion tests with decamethrin against 2 to 4 week old unfed larvae of R. appendiculatus, A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills

Fig. 40 Results of immersion tests with decamethrin against 5 to 8 week old unfed larvae of R. appendiculatus, A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills

Fig. 41 Results of immersion tests with amitraz against 2 to 4 week old unfed larvae of A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills

Fig. 42 Results of immersion tests with amitraz against 5 to 8 week old unfed larvae of A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills

Fig. 43 Results of immersion tests with carbaryl against 2 to 4 week old unfed larvae of A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills

Fig. 44 Results of immersion test with carbaryl against 5 to 8 week old unfed larvae of A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills

Fig. 45 Results of tests by the tea bag technique with permethrin against 2-, 5- and 8-week old unfed nymphs of R. appendiculatus. Regression lines relating to concentration and percentage kills

Fig. 46 Results of tests by the tea bag technique with cypermethrin against 2-, 5- and 8-week old unfed nymphs of R. appendiculatus. Regression lines relating to concentration and percentage kills

Fig. 47 Results of tests by the tea bag technique with decamethrin against 2-, 5- and 8-week old unfed nymphs of R. appendiculatus. Regression lines relating to concentration and percentage kills

Fig. 48 Results of weekly tests by the tea bag technique with permethrin against 1- to 10- and 12-week old unfed nymphs of R. appendiculatus. Regression lines relating to concentration and percentage kills

Fig. 49 Susceptibility of unfed nymphs of R. appendiculatus of different ages to permethrin. Results of weekly tests by the tea bag technique. Regression line relating to time in weeks and LC_{50} values.

Fig. 50 Results of tests by the tea bag technique with amitraz and carbaryl against 2-week old unfed nymphs of R. appendiculatus. Regression lines relating to concentration and percentage kills

Fig. 51 Results of tests by the tea bag technique with permethrin against 2-, 5- and 8-week old unfed nymphs of A. hebraeum. Regression lines relating to concentration and percentage kills

Fig. 52 Results of tests by the tea bag technique with cypermethrin against 2-, 5- and 8-week old unfed nymphs of A. hebraeum. Regression lines relating to concentration and percentage kills

Fig. 53 Results of tests by the tea bag technique with decamethrin against 2-, 5- and 8-week old unfed nymphs of A. hebraeum. Regression lines relating to concentration and percentage kills

Fig. 54 Results of tests by the tea bag technique with amitraz and carbaryl against 2-week old unfed nymphs of A. hebraeum. Regression lines relating to concentration and percentage kills

Fig. 64 Results of tests by the tea bag technique with cypermethrin and decamethrin against unfed females and males of R. appendiculatus. Regression lines relating to concentration and percentage kills at 14 days post-treatment.

Fig. 65 Results of tests by the tea bag technique with decamethrin against unfed females and males of A. hebraeum. Regression lines relating to concentration and percentage kills at 7 and 14 days post-treatment

Fig. 66 Results of tests by the tea bag technique with decamethrin against unfed females and males of A. variegatum. Regression lines relating to concentration and percentage kills at 7 and 14 days post-treatment

Fig. 67 Results of tests by the tea bag technique with decamethrin against females and males of R. appendiculatus, A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills at 7 days post-treatment

Fig. 68 Results of tests by the tea bag technique with decamethrin against unfed females and males of R. appendiculatus, A. hebraeum and A. variegatum. Regression lines relating to concentration and percentage kills at 14 days post-treatment

Fig. 69A T.S. salivary gland of R. appendiculatus + normal rabbit serum

Fig. 69B T.S. salivary gland of R. appendiculatus + R. appendiculatus rabbit serum (R 60)

Fig. 69C T.S. salivary gland of R. appendiculatus + A. hebraeum rabbit serum (R 58)

1. GENERAL INTRODUCTION

Ticks belong to the Superfamily Ixodoidea of the Order Acarina. All ticks are obligate ectoparasites and suck the blood of a variety of cold-blooded and warm-blooded vertebrates including man. The three families under the Ixodoidea are the leathery Argasidae without a scutum (the "soft" or argasid ticks) with about 140 species, the Ixodidae (the "hard" or ixodid ticks) with over 650 species and the Nuttalliellidae with a single species confined to South and South West Africa (Hoogstraal 1973). The latter two have a scutum covering part of the larval, nymphal and adult female dorsum and the entire adult male dorsum. The Argasidae and Ixodidae are cosmopolitan in distribution, but the Ixodidae are biologically more diverse than the Argasidae and occur in all natural and climatic zones including the Arctic and Antarctic regions (Balashov 1972). Ticks have been known and recognised since ancient times, but it was only when the world cattle population increased rapidly in the second half of the nineteenth century to provide beef, hide and dairy products for the human population of the great industrial centres, that the diseases they transmit and their debilitating effect on cattle became serious problems (Wellcome Research Organisation 1976). Vast grassland areas in the Americas, S. Africa and Australia were exploited for cattle farming and the considerable cattle movements during this period with susceptible exotic breeds being introduced into tick-infested areas where tick-borne diseases occur, resulted in spectacular and colossal losses. Cattle movements are also believed to have been responsible for the introduction of Boophilus microplus (Canestrini) from Java to Australia in 1872. It was the discovery during this period by Smith and Kilborne (1893) of the transmission of Babesia bigemina, the causal agent of Redwater or Texas fever in cattle, by the cattle

tick Boophilus annulatus (Say), that generated world-wide interest in ticks and indeed in arthropods as vectors of animal and human pathogens.

Blood feeding in all the developmental stages of ticks (larvae, nymphs and adults) results in complicated life cycles involving one or several hosts of the same or different species, and regular alternation of parasitic and non-parasitic phases. In the multi-host life cycles of argasid ticks there are numerous blood feeding nymphal stages and several adult gonotrophic cycles. The number of vertebrate hosts is correspondingly great and may include one or more species. In ixodid ticks the three blood feeding stages, larva, nymph and adult feed only once. Thus the maximum number of host changes is only three and the hosts may be of the same or different species. In some ixodids, each parasitic stage feeds on the same animal and only the replete fertilised female drops to the ground to oviposit. This type of life cycle appears to be adapted to the large mammal hosts which roam over large distances. Examples of such 1-host ticks are cattle ticks of the genus Boophilus. In other ixodid species, the larvae and nymphs feed on the same host, usually small mammals or birds; the engorged nymphs drop to the ground and later on feed as adults on a second large host. Hyalomma spp. show this 2-host type of life cycle. The majority of ixodid ticks however, feed on separate animals during the larval, nymphal and adult stages. The animals may be of the same species or of different species; the immatures feed on small animals and the adults usually on large animals. The animal host of ixodids may be wild animals or domestic livestock, particularly cattle and sheep; about 10% of the world's tick species frequently parasitise domestic animals (Hoogstraal 1972). Ixodid species in which all stages can utilise the large numbers of domestic animals

present, can and do build up to high levels of abundance (Hoogstraal 1973). The African species Rhipicephalus appendiculatus Neumann and Amblyomma hebraeum Koch have immature stages which are not host specific and adults and highest numbers of immatures are found on ungulates (Norval 1979); consequently levels of cattle infestation achieved by adults of these species are high. Such species are also better adapted as vectors of diseases of their adults' hosts than those in which adults and immatures feed on different groups of hosts (Norval 1979).

Ticks affect man and his economy in various ways. The neurotoxin which the female ixodid tick injects with her saliva, causes a condition known as tick paralysis in animals and humans (Stampa 1959; Knott 1961; Pearn 1966; MMWR 1979). Sweating sickness of cattle, sheep, goats and pig in Africa is caused by a toxin produced by certain species of Hyalomma. The physical damage to hides by ixodids when they are present in large numbers is a serious economic problem. The profuse bleeding from tick bites and the local irritation and severe secondary infection by bacteria may result in abscesses and suppurating wounds attractive to myiasis-producing flies and lead to lameness and foot rot. These conditions are recognised by stock owners under the name "tick worry". General unthriftiness in tick-infested animals, anaemia and even death by exsanguination resulting from heavy tick loads are known (Barnett 1961); as many as 3000 - 4000 adult females of the sheep tick Ixodes ricinus L. per animal have been recorded on red deer in Scotland (Hendrick, Moore and Morison 1938). Thus there is a need to control ticks on domestic livestock even in the absence of tick-borne diseases. But most important of all is the ability of ticks to transmit animal and human diseases, and in some regions of the world ticks and tick-borne diseases constitute the most important limiting factor for successful livestock farming.

Ticks transmit and are the invertebrate hosts of viruses (Hoogstraal 1966), rickettsiae (Rehacek 1965; Hoogstraal 1967), spirochaetes (Varma 1962), bacteria (Balashov 1972), anaplasmas, babesias, theilerias and filariae (see review by Philip 1963). Ixodid ticks as vectors of trypanosomes have recently been reviewed by Weilgama (1979). Nearly all the tick-borne disease of domestic livestock are transmitted by ixodid ticks.

The three species of ticks used in my experiments are African in distribution and are of considerable economic importance as ecto-parasites of domestic livestock, particularly cattle and sheep. Rhipicephalus appendiculatus, the Brown ear tick, is found south of the Sahara and is the chief vector of Theileria parva, the causal organism of East Coast fever in cattle, the related T. lawrenci and T. mutans causing Corridor disease in cattle and African buffalo, and mild gall sickness in cattle respectively, and also of Babesia bigemina, the cause of Redwater in cattle. It is the chief vector of the tick-borne virus disease, Nairodi sheep disease and with Amblyomma hebraeum, the Bont tick, of the rickettsial disease Boutounneuse fever or African tick bite fever caused by Rickettsia conori which affects man. Recently, Crimean-Congo haemorrhagic fever virus (CCHF) has been isolated from R. appendiculatus in Uganda (Hoogstraal 1979).

Amblyomma hebraeum is confined to parts of S. Africa, Mozambique, Bechuanaland, Zimbabwe and Malawi. Together with R. appendiculatus it is the chief vector of tick bite fever and with Amblyomma variegatum, the Tropical Bont tick, of Heartwater in cattle, sheep and goats in East, Central and S. AFrica, caused by Cowdria ruminantium, and of T. mutans in cattle.

Amblyomma variegatum (Fabricius) is widely distributed south of the Sahara with the southern limit reaching Zambia and Angola. It is

possibly the most frequently seen tick in the African fauna, but it occurs outside the African region and has even been introduced into Puerto Rico and some islands of the West Indies where it has become common (Hoogstraal 1979). Besides transmitting Heartwater in cattle, A. variegatum is also probably a vector of tick bite fever in Kenya. Several arboviruses have been isolated from A. variegatum in Africa, e.g., CCHF, Nairobi sheep disease virus, Dugbe, Bhanja, Thogoto, Jos and Somone. This species is almost certainly the vector of CCHF in Africa. The Q fever rickettsia Coxiella burneti, and T. parva and T. mutans also infect A. variegatum.

All three species are 3-host ticks, the immature stages feeding on small mammals, birds and large mammals including man and the adults on medium sized and large domestic and wild animals. All reach high levels of abundance on domestic livestock. Because of their considerable veterinary and public health importance, their control is paramount to man's economy.

The main impetus for control and eradication of ticks has come from the cattle farming industry. Indigenous breeds of cattle, although less productive, have a measure of resistance to ticks and tick-borne diseases but the importation of high quality breeding stock which had no resistance to ticks or tick-borne diseases resulted in high levels of infestation and heavy mortality in the introduced animals. The direct and indirect economic losses due to B. annulatus infestation of cattle in the USA in 1906 was estimated at US dollars 130,500,000 per year, equivalent to a billion or more 1976 dollars (Graham and Hourrigan 1977) while in Australia, losses from ticks and tick-borne disease for 1894 - 1900 have been estimated at £3,500,000 annually (Francis 1966). Such losses are rare these days, because effective control is widely practised now and losses

are measured more by lowered reproductive capacity of tick-infested animals than by deaths. Even so about 80% of the present day 1000 million world cattle population is exposed to risk of cattle tick infestation (Harrison, Palmer and Wilmshurst 1973). But when control measures are relaxed or cannot be carried out as happened during the recent guerilla warfare in Zimbabwe, heavy infestations and deaths become serious problems once again. The magnitude of the tick problem was realised as early as the end of the 19th century and attempts were made to control tick infestation by treating cattle with various oils including paraffin, but without much success. Arsenical dips introduced into S. Africa in 1893 and into Australia in 1895 gave control against ticks but successful eradication was not achieved in these countries. Cattle ticks (B. annulatus) were eradicated from the USA with the introduction of arsenical dips in 1907. However the use of chemical acaricides has been followed by the development of resistance in the ticks. Introduction of newer compounds and their widespread use has perpetuated the problem and over the years ticks have developed resistance to arsenical compounds, as well as to organochlorine and organophosphorus insecticides. The problem has been compounded by the development of cross-resistance between related or between two different types of compounds. There has therefore been considerable pressure to develop newer and newer chemical acaricides with different structures and modes of action to meet the problem of resistance, but the development of resistance to the new compounds may only be a matter of time.

Alternative methods of controlling ticks have not received the attention that they deserve. The role of host resistance in tick control has been recognised for several years but the use of resistant cattle is a more recent development. Indigenous breeds of cattle show

heritable resistance to ticks and selection within indigenous (Bos indicus) x European (Bos taurus) herds has been suggested as a method for obtaining rapid improvement of tick resistant stock within the herd (FAO 1972). Animals also acquire resistance to ticks following a previous infestation and there has been considerable interest in recent years in the induction of resistance to ticks in animals by the inoculation of antigens derived from ticks.

Management of tick-infested pastures by cultivation, clearance, burning, elimation of wild fauna and withholding of livestock has also been practised. Recently Thompson, Roa and Romero (1978) showed that molasses grass (Melinis minutiflora) had tick-repellent properties and suggested that this could be used in a tick control package within a marginal tick zone.

The use of parasites and predators is another approach that might provide useful results. Hymenopteran parasites of ticks such as Hunterellus or Ixodiphagus and predators such as ants and oxpeckers (Buphagus spp.) have not been exploited on a large scale. The use of bacterial and viral pathogens of ticks (microbial insecticides) or of parasitic nematodes for tick control also needs to be more fully investigated.

Control by sterilisation of males (by irradiation or chemosterilants) is not practical (FAO 1972) but the use of insect growth regulators (IGR) which have been found to be effective against insects is certainly worth investigating. Obenchain (1979) has reviewed the use of non-acaricidal chemicals including pheromones, juvenile hormone analogues and anti-juvenilising chemicals such as precocene II in the management of ticks.

2. OBJECTIVES OF THE WORK

Rhipicephalus appendiculatus, Amblyomma hebraeum and Amblyomma variegatum are of considerable economic importance in Africa. Control of these species on domestic livestock has become a serious problem, since all of them have developed varying degrees of resistance to organochlorine and organophosphorus compounds. The newly developed synthetic pyrethroids, permethrin, cypermethrin and decamethrin have been shown to be effective against ixodid ticks, but there has been little work on the effect of these acaricides on the three African species. One of the objectives of the work was to investigate the effect of the pyrethroids and of the newly developed triazapentadiene compound, amitraz and of the carbamate, carbaryl on all stages of the three species, to assess their relative toxicities and to evaluate their potential in tick management in Africa.

In the strategy of tick management, although chemical control is still the most important method, alternative methods have been investigated. One of these, acquired host immunity to tick infestation, has created considerable interest recently. The observation which led to the study of host immunity was the reduced feeding performance of ticks on animals which had been previously bitten by ticks. However, most of the studies have been on animals on which the same species of tick was used for the primary as well as the secondary infestation. In nature, animals are often infested by more than one species, for e.g., R. appendiculatus, A. hebraeum and A. variegatum in Africa, and one of the objectives of the investigations on host immunity was to find out if there was any cross-immunity in animals infested by different species of ticks. Another objective was to study the effect of host immunisation by tick antigens on subsequent tick infestation.

Recent work in Australia has shown the interaction between infection of cattle with tick-borne pathogens and the development of immunity to tick infestation. Under natural conditions, animals are constantly under challenge by pathogens and ticks, and to find out if a similar phenomenon occurred when animals were infected with pathogens which are not tick-borne, the effect of Trypanosoma congolense infection in rabbits on tick infestation of the animals was investigated as a laboratory model.

3. INVESTIGATIONS ON THE CONTROL OF TICKS BY CHEMICAL ACARICIDES

3.1 INTRODUCTION AND REVIEW OF LITERATURE

The main impetus for control of tick infestation on animals has come from the cattle farming industry and control by chemical acaricides is still the most economical and widely used method for management of ticks on cattle. For these reasons, most of the work done on tick control has been with chemicals and on the one-host cattle ticks, Boophilus annulatus, B. decoloratus (Koch) and B. microplus in the great cattle farming areas of the Americas, S. Africa and Australia. Handpicking of adult ticks from small herds, particularly cows which are regularly milked had been practised for several years in the USA before control by chemical acaricides (Graham and Hourrigan 1977), but the increase in cattle movement and cattle farming towards the end of the nineteenth century called for more effective and reliable methods which could be applied to large herds.

Ixodid ticks spend the major part of their life cycle in vegetational undergrowth where the unfed stages wait for a host animal and the fed stages undergo development to the next stage. Only a small part of the total life cycle, which may last from a few months to 2 or 3 years, is spent on the host sucking blood. Therefore, at any one time the number of ticks actually on the hosts represents only part of the total population of ticks in an area. The rest is widely distributed in a diffuse manner in the vegetational undergrowth. The control of the non-parasitic stages by spraying pasture or herbage, 'area control', would therefore appear to be impractical or wasteful. However application of DDT to pasture has been used successfully in reducing greatly populations of Amblyomma americanum (L) in USA (Smith and Gouck 1945) and of B. microplus larvae in Australia

(Barnett 1961) while Gorchakovskaya (1955) in Russia obtained practically complete mortality of Ixodes persulcatus Schulze in forest plots with both DDT and BHC. Because of environmental pollution with DDT, Dmitriev (1978) tested the effectiveness of OP compounds, tetramethrin and the formamidine, Galecron (chlordimeform) in forest biotopes in Russia, against Ixodes persulcatus and Haemaphysalis concinna Koch. The OP compound fenchlorphos had a high acaricidal activity, the pyrethroid was less effective, Galecron gave good and long-time control of I. persulcatus. All compounds tested were less effective against H. concinna. Application of dieldrin, heptachlor and chlordane to vegetation has also been used to control A. americanum (Lancaster 1957). The organophosphorus compound fenitrothion applied at the rate of 0.3 to 3 kg per hectare depending on the time of year gave complete control of unfed Ixodes ricinus in a forest biotope in Czechoslovakia (Rupes et al. 1977). Where wild animals are the primary hosts of a tick species, area control may be the only feasible method of control. With pasture ticks, particular vegetation associations can often be identified as areas of high tick populations and selected for acaricidal treatment. According to Barnett (1961) pasture treatment could have some application in livestock husbandry against ticks with a pronounced and short seasonal incidence and DDT with its residual effect appears at present to be the acaricide of choice applied in amounts varying from 1 to 4.5 pounds per acre. Lindane dust (BHC) at 1.12 kg per hectare effectively controlled nymphs of Haemaphysalis spinigera Neumann in the forests of S. India for up to 6 weeks and for logistic and socio-religious reasons, area control rather than cattle treatment would appear to hold greater promise in controlling this particular tick species (Drummond et al. 1969).

The host animals represent concentrations of ticks which have been picked up during their wanderings and it was realised from a very early stage that treatment of the animals with acaricides was the more successful method for controlling ticks. The objective of acaricidal application to host animals is protection from the effects of tick infestation by killing the ticks as soon as they have boarded the host and the use of animals as bait to collect ticks and then treating them with acaricides to reduce the tick population. Of the various methods of applying acaricides to animals, dipping, spraying and hand-dressing are the ones most commonly practised with livestock. Administration of acaricides by mouth (systemic acaricides) to kill the feeding ticks has also been investigated though not widely practised.

Graham and Hourrigan (1977) have reviewed the evolution of tick control practices. Lime sulphur and various oils (for e.g., Beaumont oil which contained 1.5% sulphur) were used in early attempts to control ticks on livestock, but arsenic in the form of water soluble sodium arsenite (containing 0.16% to 0.22% arsenious oxide in water) was the first acaricide to be widely used against ticks on cattle and it is still one of the cheapest and most effective agents for cattle tick control (Barnett 1961). Arsenical dips were first used in S. Africa in 1893 and in Queensland in 1895 for controlling Boophilus spp. on cattle; in 1910 it was adopted in the USA as the recommended tick control agent and remained so for nearly 60 years. It had the advantages of being readily available, cheap, and water soluble, is effective against all stages and species of ticks and is probably more effective against the adult stages than most other insecticides (Barnett 1961). Its main disadvantages are toxicity for cattle and lack of residual activity, an important consideration when animals

are constantly being exposed to ticks. The residual effect may last for only 1 or 2 days (Barnett 1961), necessitating treatment at frequent intervals. In 1941 the existence of arsenic resistant B. decoloratus was demonstrated in S. Africa by Du Toit et al. (1941) and by 1947 it was widespread in the eastern coastal cattle rearing areas of S. Africa (Whitnall and Bradford 1947). Failure to control B. microplus on cattle in Australia was noticed in 1937 and subsequently confirmed by Hitchcock and Roulston (1955). In 1945-50 arsenic resistant B. microplus was encountered in many cattle rearing countries of S. America (Harrison, Palmer and Wilmshurst 1973). It is noteworthy however that in mainland USA there was no evidence of B. annulatus or B. microplus developing resistance to arsenic (Graham and Hourrigan 1977). In any case development of resistance is inherent in the use of any insecticide. To overcome arsenic resistance, nicotine was employed in Australia and S. Africa at a strength of 0.05% provided sodium arsenite was also present at a concentration of 0.16% to 0.20% (Barnett 1961).

The remarkable success of DDT during and immediately after World War II in controlling insects led to the widespread use of chlorinated hydrocarbon (CH) insecticides in controlling ticks during the years immediately following World War II. DDT (0.5%) plus gamma BHC (0.03%) was first used successfully in USA in 1946 to control B. microplus (Graham and Hourrigan 1977). In Australia DDT became available in 1946 for the control of B. microplus, but by 1955 the tick had developed resistance to the acaricide. Gamma BHC was introduced into S. Africa in 1949 and into Australia between 1949 and 1956. But by the early to middle 1950s, cattle ticks had become resistant to both compounds in USA, S. America, S. Africa and Australia with resistance developing after only two years of field

use in S. America (Harrison, Palmer and Wilmshurst 1973). Because of the unacceptably high residues of DDT and gamma BHC in the tissues of animals that might go to slaughter and the excretion of some acaricides in the milk of dairy cows, and because of the development of resistance, the chlorinated hydrocarbon (CH) insecticides were gradually replaced by organophosphorus (OP) compounds which were effective at lower concentrations, stable in cattle dips and less likely to produce persistent residues. The OP compounds commonly used were diazinon, dioxathion, coumaphos and chlorpyrifos (Dursban). OP compounds had been used in Australia since 1956 but by 1963 B. microplus had become resistant to them as well as to the carbamate, carbaryl. Cattle ticks in S. Africa and S. America also developed resistance to OP compounds. Although arsenite resistance appeared only after several decades of use, resistance to CH and OP compounds developed rapidly.

With widespread resistance to CH and OP compounds there was an almost frantic search on for newer acaricides and this resulted in a variety of newer compounds, a formamidine (chlordimeform), an imminopyrolixine (clenpyrin), a thiourea (chloromethiuron), a carbamate (promacyl) and a formamidine-like compound, amitraz (Wharton 1976). The synthesis of amitraz, a new triazapenta-diene compound (Palmer et al. 1971; Harrison et al. 1972, 1973) was reported at a time when CH and OP resistant cattle ticks were causing considerable concern. Laboratory tests and field trials showed it to be highly effective against B. microplus in Australia (Roy-Smith 1975; Nolan 1979), Ixodes ricinus in E. Scotland (Griffiths 1975) and R. appendiculatus and A. hebraeum in S. Africa (Baker et al. 1973). The compound also had an expellent effect on attached ticks and a residual effect against larvae of B. microplus lasting 7 to 10 days after treatment.

But it had the disadvantage of being unstable unless buffered to pH 10 or above with commercial hydrated lime (calcium hydroxide). Recently Baker and Woods (1977) reported that amitraz was degraded by bacteria (Pseudomonas and Achromobacter spp.) in cattle dipping tanks in S. Africa, although tick control was excellent provided hydrated lime was added to maintain the pH, or there was total replacement of the wash at each dipping.

3.1.1 Synthetic pyrethroids with residual activity

Pyrethrum, in the form of the dried flowers of Chrysanthemum roseum, had been known as Persian Insect Powder in Western Europe since the early part of the 19th century. Flowers from a related plant, C. cinerariaefolium is now grown commercially on a large scale in Africa and are processed to give pyrethrum extract. The extract consists of a mixture of 6 closely related chemical substances, the pyrethrine. The excellent insecticidal properties of natural pyrethrins and their low mammalian toxicity are well known and they have been used in the control of arthropods including ticks for a long time. The main sources of pyrethrins are Kenya and Tanzania. Attempts to mass-cultivate the plant in other countries have not been successful in the long term and supplies of the natural product are therefore limited and used almost exclusively in domestic aerosols or in slow-burning formulations in mosquito coils. The resulting high cost of pyrethrins and the uncertainty of supplies due to crop failures led to the synthesis of piperonyl butoxide as an efficient and economical synergist for pyrethrins. The first synthetic pyrethrin, allethrin, was synthesised in 1949 by Schecter et al., but it had only less than half the activity of natural pyrethrins. Synthetic pyrethroids with considerably more activity than natural pyrethrins

were subsequently synthesised e.g., bioallethrin, resmethrin, bioresmethrin and tetramethrin. These had in addition to the knock-down activity of natural pyrethrins, considerable killing activity and bioresmethrin is probably one of the most active compounds for insect kill commercially available at present. But all the compounds are photolabile which limits their use in outdoor situations, for e.g., in cattle dips for tick control and they have virtually no residual activity. The situation has changed dramatically in the last few years by the synthesis in Great Britain By Elliott and his colleagues (Elliott et al. 1973 a,b; Elliott et al. 1974 a,b) and in Japan of a new class of pyrethroids in which the photolabile centres of the molecular framework of pyrethroids are replaced by alternative groups giving much greater stability in air and light, while retaining at the same time high insecticidal activity and low mammalian toxicity. Of these Phenothrin synthesised by Fujimoto et al. (1973) in Japan has the stable alcohol only and therefore has limited residual activity. The three synthetic pyrethroids developed by Elliott and his colleagues are; permethrin (NRDC 143), cypermethrin (NRDC 149) and decamethrin (NRDC 161). The essential components are two new acids and a set of halogen analogues of chrysanthemic acid. The alcohols are 3-phenoxybenzyl in permethrin and α-cyano-phenoxybenzyl in cypermethrin and decamethrin. The α-cyano group increases both insecticidal activity and toxicity to mammals. The chlorine in the acid moeity in permethrin and cypermethrin is replaced by bromine in decamethrin. Permethrin and cypermethrin have both the cis and trans isomers while decamethrin has only the more active cis isomer. Fenvalerate synthesised by Ohno et al. (1974) combines the α-cyano alcohol with an acid lacking the cyclopropane ring; consequently it is not a real pyrethroid but has

many properties including high insecticidal activity of the newer synethtic pyrethroids.

In summarising the properties and applications of permethrin, cypermethrin and decamethrin, Elliott (1976) mentions as their characteristics, (1) greater stability in the field than some organophosphates and carbamates, (2) rapid metabolism and elimination from mammalian systems, giving low toxicity, (3) limited persistence in the soil and (4) greater potency than other insecticides. The future of pyrethroids in control of arthropod pests and disease vectors is discussed in an excellent review by Elliott et al. (1978).

The high insecticidal activity of permethrin, cypermethrin and decamethrin against Anopheles stephensi, Glossina austeni and Stomoxys calcitrans was shown by Barlow and Hadaway (1975); decamethrin was almost 700 times as effective as DDT and about 80 times as effective as dieldrin against A. stephensi. These authors also showed that permethrin and decamethrin were photostable, comparing favourably with the more persistent insecticides of other groups. Field evaluation of permethrin showed it to be effective against larvae of the mosquitoes Culiseta inornata and Psorophora confinnis yielding almost complete control of larvae and pupae at the rate of 0.025 lb/acre (Mulla and Darwazeh 1976). Thompson and Meisch (1978) measured the residual activity of permethrin on plywood panels against Anopheles quadrimaculatus and Psorophora columbiae. At the rate of 0.125 g/m^2, permethrin was highly effective against both species over a 10-week test period. At the same rate of application malathion provided only 89.7% control during the same period. In outdoor tests, the effective rate of permethrin had increased to 0.25 g/m^2, but malathion at 1 g/m^2 gave only 67% mortality after 4 weeks. Schreck et al. (1978) tested permethrin as a potential clothing treatment against blood-sucking

arthropods. It was highly effective against 5 species of mosquitoes (Aedes aegypti, A. taeniorhynchus, Anopheles albimanus, A. quadrimaculatus and Culex nigripalpus) and the minimum effective dose for 100% kill on contact ranged from 0.063 to 0.125 mg/cm^2. Treated clothing exposed to heavy or long lasting rains or total immersion for a considerable time did not appreciably reduce the effectiveness of permethrin, but washing in hot soapy water did. In laboratory tests and semi-field trials decamethrin at the rate of 0.5 - 1.0 ppb. gave complete mortality of larvae of the mosquito species C. quinquefasciatus; C. tarsalis and C. peus larvae were more tolerant (Lewis and Tucker 1977). Field evaluation of permethrin and decamethrin against Anopheles gambiae and Anopheles funestus mosquitoes in sprayed huts and dwellings in Upper Volta and Nigeria were done by Coosemans and Sales (1977; 1978) and by Rishikesh et al. (1978) respectively. Coosemans and Sales (1977) noted that mortality observed in wild populations was very low despite good residual properties. They ascribe this to the highly irritant effect of the compounds, causing the mosquitoes to leave the treated surfaces before they have absorbed a lethal dose. In the Nigerian studies, permethrin applied to the inside of dwellings and other sprayable surfaces at the rate of 0.5 g/m^2 and decamethrin at 0.05 g/m^2 satisfactorily reduced hut resting densities of both species of mosquitoes over the 12-week evaluation period, but they did not have a satisfactory impact on the general anopheline population due to the irritability which led to the escape and survival of many mosquitoes from treated huts, confirming the earlier observations in Upper Volta (Coosemans and Sales 1977). Both compounds showed long persistence, decamethrin more than permethrin, particularly on mud surfaces. The amount of insecticides absorbed by spraymen was minimal and in any case was excreted within 24 hours and

both insecticides could be considered safe for operatives and village inhabitants.

The new synthetic pyrethroids have been tested against other groups of insects also and found to be equally effective. In laboratory simulated stream experiments, permethrin was 10 times more toxic than chlorpyrifos methyl and about 40 times more toxic than temephos (Abate) against Simulium larvae (Muirhead-Thompson 1977). 90% mortality of three species of Tabanus was obtained for up to 18 days when water emulsion sprays of permethrin were applied to horses and ponies at the rate of 0.1% (Bay et al. 1976). Permethrin was also highly effective against the house fly Musca domestica, when applied as clothing treatment (Schreck et al. 1978); when applied to various building surfaces in protected and exposed situations it was effective for up to 14 days in outside situations but for up to 123 days when protected from the environment (Combs 1978). Permethrin was also highly effective as an outdoor ultra low volume spray against house flies in Egypt (Taha et al. 1979). Clothing treated with permethrin at the rate of 0.016 mg/cm^2 gave 100% control of the stable fly, Stomoxys calcitrans (Schreck et al. 1978). Barlow and Hadaway (1975) found that decamethrin applied topically to stable flies was 160 times as effective as DDT, but only 14 times as effective as dieldrin. The same compound at a concentration of 0.005% on a steer repelled 100% of exposed S. calcitrans; a concentration of 0.0001% killed 92% of the exposed flies and 30 - 50 mg of the technical material applied as spots on animals acted systemically against stable flies (Schmidt and Matter 1978).

Permethrin and decamethrin applied topically to tsetse flies, Glossina austeni were potent insecticides and decamethrin was 1000 times as effective as DDT and about 125 times as effective as dieldrin

against this species (Barlow and Hadaway 1975). In further tests Hadaway (1978) found permethrin, cypermethrin and decamethrin more effective than endosulfan against G. austeni and G. morsitans following topical application, but whereas endosulfan mortality rates increased as the post-treatment temperature increased from 18 - 20 C through 25 C to 30 C, mortality rates with the synthetic pyrethroids decreased. Barlow et al. (1979) found that permethrin, cypermethrin and decamethrin applied as a residual spray at 0.001 g/m^2 gave persistent mortality of G. austeni for up to 8 weeks and proved highly superior to dieldrin at 0.1 g/m^2. FAO (1977) recommended that permethrin and decamethrin because of their outstanding toxicity to tsetse flies and their persistence in aerial and ground spray residues, should be evaluated in the field.

Permethrin, cypermethrin and decamethrin have been evaluated in field trials against Glossina palpalis and G. tachinoides. In the Ivory Coast, blue tissue screens impregnated with 75 mg/m^2 of decamethrin and spraying of vegetation belt with 30 mg/km of decamethrin caused a significant immediate and long term reduction in G. palpalis populations (Laveissiere et al. 1979). The residual effect against G. palpalis gambiensis of the three pyrethroids and of the CH compound endosulfan was compared by spraying leaves in a test zone in Upper Volta (Challier et al. 1977). Decamethrin at 0.1% was effective for 5 months, almost twice as long as endosulfan; cypermethrin at 0.5% was effective for 13 - 15 weeks and permethrin at 0.1% was effective for only 1 week. A single helicopter application of decamethrin at the rate of 12.5 g/ha in the forest habitats of G. tachinoides in Upper Volta was extremely effective in controlling the flies for at least 38 days (Baldry et al. 1978). A dose as low as 0.36 g/ha of decamethrin also gave good control while permethrin at 1.9 g/ha gave

promising results (Wettere et al. 1978). In field trials in Chad, decamethrin applied at concentrations above 7.5 mg/l, although giving immediate total control of G. tachinoides had limited residual toxicity (Gruvel and Taze 1978).

Permethrin and decamethrin both have some effect on non-target organisms also. Dipteran populations in water were considerably reduced by residual application of decamethrin at 12.5 g/ha and the number of fresh water shrimps was reduced by 90%, although there was no recognisable effect on birds and fish (Takken et al. 1977). Wettere et al. (1978) therefore recommended that in spite of its high activity against tsetse flies, decamethrin should not be field tested on a large scale until more information was available on its impact on aquatic food chains.

Kamel in Egypt (quoted by Brooke 1976) found that permethrin had a level of activity against the body louse Pediculus humanus humanus at least comparable to that of temephos and malathion. Studies in Australia have shown that cypermethrin, the least tested synthetic pyrethroid, at 5 and 10 ppm in dips killed the sheep louse Damalinia ovis on sheep and prevented reinfestation for up to 19 weeks; permethrin at the same concentration was less effective (Hall 1978).

Minimum effective dose of permethrin against the bed bug Cimex lectularis causing 100% mortality when exposed to treated clothing, was > 1.0 mg/cm^2, the highest among several species of mosquitoes, flies, fleas and ticks (Schreck et al. 1978); but against the triatomine bugs Panstrongylus megistus and Triatoma infestans, vectors of Chagas' disease in Brazil, permethrin and decamethrin were more effective than BHC, the insecticide currently used in triatomine control; decamethrin at an average dose of 3 ng/insect of a 0.01% dust gave 100% mortality of P. megistus while BHC at 30 ng/insect had

no effect at all (Gilbert et al. 1979; Pinchin et al. 1979).

Clothing impregnated with permethrin at the rate of 0.032 mg/cm^2 caused 100% mortality of tropical rat fleas, Xenopsylla cheopis exposed to it (Schreck et al. 1978).

Roush and Hoy (1978) investigated the effect of permethrin on the spider mite Tetranychus urticae and its predator mite Metaseiulus occidentalis and found that application at ca 1 g/100 l. preserved 50% of the predators, but effectively controlled the spider mites. Permethrin at 0.01% was only marginally effective against the sheep scabies mite Psoroptes ovis and failed to eradicate all mites in 2 successive dippings (Maleney and Roberts 1979).

The high potency, long residual effect and photostability of permethrin, cypermethrin and decamethrin are prime considerations in their choice as acaricides, particularly as there is increasing evidence of resistance to organochlorine, organophosphate and carbamate compounds in ixodid ticks. Gladney and Dawkins (1976) found permethrin to be highly effective against nymphs of the brown dog tick Rhipicephalus sanguineus (Latreille), but less so than another synthetic pyrethroid, FMC 26061 and two OP compounds phoxim and chlorphoxim. Coumaphos was one of the least effective of 17 insecticides sprayed on to cattle to control adults of the lone star tick, Amblyomma americanum; 0.025 - 0.1% permethrin gave nearly 100% control after 1 day and 76% to 90% after 1 week and 33% to 45% after 3 weeks (Drummond and Gladney 1978); the standard treatment, toxaphene at 0.5%, gave 92% control after 1 day, 76% after 1 week and 18% after 3 weeks. The 25% cis : 75% trans isomer of permethrin was slightly less effective than the 40% cis : 60% trans ratio. Clothing impregnated with $>$ 1.0 mg/cm^2 of permethrin killed 100% of adult and nymphal lone star ticks in 15 min. following a 30 sec. exposure period; when the exposure period was increased to 2 min., 100% mortality was

obtained with an impregnation rate of 0.016 g/cm^2 (Schreck et al. 1978). Nolan et al. (1977) found decamethrin to be one of the most active acaricides ever tested against Boophilus microplus in Australia. OP-resistant strains of B. microplus were effectively controlled by permethrin and decamethrin (Nolan and Bird 1977); furthermore, the addition of small quantities of both compounds to OP acaricides produced efficient mixtures giving control of the OP-resistant strains. Breese (1977) found that cypermethrin was more effective than permethrin against larvae of a susceptible strain of B. microplus; cypermethrin and fenvalerate at 0.02% gave better than 99% control of B. microplus on cattle for 21 days and prevented reinfestation for 9 - 12 days. Nolan et al. (1977) found fenvalerate, pyrethrum extract, permethrin, cypermethrin and decamethrin, 20, 23, 33, 63 and 262 times respectively as effective as DDT against susceptible larvae of the cattle tick B. microplus. These authors concluded that the synthetic pyrethroids were highly effective in controlling susceptible and organophosphate and carbamate resistant strains of B. microplus.

Cross-resistance between insecticides in the same or different groups is one of the most difficult problems in pest management. As early as 1959, Whitehead observed cross-resistance to pyrethrum in DDT-resistant blue ticks (B. decoloratus) in South Africa. Cross-resistance between pyrethroids and DDT and its analogues has also been reported in house flies, Musca domestica in Denmark and Sweden (Keiding 1976), the mosquito Culex tarsalis (Plapp and Hoyer 1968), and in the stable fly Stomoxys calcitrans (Stenersen 1985). A DDT-resistant strain of Anopheles quadrimaculatus was nearly 4 times as tolerant to permethrin as a susceptible strain (Schreck et al. 1978). Nolan et al. (1977) demonstrated resistance to permethrin, cypermethrin and decamethrin in both larvae and engorged females of DDT-

resistant strains of B. microplus in Australia, although the more active decamethrin effected satisfactory control of the resistant strain at relatively low concentrations. According to Wilson (1978), although ticks can degrade permethrin quite rapidly, its prolonged persistence makes it a viable acaricide. Cross-resistance between OP compounds and pyrethroids has not arisen so far. Negative cross-resistance between OP compounds and pyrethroids was demonstrated by Chapman and Penman (1979) who found an OP-resistant strain of the spider mite, Tetranychus urticae more susceptible to the synthetic pyrethroid fenvalerate, than a strain which had not been exposed to insecticides.

3.1.2 Acaricide resistance in the African ticks, Rhipicephalus appendiculatus, Amblyomma hebraeum and Amblyomma variegatum

The number of tick species which have developed resistance to acaricides is few, but since they are of considerable veterinary importance, their existence is a threat to livestock economy. While resistance shows its highest incidence in one-host ticks of the genus Boophilus, probably because a large proportion of the total population is under constant chemical challenge, resistance has nevertheless developed in multi-host ticks (Wharton and Roulston 1970). The current status of resistance to acaricides in ticks of Africa south of the equator has been reviewed by Baker (1978), who points out that the development of chemical resistance in ticks has been accelerating in Africa for the past decade or more. Toxaphene and BHC have been highly effective in controlling ticks in Africa for many years, until Baker and Shaw (1965) showed toxaphene and BHC resistance in R. appendiculatus in equatorial and southern Africa. These authors suggested that there might be a linkage between toxaphene and BHC

resistance and that the resistant strains were not cross-resistant to OP insecticides such as delnav (dioxathion) and Supona (chlorfenvinphos) although resistance to some OP compounds has been recorded in South Africa (Wharton 1976). Resistance to toxaphene and BHC in this species was shown to be widespread. Resistance to both chemicals was reported in Rhodesia (Jones-Davies 1972), and to toxaphene in Uganda (Kitaka et al. 1970), Swaziland (Baker 1978), Malawi (Baker 1978), Zambia (Baker 1978), Kenya (Crampton and Gichanga 1979) and Tanzania (Wharton 1976). Arsenic resistant R. appendiculatus has been reported in South Africa and Swaziland (Mathewson and Baker 1975), in Rhodesia (Baker 1978), Malawi (Baker 1978) and Zambia (Baker 1978).

Acaricide resistance in A. hebraeum in S. Africa, Swaziland, and Rhodesia has previously been shown only to arsenic (Mathewson and Baker 1975), but in 1977 Baker et al. (1977) showed that larvae, nymphs and adults of several strains of this species were resistant to toxaphene also, and resistance to certain OP compounds has also been observed (Baker et al. 1978b).

Nayar and Isa (1973) observed resistance to toxaphene and BHC in A. variegatum in Tanzania and arsenic and toxaphene resistance in this species has also been reported from Zambia (Baker 1978) and Kenya (Crampton and Gichanga 1979).

Toxaphene and certain OP acaricides have been banned in Kenya because of resistance. Nevertheless toxaphene is still used in many African countries and may continue to be effective where resistance fails to develop or where resistant individuals comprise only a small proportion of the population.

Baker (1978) is of the opinion that chemical treatments used at present are unlikely to cope in the future with the continuing problem of resistance in Africa. Although the estimated research cost for

finding a new acaricide is in excess of US$ 20 million and likely to increase (Baker 1978), there may be no alternative to using newer acaricides. Baker et al. (1973) have described the effectiveness of the new compound amitraz against highly OP-resistant B. decoloratus. However, there is the perennial problem of resistance developing to any new chemical compound including amitraz and it is in this context that the new synthetic pyrethroids have a future in tick control.

When I started my investigations there was no published information on the effect of permethrin, cypermethrin and decamethrin on Rhipicephalus appendiculatus, Amblyomma hebraeum and A. variegatum.

3.2 MATERIALS

3.2.1 Ticks

The three species of ticks used in my experiments are African in distribution (Fig. 1) and were from laboratory colonies established in the Department of Entomology from OP-susceptible material from the Wellcome Research Laboratories (WRL), Berkhamsted. The original source of Rhipicephalus appendiculatus and Amblyomma variegatum was Kabete, Kenya and of Amblyomma hebraeum, Gulu Farm, East London, S. Africa. All were obtained from WRL as unfed larvae. The WRL R. appendiculatus maintained here for about 8 years had to be abandoned because the females became reluctant to feed on rabbits and guinea pigs. A new colony of R. appendiculatus was started in August 1977 from engorged females obtained from the Veterinary Investigation Laboratories, Kabete, Kenya. The A. hebraeum colony was started in September 1976 and the A. variegatum colony in October 1977. In some of the host immunity experiments, Dermacentor marginatus (Sulzer) from a colony established here in August 1978 from engorged females collected near Bratislava, Czechoslovakia, were also used.

FEMALE MALE

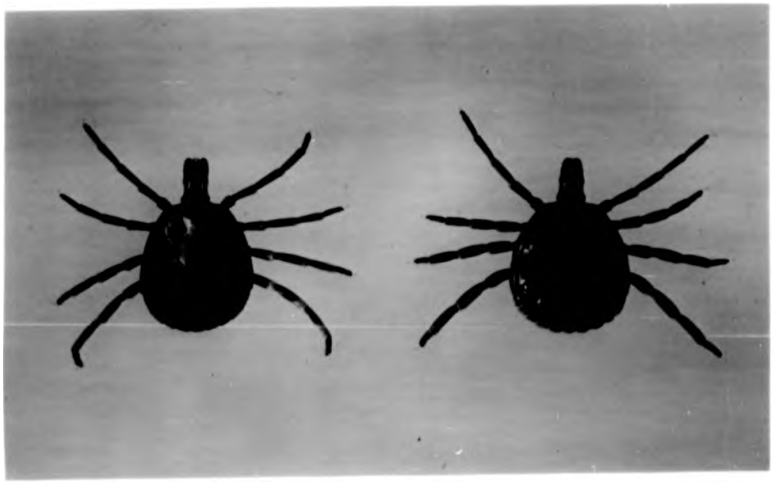
FIG. 1A
RHIPICEPHALUS APPENDICULATUS

FEMALE MALE

FIG. 1B
AMBLYOMMA HEBRAEUM

FEMALE MALE

FIG. 1A
RHIPICEPHALUS APPENDICULATUS

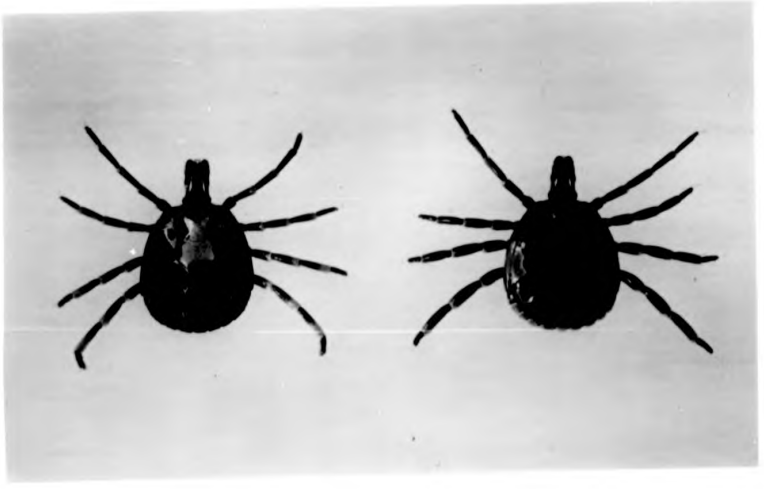


FEMALE MALE

FIG. 1B
AMBLYOMMA HEBRAEUM

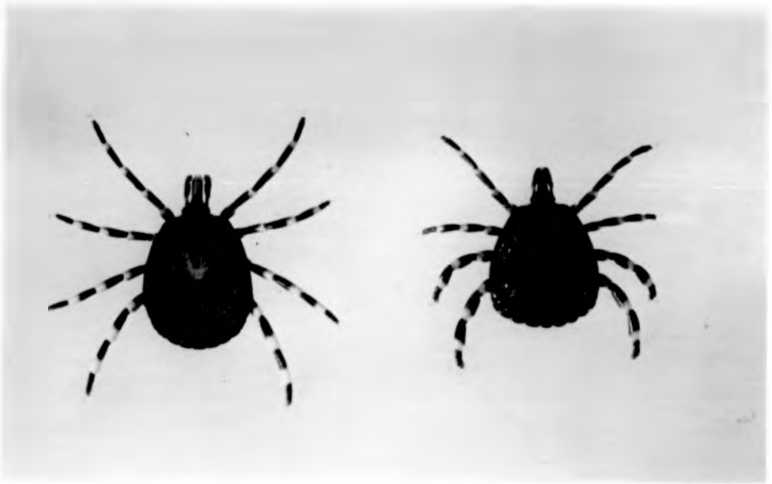
FEMALE MALE

FIG. 1A
RHIPICEPHALUS APPENDICULATUS



FEMALE MALE

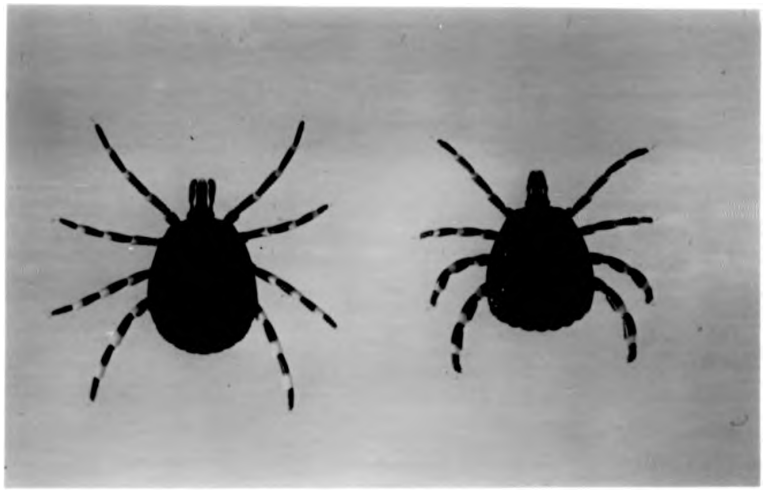
FIG. 1B
AMBLYOMMA HEBRAEUM



FEMALE MALE

FIG. 1C
AMBLYOMMA VARIEGATUM

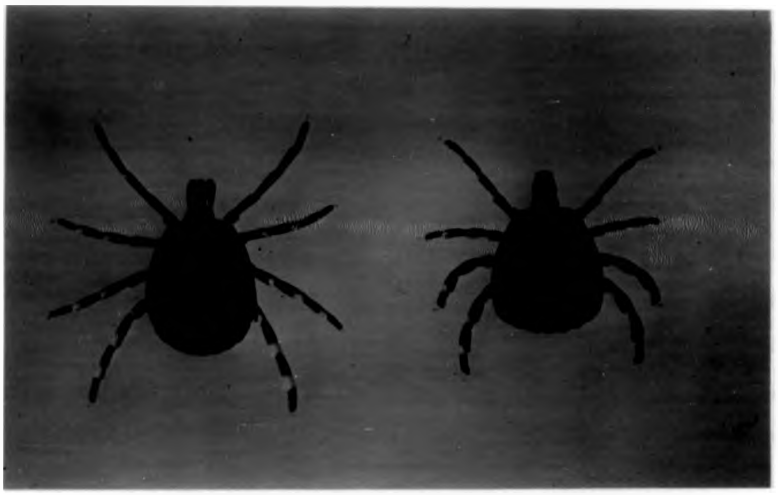
FIG. 2
TUBES CONTAINING TICKS COLOUR CODED FOR EASY IDENTIFICATION



FEMALE MALE

FIG.1C
AMBLYOMMA VARIEGATUM

FIG.2
TUBES CONTAINING TICKS COLOUR CODED FOR EASY IDENTIFICATION



FEMALE MALE

FIG.1C
AMBLYOMMA VARIEGATUM

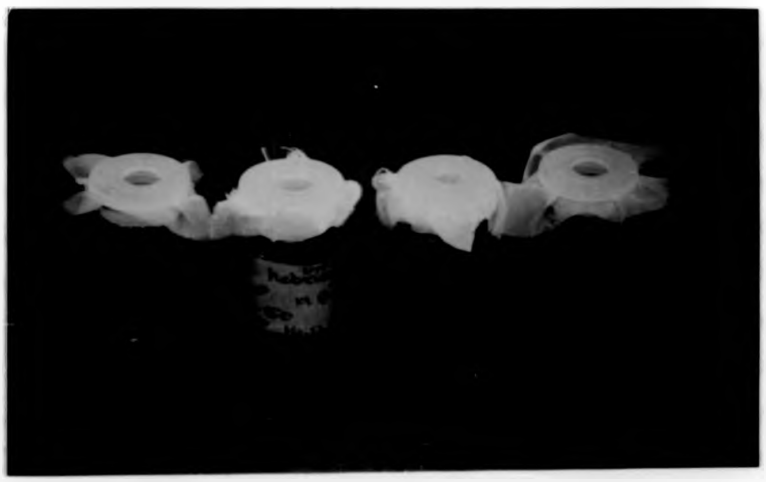


FIG.2
TUBES CONTAINING TICKS COLOUR CODED FOR EASY IDENTIFICATION

3.2.1.1 Maintenance of non-parasitic stages

Non-feeding ticks of all stages as well as engorged ticks which had detached from animals were kept in glass specimen tubes, 5 cm x 2 cm internal diameter (ID) or 3.5 cm x 2 cm ID closed with 4.5 cm^2 pieces of gauze (Oratex Dental Napkins No. 3) and stoppered with polythene caps with a hole approximately 1 cm in diameter in the middle for aeration (Fig. 2). Tubes containing the four species were colour coded for easy identification and to prevent mix-up. Fine nylon material instead of gauze was used for closures on tubes containing egglaying females, eggs and fed larvae to prevent escape of unfed larvae and unfed nymphs which could pass through the coarse meshes of the gauze. For obtaining mixed pools of eggs, fed females were placed in 14 cm diameter glass or plastic petri dishes, usually 5 to 10 females per dish depending on their size. After cessation of oviposition the eggs were pooled and distributed into tubes. By weighing egg masses from individual females of R. appendiculatus and counting the number of larvae emerging from egg masses of different weights, a regression line correlating number of larvae to egg mass was obtained. The number of larvae released on experimental animals could then be estimated by intrapolation of the weight of the egg mass from which the larvae had emerged, onto the regression line. Fed larvae of all species were collected and tubed in bulk; when they had entered the quiescent premoult phase, they were easier to handle and were tubed for emergence of nymphs in batches of 200 for R. appendiculatus and D. marginatus and 150 for A. hebraeum and A. variegatum.

In the initial period of the studies, the ticks were kept in a specially designed insectary with a moat surround containing Risella oil, and maintained at a constant temperature of 25 - 26 C and a photoperiod of 12 hours provided by one 80 watt and one 30 watt

fluorescent tube lights connected to a Venner daily cycle time switch. The high relative humidity of 80% required by the ticks was provided by 20% potassium hydroxide in distilled water in the lower chambers of glass desiccators and the ticks were kept in the upper chambers. Later on, non-feeding ticks were kept in a separate insectary, thermostatically and hygrostatically controlled at 25 - 26 C and 80% relative humidity respectively, and the first insectary used solely for feeding ticks on animals. The constant humidity in the second insectary was provided by a "Defensor 500" humidifier connected to a hygrostat and fed with distilled water. The 12-hour photoperiod in this insectary was provided by 2 80 watt and one 40 watt fluorescent tube lights controlled by a Venner daily cycle time switch. The working area where ticks were handled was protected by a moat surround containing Risella oil to prevent the escape of ticks.

Ticks treated with acaricides were kept in a separate laboratory, in desiccators with 20% potassium hydroxide to provide 80% relative humidity; these were placed in a cooled incubator (Gallenkamp, IH-287) maintained at 25 - 26 C. Timed cycling of illumination in the incubator was by a battery of six 8 watt fluorescent tube lights providing a photoperiod of 12 hours, thus ensuring that treated ticks were maintained in as near an identical regimen of temperature, humidity and photoperiod as stock ticks.

3.2.1.2 Maintenance of parasitic stages

Outbred female albino guinea pigs (Dunkin Hartley strain) and female New Zealand white rabbits obtained from commercial suppliers were used for feeding ticks. The guinea pigs ranged in weight from 600 to 800 g and the rabbits from 2 to 3 kg. Guinea pigs were usually used for feeding all stages of D. marginatus and larvae and nymphs

of R. appendiculatus, A. variegatum and A. hebraeum and rabbits were used for feeding adults of R. appendiculatus, A. variegatum and A. hebraeum. Unless otherwise stated, each animal was used only once and painlessly killed after completion of tick feeding by injection of veterinary "Euthatal" (Pentobarbitone Sodium BP, 200 mg in 1 ml, from May and Baker Ltd., Dagenham, England).

The animals were prepared for tick feeding as follows: they were anaesthetised by injection of "Saffan" Glaxo Laboratories, Greenford, England) at the dose of 1 ml per kg of body weight, intramuscularly into the dorsum of the upper hind leg. The feeding area was prepared by shaving the dorsum of the animals with an electric hair clipper to provide a clear area of approximately 25 cm^2 in guinea pigs and 50 cm^2 in rabbits. The shaved skin was disinfected with methanol to kill any surface bacteria which might cause secondary infection of the tick bite wounds. Cylindrical feeding sleeves (12 cm long and 11 cm in diameter for guinea pigs, and 16 cm long and 14 cm in diameter for rabbits) made of heavy duty drill were fixed to the shaved area with a proprietary adhesive, "Bostik 1" (Bostik Ltd., Leicester, England) and secured to the body with Zinc oxide adhesive tape (Vestric Ltd., London, England). "Elizabethan ruffs" or collars made from rigid polythene (ICI, England) round the neck of the animals prevented the feeding sleeves from being chewed or scratched off (Fig. 3). The animals were then placed in cages and left overnight for the fumes of the adhesive to evaporate. Although the feeding ticks were confined inside the sleeves, the cages with the animals were placed in trays of water to ensure that any escaping ticks were trapped. R. appendiculatus adults were fed on the unshaved ears of rabbits; the feeding bags in this case were conical (14 cm long, 8 cm in diameter at the bottom and 12 cm in diameter at the

FIG.3
RABBIT WITH SLEEVE AND COLLAR FOR FEEDING TICKS

FIG.3
RABBIT WITH SLEEVE AND COLLAR FOR FEEDING TICKS

top), totally enclosed both ears and were fixed to the head just posterior to the base of the ears.

The number of larvae released on the guinea pigs ranged from approximately 2000 of the larger A. hebraeum and A. variegatum to about 4000 of the smaller R. appendiculatus and D. marginatus. Up to 400 nymphs of R. appendiculatus (usually 200), 200 nymphs of D. marginatus, 150 nymphs of A. hebraeum and 150 nymphs of A. variegatum were released on each guinea pig or rabbit. 25 females and 30 males of R. appendiculatus or 10 females and 30 males each of A. hebraeum or A. variegatum were usually released on a rabbit. Males and females of R. appendiculatus were released simultaneously. Males of A. hebraeum and A. variegatum were released first and allowed to attach and start feeding, the females were released 3 to 5 days later.

It was simpler and safer to release ticks on animals by quickly opening the tube containing them and dropping it into the feeding bag through the open top rather than by transferring the ticks to the skin with a brush. The top of the bag was then quickly gathered together and secured tightly with tape. A couple of days later by which time the ticks would have attached, the feeding sleeve was opened, the empty tube removed and the sleeve closed again. The sleeves were opened every subsequent day and the engorged ticks collected and tubed.

3.2.2 Acaricides

I have followed Wharton and Roulston (1970) in using the term acaricide for chemicals used for the control of Acarina (ticks and mites) in the same way that the term insecticide has been used for Insecta and nematocide for Nematoda.

3.2.2.1 Compounds tested

The physical properties of the two pyrethroids, permethrin and cypermethrin are very similar; both are brown viscous liquids, with high boiling points (permethrin 200 C), barely soluble in water (permethrin ca 2 mg/L) but freely soluble in most organic solvents. Decamethrin is a white crystalline powder insoluble in water, but soluble in organic solvents, but to a lesser extent than permethrin and cypermethrin. Permethrin is commercially available as a 25% emulsifiable concentrate, a 15% transparent emulsifiable concentrate, a 25% wettable powder and a 0.5% dust. Cypermethrin is also available commercially as 2% emulsifiable concentrate and decamethrin as 2.5% wettable powder.

Amitraz is a crystalline non-hygroscopic solid. Solubility in water is very low, about 1 mg/l.; in acetone and toluene solubility is more than 300 g/l. It is stable at neutral pH but at acid pH 80% to 86% is decomposed within about 2 weeks (Harrison et al. 1973). It is available commercially as 25% and 50% wettable powder and 12.5% and 20% emulsifiable concentrate.

Carbaryl is a white crystalline compound. Its solubility in water is 40 mg/l. and it is soluble in most polar organic solvents. It is available in a variety of formulations, including: wettable powder, dust, bait pellets and suspensions in molasses and non-phytotoxic oil.

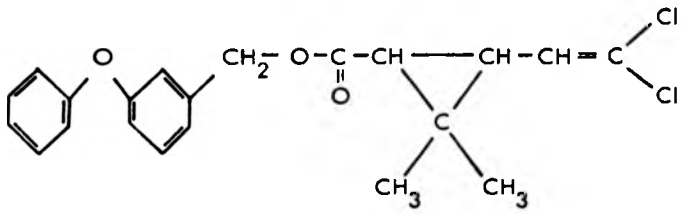
The compounds are listed in Table 1 and their structural formulae are shown in Figs. 4 and 5.

TABLE 1 - LIST OF COMPOUNDS TESTED

Common name	Code number	Proprietary name	Purity	Formulation	Source
permethrin 40% cis isomer 60% trans isomer	NRDC-143 OMS 1821 FMC 35297 PP 557 WL 43, 479	Ectiban Ambush Eksmin Kafil, Kestrel Perthrine	94% 15%	Pure liquid transparent emulsifiable concentrate	ICI England S.B. Penick & Co. USA
cypermethrin 40% cis isomer 60% trans isomer	NRDC-149	Barricade Ripcord	90%	pure liquid	ICI England
decamethrin* 100% cis. isomer	NRDC 161 OMS 1998	Roussel-Uclaf 22974 K-othrin Decis Deccotane	100%	technical grade powder	Wellcome Research Laboratories England
amitraz	BTS 27419 RD 27419	Tactik, Mitac Azaform Triatox, Baam Triazid	12.5%	emulsifiable concentrate	Boots Drug Co. England
carbaryl	US 7744	Sevin, Septon Dicarbam + 20 other names	85%	wettable powder	Duphar-Midox Ltd., Kent, England

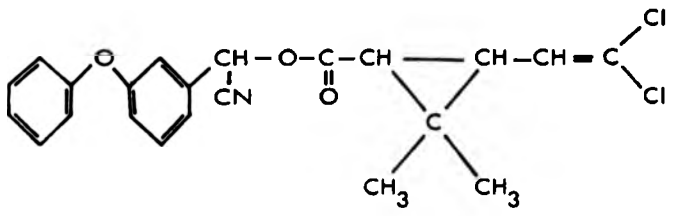
* decamethrin although widely used, has not been accepted as a common name for NRDC 161 by the International Organization for Standardization (ISO).

3-phenoxybenzyl (±) -cis, trans-3-(2,2-dichlo rovinyl -2,2-dimethylcyclopropane carboxylate



PERMETHRIN

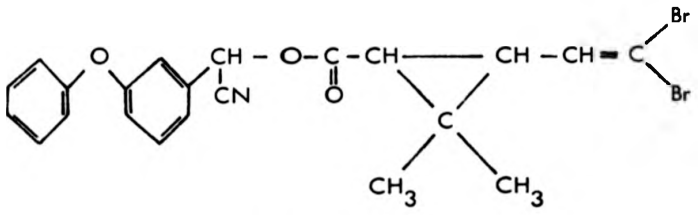
(±) -α-cyano-3-phenoxybenzyl (±) -cis ,trans -3- (2,2- dichlorovinyl) -2,2 -dimethylcyclopro panecarboxylate



CYPERMETHRIN

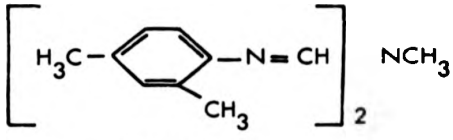
FIG.4
CHEMICAL NAMES AND STRUCTURAL FORMULAE OF PERMETHRIN AND CYPERMETHRIN

(-)- α- cyano-phenoxybenzyl (+) -cis-3- (2,2-dibromovinyl) -2,2 dimethyl cyclopropane carboxylate



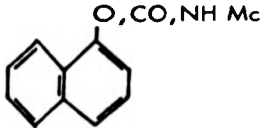
DECAMETHRIN

NN -di - (2,4 -xylyliminomethyl)-methylamine



AMITRAZ

1 - naphthyl methylcarbamate



CARBARYL

FIG.5
CHEMICAL NAMES AND STRUCTURAL FORMULAE OF DECAMETHRIN AMITRAZ AND CARBARYL

3.2.2.2 Preparation of acaridical solutions

3.2.2.2.1 Preparation of test solutions for immersion, dipping and tea bag technique

10% and 20% solutions of cypermethrin and decamethrin were prepared on a weight/volume basis in a 1:1 mixture of acetone and ethanol. From these a series of stock solutions ranging from 0.00001% to 5% were made with the same acetone-ethanol mixture as above. All stock solutions were kept at 4 C in a domestic refrigerator.

Before each test, a range of 4 to 6 working concentrations were made from the appropriate stock solutions as follows: 1.25 ml of each stock solution was mixed with 23.75 ml glass distilled water (containing 0.1% Tween 80) to give the desired working concentration which would be a 1:20 dilution of the stock solution.

1% solutions of permethrin and amitraz were made from emulsifiable concentrates on a volume/volume basis and of carbaryl from wettable powder on a weight/volume basis, in glass distilled water (containing 0.1% Tween 80). From these solutions a series of working concentrations were made in 25 ml glass distilled water (containing 0.1% Tween 80). To ensure thorough mixing and uniform dispersal of the acaricides, the concentrations were prepared in drinking cups ("Monogloss" PV7 squat white, Mono containers Ltd., England) cut down to hold 100 ml, or in "Thorpac" aluminium foil pie pans (capacity 60 ml, Thorpac Ltd., Cirencester, England) and kept constantly agitated till used with a magnetised follower on a stirrer. The "Thorpac" aluminium foil pie pans were usually used for immersion and dipping techniques and the plastic drinking cups for the tea bag technique.

Control solutions for tests involving permethrin, amitraz and carbaryl were 25 ml of glass distilled water containing 0.1% Tween 80.

In tests with cypermethrin and decamethrin the controls were immersed in a total volume of 25 ml glass distilled water with 0.1% Tween 80 containing 1.25 ml of 1:1 mixture of acetone and ethanol.

3.2.2.2.2 Preparation of test solutions for impregnated packet technique.

20% stock solutions of pure technical grade permethrin and cypermethrin, and 10% stock solution of technical grade decamethrin were made up on a weight/volume basis in chloroform. The solvent for making up the test solutions was a mixture of 2 parts of chloroform to 1 part of dry-heat sterilised pure olive oil to which an antioxidant, Ionol CP was added to a concentration of 0.02%.

3.2.2.2.3 Preparation of concentrations for topical application.

20% stock solutions of pure technical grade permethrin and cypermethrin, and 5% stock solution of decamethrin were prepared in acetone, on a weight/volume basis. From these serial dilutions were made to give a range of concentrations between 0.0008% and 0.1% of the acaricides.

3.3 METHODS OF TESTING

Evaluation of acaricidal efficiency in the field is virtually impossible and this is usually done in laboratory tests; however, the habits and life cycle of ixodid ticks are different from those of other blood-sucking arthropods and the methods of testing acaricides have to be modified accordingly.

Control of multi-host ticks present special problems. These ticks apart from parasitising domestic livestock, infest a variety of hosts, domestic and wild, from small mammals and ground frequenting birds to large herbivores and carnivores which may be difficult to

reach and treat. Wild animals will therefore provide a reservoir population for fresh infestation of domestic animals on which infestation could be controlled by acaricidal treatment. Treatment of domestic stock which can be gathered and treated is accepted as the most feasible method of tick control, since they represent concentrations of ticks which have been picked up during their wanderings.

Larvae, nymphs and adults of some species of multi-host ticks may have peak infestation on animals at different times of the year. It takes progressively higher doses of acaricides to kill larvae → nymphs → adults. Ticks in different stages of engorgement may be found attached to animals at the time of treatment. Since they may be more tolerant to acaricides than the unfed stages it is important to test fed larvae, nymphs and adults as well as unfed stages, and the dose used in field control should be one which has the most effect on fed adults. Although adult males do not suck blood or drink only small quantities, they can nevertheless transmit pathogens by bite through saliva. Therefore it is important to include males also in acaricidal tests.

There is no certainty that questing ticks would encounter a host animal immediately and there may be a considerable delay before this happens. It is therefore important to test ticks of different calendar ages for susceptibility to acaricides. Shaw (1966) found that 3-week old larvae of B. microplus were more resistant to dioxathion than younger and older larvae and suggested that tests should therefore be done with 2- to 3-week old larvae. Uspenskij and Lavikov (1974) tested the effect of DDT on natural populations of unfed I. persulcatus adults in Russia during the active season and found that the susceptibility increased continuously from the beginning to the end of the active season. Similar results were obtained by Rupes et al. (1977)

with fenitrothion on unfed I. ricinus larvae and nymphs in Czechoslovakia. Based on laboratory experiments and field trails, they found a marked correlation between increasing age of the ticks and increased susceptibility to the acaricides. Mathewson and Hughes (1978) found that there was a small change in susceptibility of larvae of R. evertsi Neumann, R. appendiculatus, Hyalomma rufipes Koch and A. hebraeum to chlorfenvinphos, dioxathion and carbaryl over a 4-week period, the older larvae being more susceptible than younger larvae.

I investigated the effect of ageing on susceptibility to permethrin, cypermethrin and decamethrin with unfed larvae of R. appendiculatus and A. hebraeum by testing them every week from 2 to 8 weeks after eclosion. In similar tests nymphs of R. appendiculatus and A. hebraeum were tested at 2, 5 and 8 weeks after moulting for susceptibility to permethrin, cypermethrin and decamethrin. In a more comprehensive experiment, R. appendiculatus nymphs were tested every week from 1 to 10 weeks and then at 12 weeks after moulting for susceptibility to permethrin.

Acaricides may affect ticks by straightforward kill, by arresting or delaying moulting of engorged stages, by expelling attached feeding ticks, e.g., chlordimeform (Gladney et al. 1974) and amitraz (Baker et al. 1973; Roy-Smith 1975), by preventing or reducing egg laying or by reducing egg viability. Some acaricides may also have a repellent/irritant effect which may actively repel questing ticks or make them fall off the treated animals which they have boarded. The residual effect of acaricides is also an important consideration in treatment of livestock since this will prevent reinfestation and extend the time between treatments. Depending on what stage is tested, the effect of acaricides on all these aspects will have to be considered although in

practice, it is usually only straightforward kill which is assessed.

In my experiments unfed larvae, unfed nymphs and unfed adult males and females were tested for the lethal effect of the acaricides and fed nymphs were tested for mortality as well as effect on moulting. The usual method for controlling attached ixodid ticks on domestic animals is by totally immersing or "dipping" them in an acaricidal bath (dip wash) or taking them through an acaricidal spray race (spray wash). Three of the widely used laboratory methods for testing acaricides against ticks parallel field treatment most closely and are: immersion, tea bag technique and dipping. The principle underlying all three is the same i.e., total immersion of the ticks in the acaricidal solutions, although the methods differ in detail.

3.3.1 Immersion technique for unfed larvae

In this technique for testing unfed larvae, the ticks are kept confined for a few minutes between a "sandwich" of two filter papers which is under the acaricidal solution in a petri dish (Shaw 1966). This is now one of the standard methods for testing unfed larvae and was used by me for R. appendiculatus, A. hebraeum and A. variegatum. Larvae were tested at varying intervals after hatching; in the majority of cases at weekly intervals from 2 to 8 weeks. Each compound was tested over a range of 4 to 6 concentrations and each concentration was tested in duplicate. The number of larvae tested per concentration usually varied from 100 to 200.

During the tests the larvae were handled over a white enamelled tray (30 cm x 35 cm) containing water to prevent their escape. The tube with larvae was placed in a 5 cm petri dish in the tray. Two additional petri dishes, 15 cm in diameter with an 11 cm Whatman No. 1 filter paper were also placed in the tray (Fig. 6A). The closure was

removed from the tube of larvae and a glass funnel 2.5 cm ID and with a stem approximately 7 cm long and 5 mm ID quickly inverted over the open end. The larvae rapidly moved up the sides of the tube into the glass funnel and aggregated in clusters at the open top of the stem (Fig. 6B). This method was safer and easier than aggregation of larvae at the rim of the larval tube for handling the fast moving larvae of the 3 species used in my experiments. Approximately 100 larvae were removed from the cluster with No. 2 paint brush and placed on the filter paper in the 15 cm petri dish. 10 ml of the acaricidal solution, which was kept constantly agitated on a magnetic stirrer, was withdrawn with a pipette and 3 ml delivered into the petri dish after lifting the edge of the filter paper. The movement of the larvae was slowed down when the paper absorbed the solution. A further 4 ml was poured over the larvae and a second 11 cm filter paper placed over them so that they were sandwiched between the two filter papers. The remaining 3 ml of the solution was poured over the "sandwich" which was thus saturated with 10 ml of the solution. The immersion period was 3 minutes instead of 10 minutes used by Shaw (1966). At the end of the immersion period the "sandwich" was removed from the petri dish with forceps and placed on a double thickness of 24 cm Whatman No. 1 filter paper. After initial absorption of the excess solution, the "sandwich" was moved to a dry area of the filter paper base. The top layer of the "sandwich" was lifted and the larvae carefully removed with a No.3 paint brush and placed in the apex of a 15 cm Whatman No. 1 filter paper folded twice to make a cone, which had been kept in a desiccator at 25 C and 80% relative humidity for a few days before use to absorb moisture. This was very important as larval mortality could be high due to desiccation following handling and consequent dispersal from the larval cluster. The open end of the filter paper cone was closed

FIG. 6A
IMMERSION TEST FOR UNFED LARVAE

FIG. 6A
IMMERSION TEST FOR UNFED LARVAE

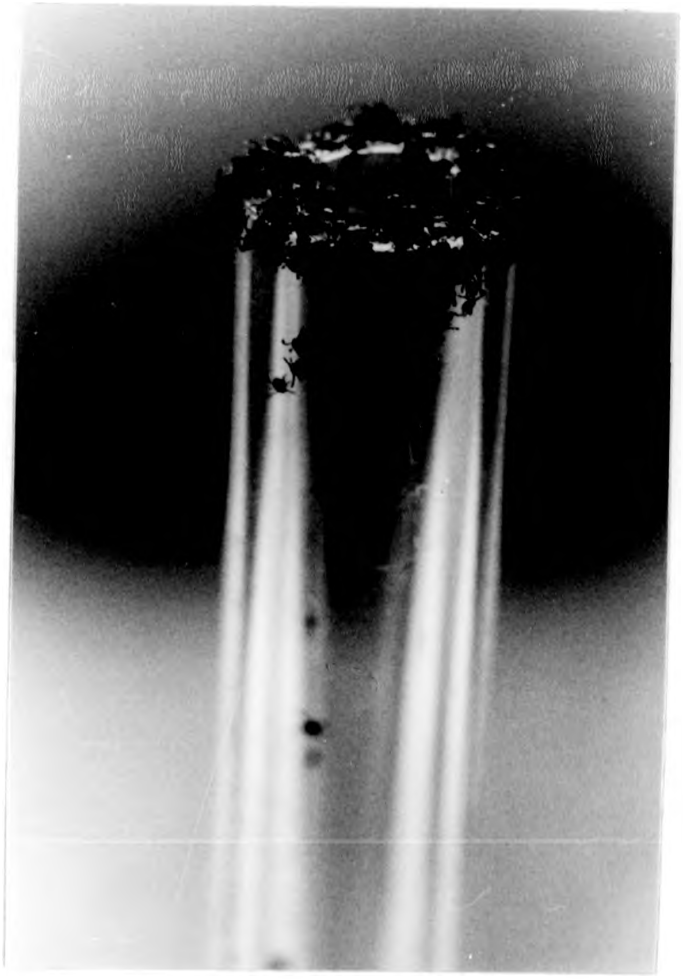


FIG.6B
RHIPICEPHALUS APPENDICULATUS LARVAL AGGREGATES
ON RIM OF FUNNEL

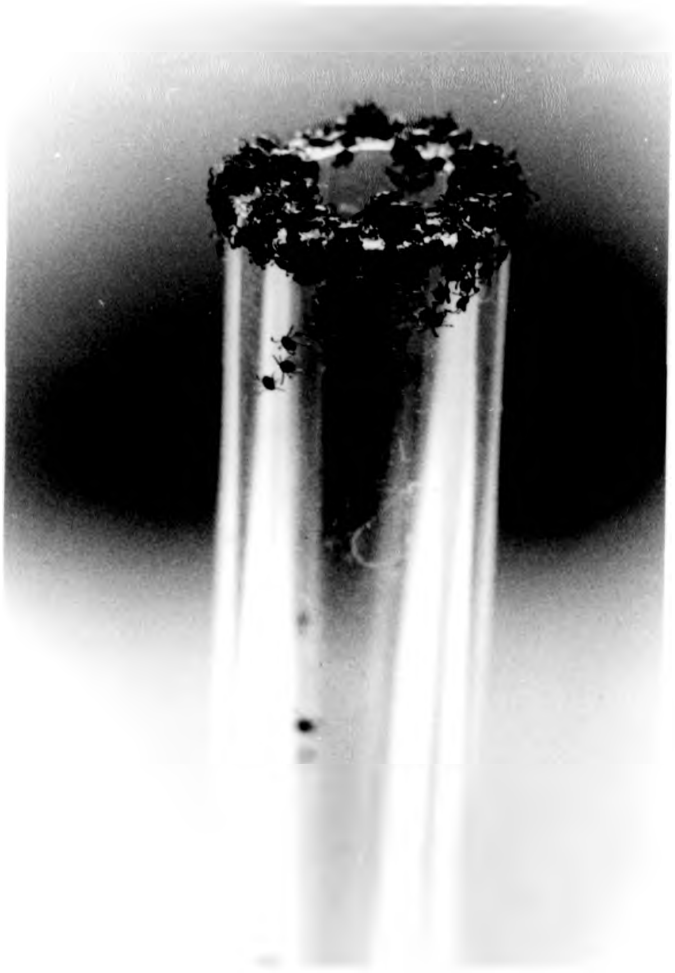


FIG. 6B
RHIPICEPHALUS APPENDICULATUS LARVAL AGGREGATES
ON RIM OF FUNNEL

by means of a crimping machine or by bulldog clips. The sealed filter paper cones were placed in desiccators at 25 C and 80% relative humidity.

The assessment of mortality was made 24 hours after immersion by opening the sealed end of the filter paper cone and counting the dead and live larvae. The actively moving larvae were crushed with a spatula with the flat end bent at an angle, and counted during the process. The filter paper with motionless larvae was placed on a warm plate (ca 35 C) for 15 seconds. Obviously dead larvae and those which did not walk away were treated as dead, although some of them showed feeble movements. These were separated and counted as dead. Tests were carried out at room temperature (22 - 25 C) and 44 - 48% relative humidity.

3.3.2 Impregnated packet technique for unfed nymphs

While total immersion of the animals in acaricidal solutions might kill attached ticks, treatments which will kill them before attaching is infinitely more preferable since it will prevent infestation and the transmission of pathogens by the bite of infected ticks.

Ticks moving around on animals treated with residual acaricides may absorb lethal doses. It is in this context that the impregnated packet technique devised by Stone and Haydock (1962) for unfed larvae of B. microplus, is useful. This is the method of "selfdosing" in which ticks are confined and allowed to move around in filter paper packets impregnated with acaricidal solutions, a situation analogous to ticks walking on the bodies of animals which have been treated days previously with residual acaricides. This method has been recommended by FAO (1971, 1972) for testing acaricidal resistance in larval ticks.

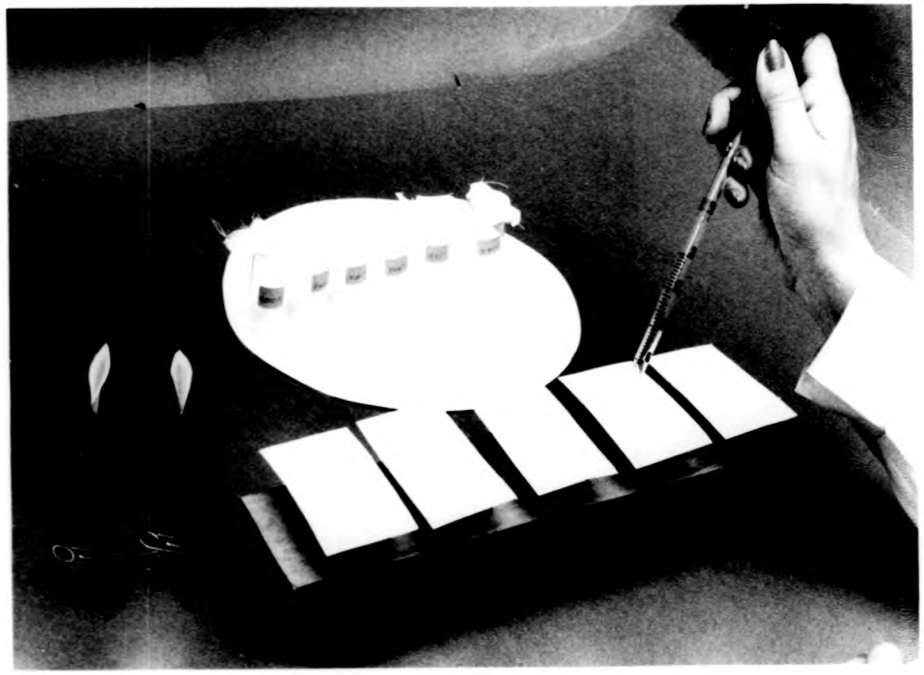


FIG.7
IMPREGNATED PACKET TECHNIQUE

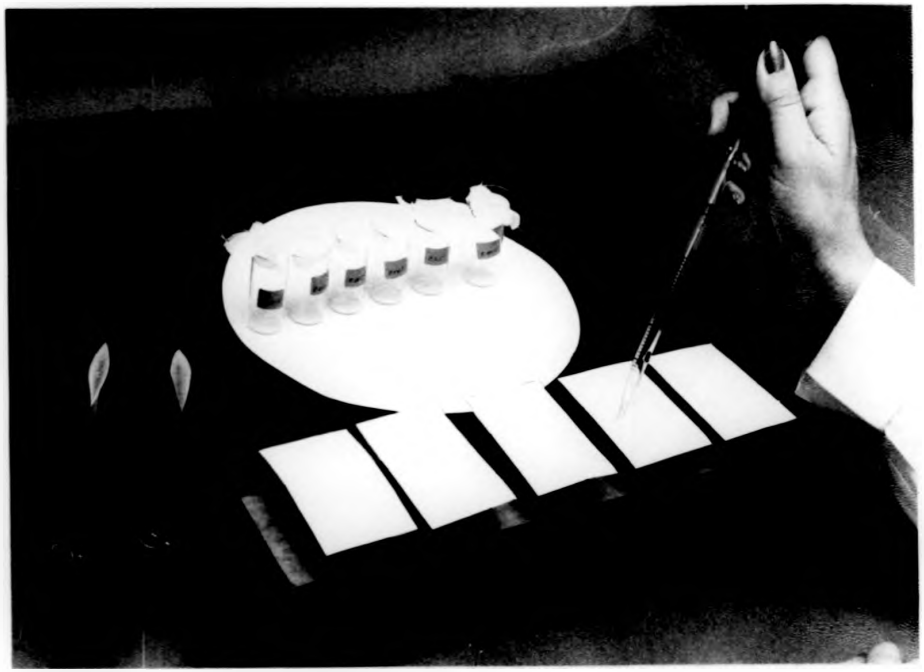


FIG. 7
IMPREGNATED PACKET TECHNIQUE

by means of a crimping machine or by bulldog clips. The sealed filter paper cones were placed in desiccators at 25 C and 80% relative humidity.

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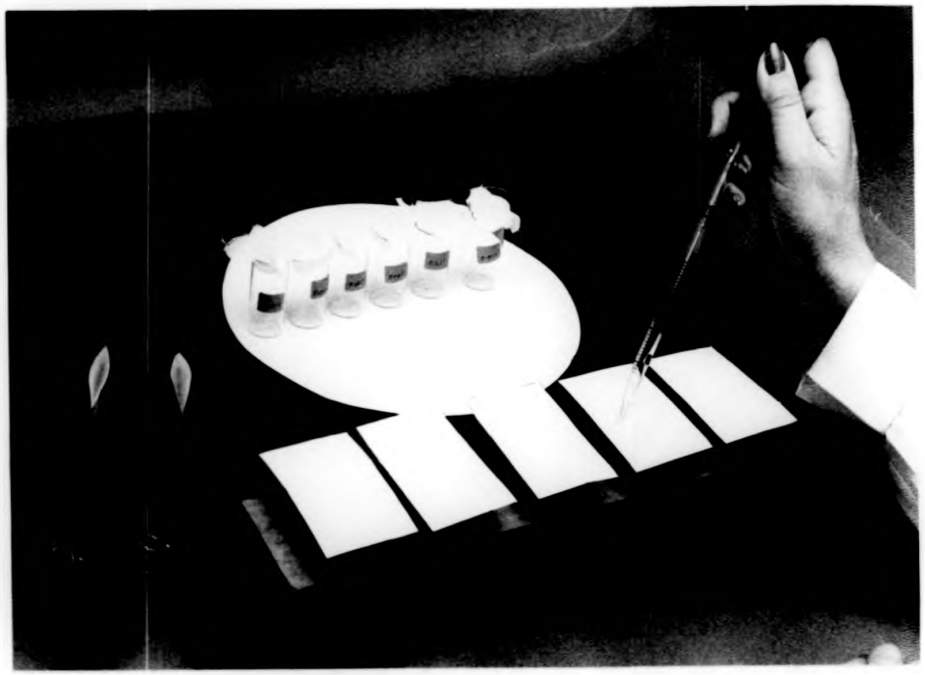


FIG. 7
IMPREGNATED PACKET TECHNIQUE

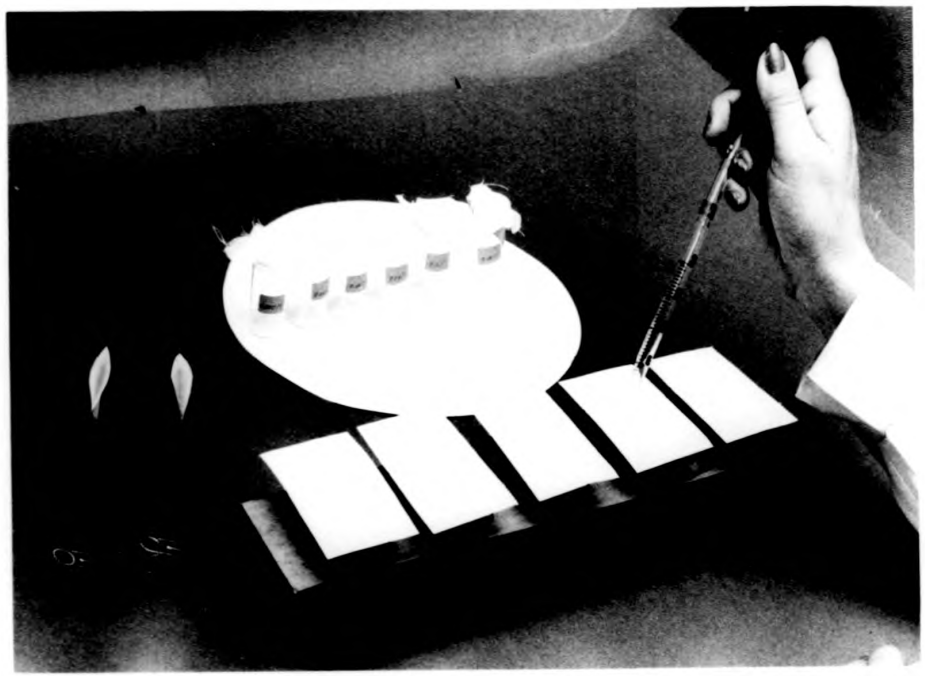


FIG.7
IMPREGNATED PACKET TECHNIQUE

I used this method in a limited series of experiments with unfed nymphs. Pieces of Whatman No. 1 filter papers (8 cm x 5 cm) supported horizontally at the corners on four pins were impregnated with 0.5 ml of different concentrations of the acaricidal solutions (Fig. 7) to give dose rates ranging from 10 mg/m^2 to 250 mg/m^2. After about half an hour the papers were hung up by "bulldog" clips at room temperature for 72 hours before testing. For the test they were folded lengthwise and the two opposite sides closed with "bulldog" clips to give 4 cm x 5 cm packets. 15 to 20 unfed nymphs, 1 to 4 weeks old, which had previously been chilled on wet ice to make them immobile and easier to handle, were transferred into the packet with a No. 3 paint brush, the packet closed with a third "bulldog" clip and placed in a desiccator (maintained at 80% relative humidity) at 25 C.

24 hours later the packets were opened and the nymphs transferred into glass specimen tubes maintained under the same conditions as above. A further 24 hours later (i.e., 24-hour exposure + 24-hour holding period) mortality counts were made using the same criteria as for the tea bag technique (see Section 3.3.3). Since it was not always easy to assess mortality after 24-hour exposure + 24-hour holding period, these periods were extended to 48 and 72 hours.

The impregnated packet technique was later discarded in favour of the tea bag technique.

3.3.3 Tea bag technique for unfed nymphs

The tea bag technique developed by Gladney *et al.* (1972) for testing unfed nymphs of *R. sanguineus* has certain advantages over the immersion technique. The ticks are kept enclosed during treatment thus reducing the chances of accidental escape and they absorb the acaricidal solution during the actual treatment as well as from the residual film on the bag during the draining and slow drying-off period.

This to me would be a more cogent reason for adopting this technique as it parallels field treatment closely. I used this method for unfed nymphs, fed nymphs and unfed adult males and females.

Nymphs were usually tested when 2 weeks old; they were also tested at weekly intervals from 1 to 10 weeks and then at 12 weeks. The age of the nymphs was calculated from the day when molting was completed.

Tea bags or envelopes were prepared from heat sealable tea bag paper (STD heat sealable tea bag 16.5g G.S.M. from James R. Crompton and Bros., Lancashire, England) by cutting 10 cm x 4 cm strips, folding them lengthwise and sealing them on two sides with a soldering iron 0.4 mm in diameter (Antex Precision, England) to make a flat envelope (4 cm x 5 cm) closed on three sides. Twenty fed larvae in the pre-moult phase, when they are inactive and easy to handle and count, were placed in each envelope and the open end sealed. These envelopes were kept at constant temperature, humidity and photoperiod in the tick holding room. Newly emerged nymphs could be seen through the translucent bags and were used as required.

Solutions for immersion of the tea bags containing ticks were prepared as previously described in section 3.2.2.2.1. Each compound was tested over a range of 4 to 6 concentrations and two envelopes used per concentration. The solutions were constantly agitated on a magnetic stirrer till used. Before the test, miniature "bulldog" spring hooks (No. 31 from M. Myers & Sons Ltd., England) were attached to each bag; the bags were completely immersed for 3 seconds in 25 ml of the acaricidal solution in disposable paper cups, using a pair of forceps. At the end of the immersion period the tea bag was lifted out of the solution and the bottom edge blotted immediately on a double thickness of filter paper to remove excess fluid. Each

FIG.8
TEA BAG TECHNIQUE

FIG.8
TEA BAG TECHNIQUE

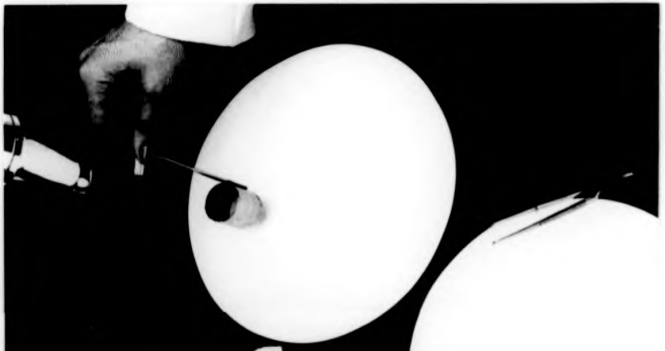
envelope was then hung by the hook on a wire rack resting in a tray of water (Fig. 8). The treated bags were held at room temperature (22 - 25C) and 44 - 48% relative humidity. Mortality was recorded 24 hours after treatment. The tea bag was cut open and the unaffected live nymphs which crawled out immediately were counted. Those which were obviously paralysed, i.e., inactive or showing feeble movements were put on a filter paper disc with the ventral side up and placed on a warm plate (ca. 35 C) for 15 seconds. Nymphs which did not right themselves and walk away after the warm-up period were considered dead.

3.3.4 Dipping technique for fed nymphs

Whitehead (1958) used a dipping technique for larvae and fed adults of B. decoloratus in which the ticks were immersed in acaricidal solutions in a small tube which was kept agitated. After treatment the adult ticks were removed, allowed to drain and transferred to clean tubes to determine whether they could lay fertile eggs if they survived. A similar method was used by Hazeltine (1959) in which engorged females of R. sanguineus were confined in a cheesecloth pouch and dipped in acaricidal solutions. Dipping engorged females confined in small open topped wire mesh baskets in acaricidal solutions has since been widely practiced for testing their egg laying potential following acaricidal treatment. Dipping also parallels field treatments closely and I used it for fed nymphs to determine the effect of acaricides on moulting, before the tea bag technique was adopted as standard procedure. Dipping using open top baskets can be used only for the slow moving fed stages and is unsuitable for active unfed stages.

Dipping baskets were made from stainless steel and measured

FIG.9
DIPPING TECHNIQUE



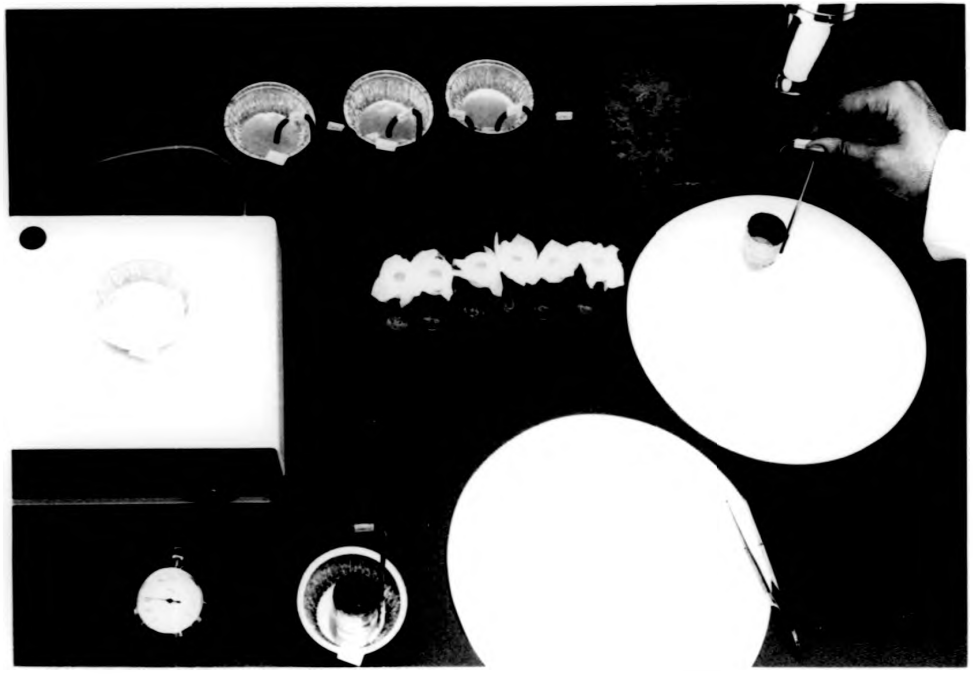


FIG. 9
DIPPING TECHNIQUE

2.5 cm deep and 3 cm in diameter (Fig. 9). The bottom of the basket was made of stainless steel wire (32 mesh x 35 S.W.G.). A handle 12 cm long and curved at the top attached to the outside of the basket enabled it to be dipped in the solution and lifted to drain.

Fed nymphs in batches of 10 immediately after detachment from animals were placed inside the basket and lowered into 25 ml of the acaricidal solution in an aluminium foil pie dish and kept constantly agitated. Care was taken to ensure that all the ticks were totally immersed. After three minutes the basket was lifted out of the solution, allowed to drain and excess solution removed by thorough blotting on filter paper. The ticks still in the basket were dried in a stream of warm air (ca 28 C) from a hair dryer (Haartrockner 855 from Knauer-Druck-Verpackungswerke, Dettingen/Wurtt., West Germany) before removal into glass specimen tubes. The tubes were placed in a desiccator (80% relative humidity) in a cooled incubator at 25 C and 12-hour photoperiod.

In these preliminary experiments mortality counts were based on the failure of ticks to moult. In later experiments a more comprehensive scoring system was used (see Section 3.3.5).

3.3.5 Tea bag technique for fed nymphs

In a few experiments this method was used for testing fed nymphs. Fully engorged nymphs immediately after detaching from animals were put in tea bags in batches of ten. The protocol for testing was the same as for unfed nymphs.

In scoring for mortality three different criteria were combined; ticks which died without entering the premoult phase, those which entered the premoult phase but died before moulting and those which moulted but died subsequently. These rates have been combined to give

the cumulative mortality. The effect of the acaricide on fed nymphs also manifested itself in an extended premoult period. The numbers moulting in the control group and groups treated with each concentration were recorded from the day the first moult was seen in the control batch, and from then on every other day. From these data the average premoult period was calculated for each concentration.

3.3.6 Topical application for unfed adults

While immersion and the dipping technique are relevant in implementation of field control measures, they do not enable one to determine the actual dose per tick. Topical application is unique in that it is the only one of the various test methods which applies a known dose (Busvine 1968a). While it is regarded as a sound method it has sometimes been regarded as inconvenient because of the difficulty in handling and treating arthropods separately; in any case it can be considered only in a laboratory context and does not parallel any treatment in the field. Since the actual dose per tick can be determined with some accuracy I used this method also for testing unfed males and females.

Adult male and female ticks usually from two weeks to two months after emergence were weighed, so that the dose per milligram of tick body weight (tbw) could be calculated for comparing results from the three species which differ in weight. The ticks were stuck by their ventral side to double-sided adhesive tape. 10 females and 10 males were used per concentration and each compound was tested over a range of 4 to 6 concentrations. One ml of each concentration was applied to the dorsal side of the alloscutum just behind the scutum of each tick with a SMI Micropettor (Scientific Manufacturing Industries, California) (Fig. 10). The dose in mg was calculated on the basis of

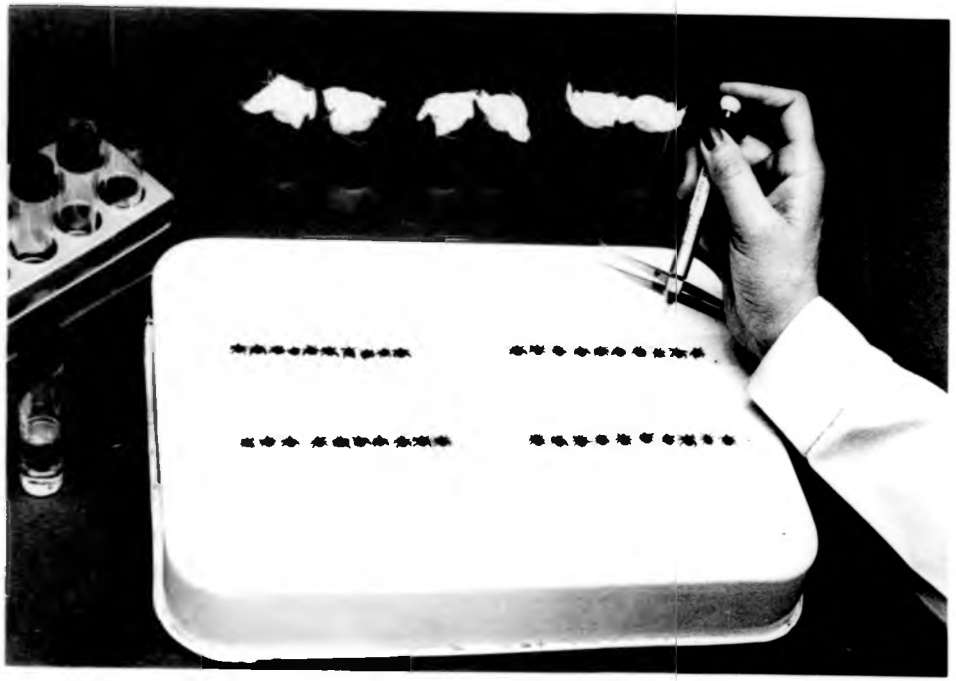


FIG. 10
TOPICAL APPLICATION OF ACARICIDES

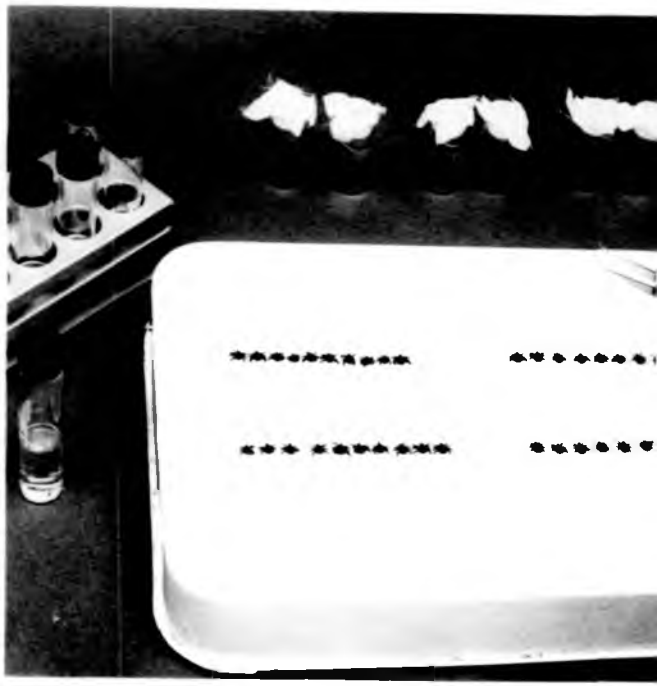


FIG. 10
TOPICAL APPLICATION OF ACARICIDES

1 µl of the different concentrations. The control group of ticks were treated with 1 µl of acetone. The treated ticks were removed into glass specimen tubes and held at 25 C, 80% relative humidity and 12-hour photoperiod. Mortality was scored 7 days post-treatment, using the same criteria as for the tea bag technique (see Section 3.3.7).

3.3.7 Tea bag technique for unfed adults

The tea bag technique of Gladney et al. (1972) was slightly modified for testing unfed adults. The modification was not in the actual testing technique but in the handling of ticks, the number of ticks per bag and an extended holding period after treatment. In most of the tests, 10 females or 10 males, three weeks to two months after emergence, were placed in each bag before the test and not in the premoult stage as with unfed nymphs. Two bags (total of 20 ticks) were used per concentration. 24 hours after treatment the ticks were removed from the bag, put into glass specimen tubes and held at 25 C, 80% relative humidity and 12-hour photoperiod. Mortality was not scored at 24 hours, but at 7 days after treatment. Live ticks usually were at the top of the tube and could easily be counted. Those which were paralysed i.e., inactive or showing feeble movements were placed ventral side up on a filter paper disc in a petri dish. Ticks which could not right themselves and walk away after a 15 to 30-seconds warm up period and after gently blowing on them were scored as dead.

3.4 SCORING OF TESTS

Since insecticides are intended to kill, the response usually chosen to assess their effects is death following treatment. Unfortunately, the point of death in arthropods is much less obvious than in higher forms of life (Busvine 1971).

Insects of ticks treated with different amounts of poison may exhibit various degrees of intoxicatin. The effects may vary, with the amount and formulation as well as by the period of contact with the compounds, from trivial temporary effects from which the arthropods may subsequently recover to complete death. The time for assessment of mortality after treatment is therefore important; for instance chemicals with rapid knock-down action such as pyrethroids may allow some individuals to recover from temporary paralysis, compared to a slow acting poison causing irreversible effects. Consequently the conclusions reached will vary greatly according to the time chosen after treatment to assess their effects.

Variations in the criteria for determining mortality also greatly affect the results of the experiment. Most of the acaricidal tests on ixodid ticks are done on unfed stages and are assessed on the basis of death, but to set clear cut criteria for determining mortality is rather difficult. There is a progressive slowing down of activity from larvae → nymphs → adults and it may often be hard to separate the natural sluggishness of the ticks particularly of adults of some species from the toxic effects of insecticides. This is particularly true in the case of pyrethroids with their excellent knock-down properties where knock-down which is the beginning of the toxic process may be temporary and reversible with lower concentrations. This temporary effect may be mistaken for true death thus giving an exaggerated mortality rate. If sufficiently high concentrations have

been applied, the toxic process proceeds further ending in the death of the arthropod (Burt & Goodchild 1974). The time chosen for assessing mortality is vital. Mortality in larvae and nymphs of ixodid ticks is comparatively easy to assess 24 hours after treatment, but the very sluggish nature of the adult ticks makes this difficult and the effect after a 24-hour observation period may not be clear enough for a final analysis of their condition to be made. A minimum observation period of seven days has therefore been adopted for adults, since a long delay may elapse before death occurs. In most cases, a second mortality reading was made 14 days after treatment also.

The toxic effects of acaricides on ixodid ticks range from slight disturbance of motor function (such as incoordinated movements and inability to stay on vertical surfaces) through severe disturbances of motor function shown by partial or complete paralysis of one or more legs, inability to right themselves when placed dorsal side down, and splaying of the palps, to a total loss of movement, desiccation and absence of any sign of life even after stimulation by gentle blowing and/or placing on a warm surface. For all practical purposes ticks which have lost the powers of movement may be regarded as dead and included in mortality rates since they will be unable to proceed with essential activities such as host seeking and feeding to complete their life cycle.

3.5 STATISTICAL ANALYSIS OF DATA

The mortality rates of the ticks for different concentrations of each compound were plotted against the log - concentration on log - probit paper. The results were analysed by a CDC 7600 computer using Genstat package which fits a probit model for bioassays using regression facilities. The probit transformation was used in order to

linearise the relationship between the rates of mortality and different concentrations of the compounds. The Genstat package gives estimates of log - concentration percentages together with 95% fiducial limits, regression coefficient (i.e., slope and intercept of the line) and their standard errors, and the value of Chi^2 with its associated degrees of freedom which was used to assess how well the data fitted the probit model. The LC_{50} and LC_{90} with 95% fiducial limits, slope ± SE, Chi^2 and P plus the average number of ticks are given in tabular form. In all cases where there was mortality in the control, the subsequent rates of kill for different concentrations were adjusted using Abbott's formula (Busvine 1971) before the probit analysis was carried out.

The potency of each compound was evaluated on the basis of the concentration which gave 50% mortality of the ticks (LC_{50}); the relationship between LC_{50} and LC_{90} (concentration required to obtain 90% mortality) can be estimated from the steepness of the slope of the line. The goodness of fit of the probit is based on the value of Chi^2 for a given number of degrees of freedom. An increase in the value of Chi^2 indicates that fiducial limits are less reliable, and when it reaches a figure corresponding to a probability (P) level of <0.05, we conventionally consider fiducial limits not sufficiently reliable to be of any use.

3.6 RESULTS

3.6.1 Developmental periods and feeding behaviour of Rhipicephalus appendiculatus, Dermacentor marginatus, Amblyomma hebraeum and Amblyomma variegatum

Observations on the life cycle and feeding behaviour of all four species were made under controlled and identical laboratory conditions. During the feeding phase the ticks were at a temperature of 25 - 26 C and 12-hour photoperiod and during the non-parasitic stages, at 25 - 26 C, 80% relative humidity and 12-hour photoperiod. Duration of preoviposition period, egg stage, nymphal and adult premoult and of larval, nymphal and adult female feeds of the four species, are given in Table 2.

3.6.1.1 Preoviposition period, egg stage, nymphal premoult and adult premoult

The minimum duration of the preoviposition period, 3 - 6 days in R. appendiculatus and 4 - 7 days in D. marginatus, was shorter than in the two species of Amblyomma, 8 - 14 days in A. hebraeum and 11 - 16 days in A. variegatum. The egg stage was shortest in D. marginatus with a minimum duration of 16 - 17 days and longest in the Amblyomma spp. with a minimum of 61 - 68 days. In R. appendiculatus, the minimum duration was 24 - 34 days. The nymphal and adult premoults were shortest in D. marginatus with a minimum duration of 6 - 7 days and 12 - 13 days respectively and longest in the Amblyomma spp., 16 - 21 days and 15 - 20 days and 24 - 29 days and 22 - 28 days respectively, and intermediate in R. appendiculatus with 8 - 10 days and 14 - 20 days respectively.

TABLE 2 - MINIMUM DURATION IN DAYS OF PREOVIPOSITION, EGG STAGE, NYMPHAL PREMOULT AND ADULT PREMOULT, AND MINIMUM AND MAXIMUM DURATION OF LARVAL, NYMPHAL AND ADULT FEMALE FEEDS

	Pre-oviposition	Egg stage	Larval feed	Nymphal premoult	Nymphal feed	Adult premoult	Female feed
R. appendiculatus	3 - 6	24 - 34	3 - 6	8 - 10	4 - 11	14 - 20	6 - 14
D. marginatus	4 - 7	16 - 17	3 - 6	6 - 7	5 - 10	12 - 13	9 - 19
A. hebraeum	8 - 14	61 - 68	4 - 8	16 - 21	6 - 13	24 - 29	9 - 20
A. variegatum	11 - 16	61 - 68	5 - 10	15 - 20	5 - 12	22 - 28	12 - 17

3.6.1.2 Duration of larval, nymphal and adult female feeds

As with most ixodid ticks, larval feeds of the four species had the shortest duration and adult females took the longest time to engorge. Larval feeds of R. appendiculatus and D. marginatus lasted from a minimum of 3 days to a maximum of 6 days; larvae of the Amblyomma spp. fed from a minimum of 4 days to a maximum of 10 days. Nymphal feeds were also shorter and similar in R. appendiculatus and D. marginatus (minimum of 4 days and maximum of 11 days) and longer and similar in the two Amblyomma species (minimum 5 days, maximum 13 days). Adult females of R. appendiculatus engorged in 6 to 14 days and D. marginatus in 9 to 19 days; females of A. hebraeum and A. variegatum fed from a minimum of 9 days to a maximum of 20 days.

3.6.1.3 Sex ratio in the laboratory colonies

The male to female ratio was 1:0.9 in R. appendiculatus (250 adults) and D. marginatus (112 adults). There were more males than females in the Amblyomma spp., the male to female ratio being 1:0.8 in A. hebraeum (480 adults)and 1:0.7 in A. variegatum (620 adults).

3.6.1.4 Feeding behaviour

Larvae, nymphs and adults of A. hebraeum and A. variegatum attached to the host animals and fed in tight clusters (Fig. 11). Cluster feeding was particularly marked with adults. Males which were released first attached in one or two tight clusters and the females attached in and around the cluster of feeding males (Fig. 12). This was in marked contrast to the feeding behaviour of R. appendiculatus, where particularly the larvae and nymphs, attached on the feeding area more evenly; adults of this species fed in clusters although they were not so compact as with Amblyomma spp. During feeding, there was very

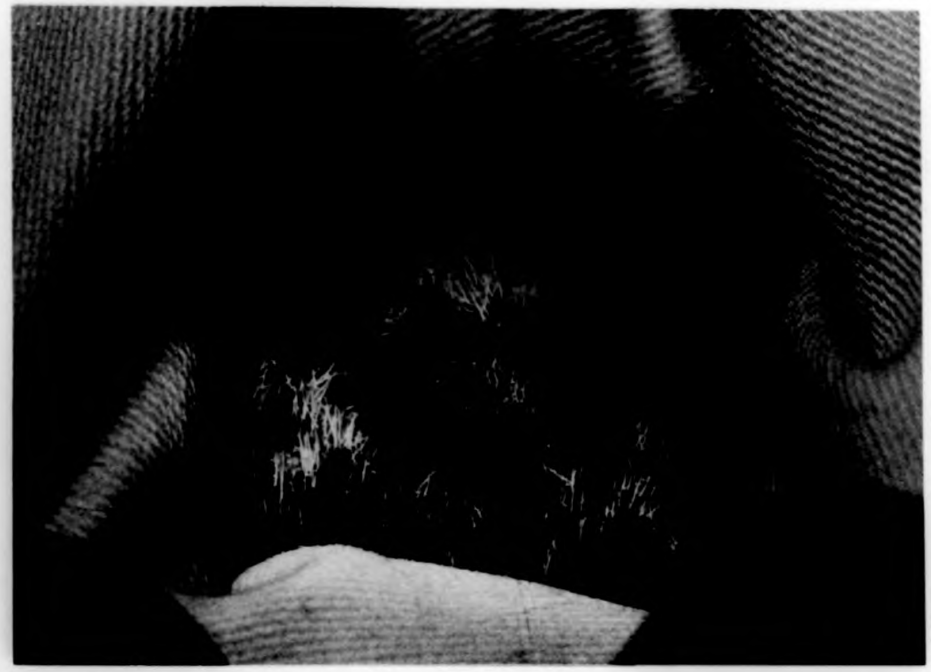


FIG.11
CLUSTER FEEDING OF AMBLYOMMA VARIEGATUM LARVAE ON GUINEA PIG

FIG.11
CLUSTER FEEDING OF AMBLYOMMA VARIEGATUM LARVAE ON GUINEA PIG

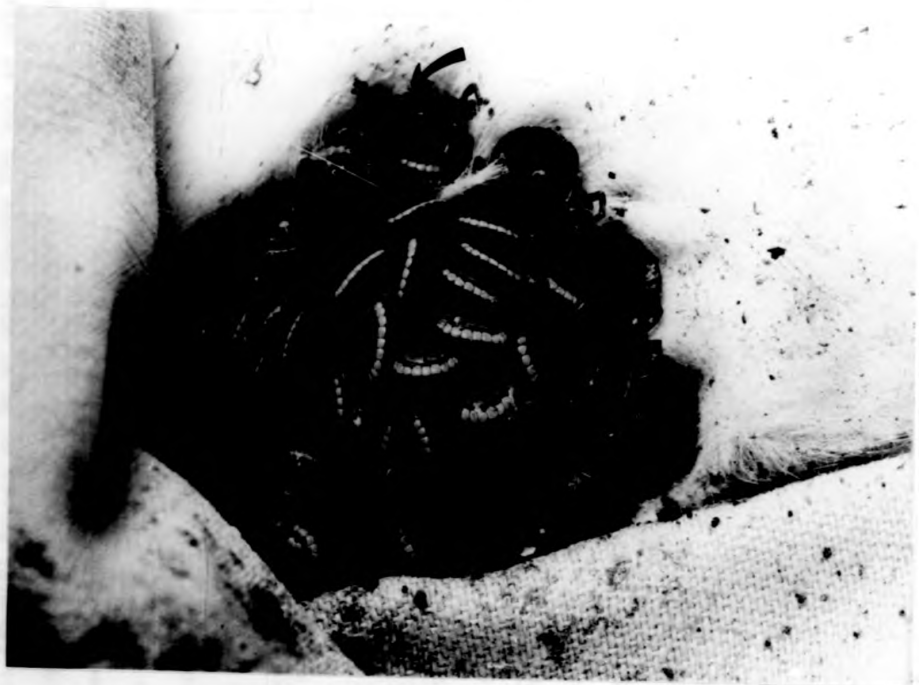


FIG. 12
CLUSTER OF FEEDING MALES OF AMBLYOMMA HEBRAEUM ON RABBIT WITH FEMALES

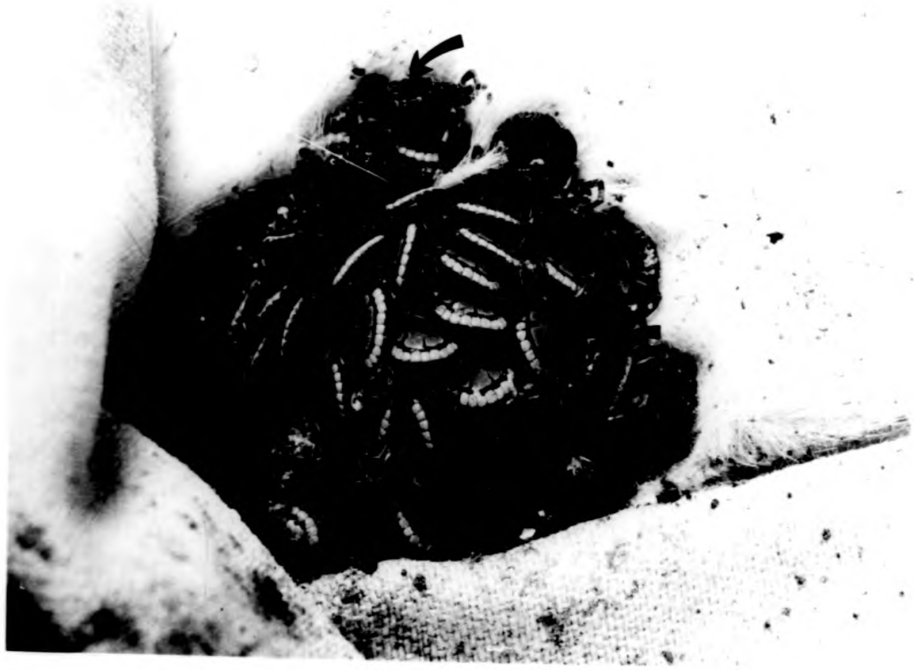


FIG. 12
CLUSTER OF FEEDING MALES OF AMBLYOMMA HEBRAEUM ON RABBIT WITH FEMALES

little or no excretion of digested blood, but only of white guanin granules by the Amblyomma spp., while R. appendiculatus and D. marginatus, particularly nymphs and adults, excreted copious amounts of faecal pellets of digested blood.

The number of larvae released was not known or estimated in all cases; but judging by the absence of dead unfed larvae on the animals, recovery of fed larvae of all species approached 100%. Recovery of fed nymphs was highest with R. appendiculatus (average 92%, range 86% to 98%). The average recovery rate of fed nymphs of A. hebraeum was 81% (range 50% to 100%) and of A. variegatum 78% (range 68% to 89%). The recovery rate of fed females of D. marginatus from guinea pigs, 66% (range 33% to 90%), was the lowest among the four species. The highest recovery rate of fed females was obtained with R. appendiculatus from rabbits' ears (average 94%, range 80% to 100%). On an average 81% (range 50% to 100%) of A. hebraeum and 86% of A. variegatum (range 60% to 100%) females released on the back of rabbits fed.

3.6.1.5 Relationship between weight of engorged females and egg output, and between weight of egg mass and number of larvae

These were determined for R. appendiculatus only. 30 engorged females were weighed individually immediately after feeding on an "Oertling" R52 analytical balance. After completion of oviposition, the egg mass laid by each female was also weighed. The weight of the engorged females ranged from 90 mg to 600 mg (average 369 mg) and the weight of the egg masses from 28 mg to 364 mg (average 194 mg). The % conversion efficiency index (CEI = wt. of egg mass/wt. of fed female) which is a measure of the ability of the female to convert body weight to egg weight (Drummond and Whetstone 1970) was on average about half (52%, range 31% to 61%). There was a positive correlation

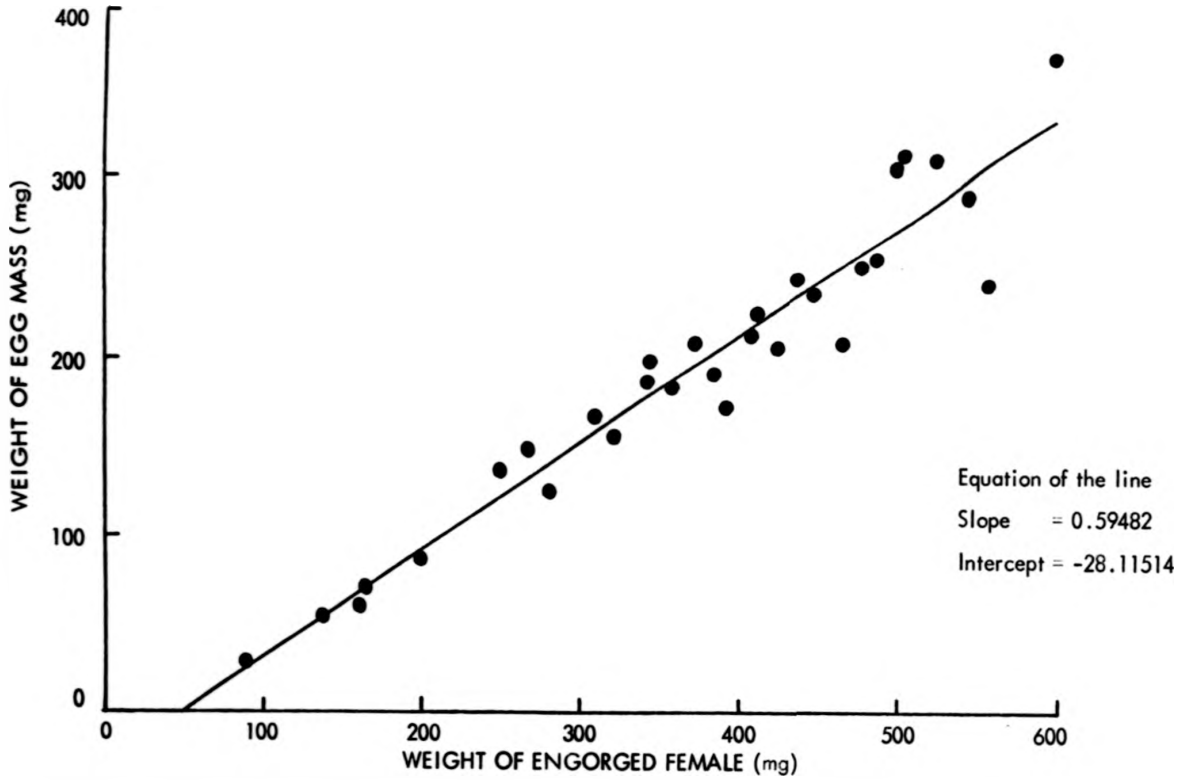


FIG.13
RELATIONSHIP BETWEEN WEIGHT OF ENGORGED FEMALES OF R.APPENDICULATUS AND WEIGHT OF EGG MASS LAID

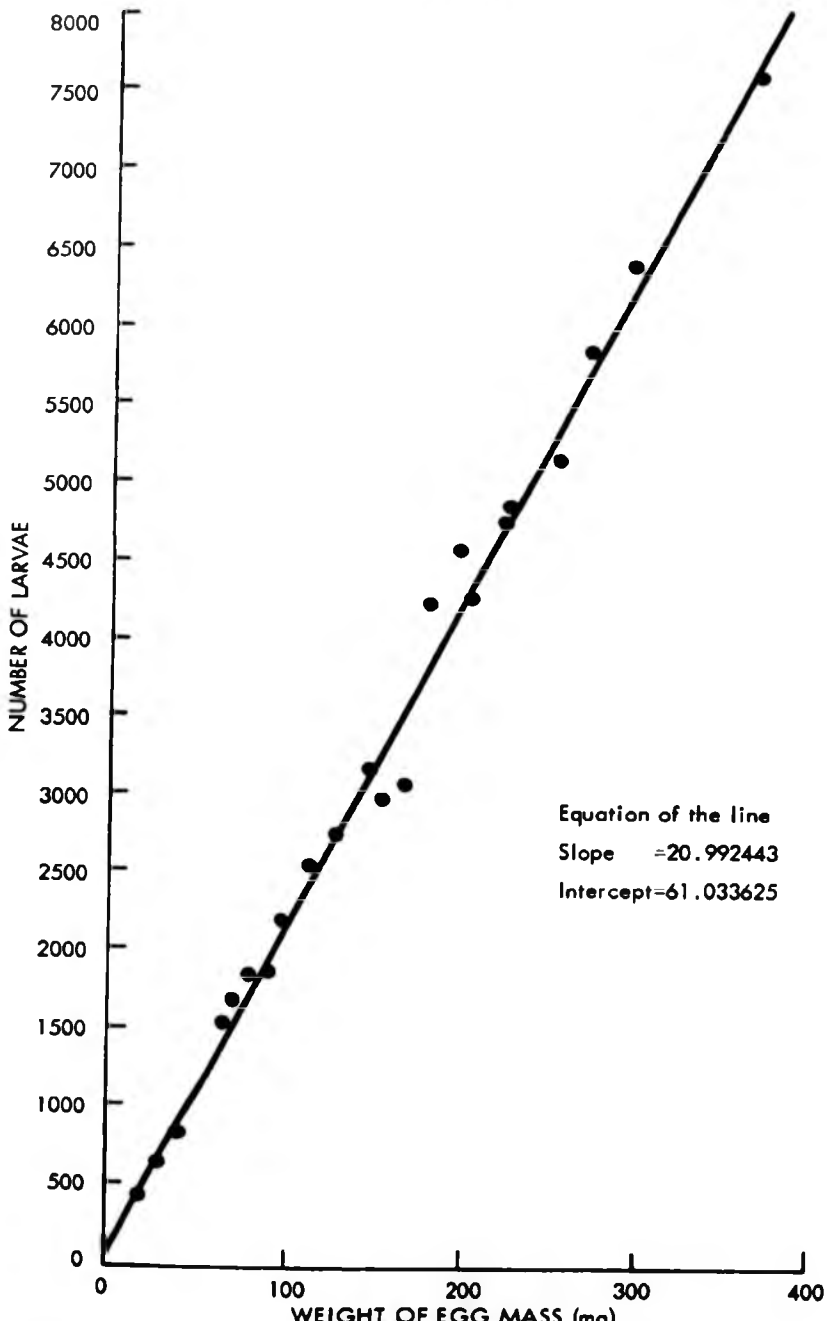


FIG. 14

RELATIONSHIP BETWEEN WEIGHT OF R. APPENDICULATUS EGG MASS AND NUMBER OF LARVAE

between weight of engorged females and the weight of the egg masses laid by them (Fig. 13).

The number of larvae emerging from egg masses ranging in weight from 20 mg to 364 mg laid by 22 individual females, ranged from 438 to 7621. The number of larvae per mg egg mass ranged from 18 to 24, with an average of 21. The correlation between weight of egg mass and number of larvae was highly significant (Fig. 14) and the number of larvae emerging from egg mass of known weight could be estimated by interpolation of the weight on to the regression line. There was good agreement between estimated and actual number of larvae and the percent error was only 7% to 8%.

3.6.2 Results of tests with larval ticks

Larvae of ixodid ticks after hatching from eggs are unable to feed immediately. A certain interval, referred to as the prefeeding period, elapses before they are ready to feed. During this period which may vary with species and environmental conditions, the cuticle hardens and the ticks usually remain at the bottom of the tubes or at the gottom of vegetation in nature. This is followed by excretion of guanin. The ticks in the laboratory colonies would then ascend the sides of the tubes to gather at the top. Under natural conditions, they would at this stage ascend the stems of grass or other vegetation and quest for host animals. Testing ticks before they have attained this physiological age would be irrelevant to control measures since control by treatment of host animals or by treatment of vegetation by residual insecticides would be ineffectual unless the ticks are actually questing or have passed beyond this stage and boarded a host animal.

All the eggs in a batch do not hatch simultaneously. Larval

eclosion (from emergence of first larva to emergence of all larvae) takes several days and is usually complete in about one week, so that batches removed for testing at any one time contain a mixture of larvae whose ages differ by about one week. The youngest larvae to be tested were 1 - 2 weeks old, i.e., 7 - 14 days after emergence, but for convenience they are referred to as 2-week old larvae; from then on tests were done usually every week til the 8th week. For comparing the potency of the different compounds on the same species and the effect of each compound on the three species of ticks, results from 2-, 3- and 4-week old larvae have been pooled to represent "young" larvae (2 - 4 weeks) and those from 5-, 6-, 7- and 8-week old larvae pooled to represent "old" larvae (5 - 8 weeks).

The effect of ageing on susceptibility of larvae of R. appendiculatus and A. hebraeum to permethrin, cypermethrin and decamethrin was investigated by comparing the values of LC_{50} obtained for larvae of successive age groups starting from 2 weeks upto 8 weeks.

3.6.2.1 The effect of three synthetic pyrethroids on unfed larvae of Rhipicephalus appendiculatus

The average number of larvae tested per concentration of the three compounds varied from 182 to 922. Results are presented in Table 3 and Fig. 15. Based on LC_{50} values, decamethrin was slightly more effective against this species irrespective of whether the larvae were 2 to 4 weeks old or 5 to 8 weeks old. The LC_{50} of permethrin and cypermethrin for 2- to 4-week old larvae were similar, i.e., 0.000082% and 0.000096% respectively, but the value was lower, 0.000065%, for decamethrin showing that this compound was 1.2 to 1.5 times as effective as permethrin and cypermethrin. Values for 5- to 8-week old larvae were 0.000069% for permethrin, 0.000071% for cyper-

TABLE 3 - SUSCEPTIBILITY OF R. APPENDICULATUS UNFED LARVAE, 2 to 4 WEEKS AND 5 to 8 WEEKS OLD, TO 3 SYNTHETIC PYRETHROIDS. RESULTS OF IMMERSION TESTS. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	2 - 4	0.000082	0.000078 - 0.000086	0.000142	0.000130 - 0.000159	182	5.370 ± 0.39	3.84	>0.75
	5 - 8	0.000069	0.000067 - 0.000070	0.000119	0.000113 - 0.000125	700	5.397 ± 0.23	12.14	<0.01
cypermethrin	2 - 4	0.000096	0.000093 - 0.000099	0.000171	0.000162 - 0.000181	584	5.109 ± 0.18	1.067	>0.75
	5 - 8	0.000071	0.000069 - 0.000073	0.000120	0.000115 - 0.000127	639	5.599 ± 0.25	22.45	<0.01
decamethrin	2 - 4	0.000065	0.000064 - 0.000066	0.000108	0.000104 - 0.000112	922	5.883 ± 0.21	1.276	>0.5
	5 - 8	0.000050	0.000047 - 0.000051	0.000105	0.000099 - 0.000111	483	3.963 ± 0.16	18.50	<0.001

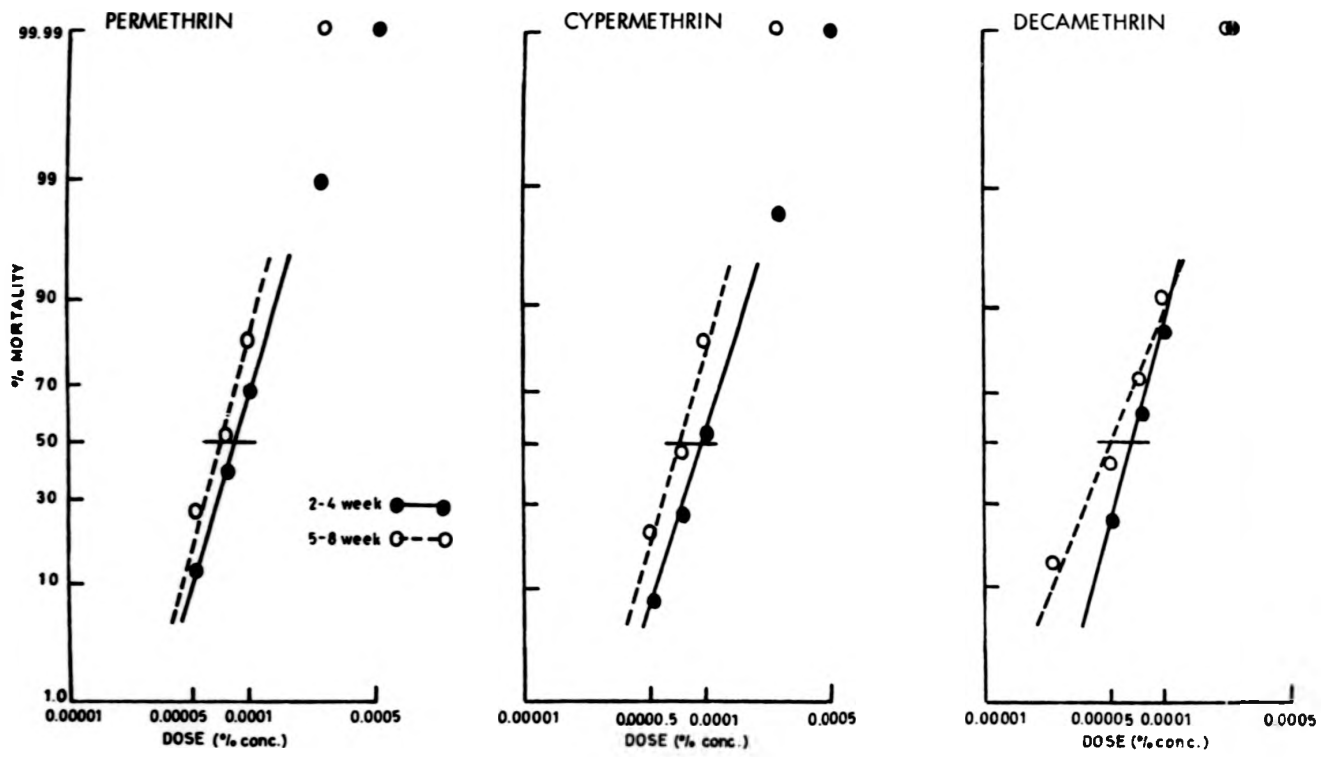


FIG. 15

RESULTS OF IMMERSION TESTS WITH 3 SYNTHETIC PYRETHROIDS AGAINST 2 TO 4 WEEK AND 5 TO 8 WEEK OLD UNFED LARVAE OF R. APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

methrin and 0.00005% for decamethrin, the latter compound again being about 1.4 times as effective as permethrin and cypermethrin. The LC_{90} values of decamethrin were 0.000108% for 2- to 4-week old larvae and 0.000105% for 5- to 8-week old larvae, an increase in effectiveness by a factor of 1.3 to 1.6 compared to LC_{90} values for permethrin and cypermethrin. The slope of the regression line was steep with all three compounds (Fig. 15), suggesting that the concentration required to effect 90% kill was close to the concentration required to obtain 50% kill. On the basis of the LC_{50} and LC_{90} values for all three compounds and the two age groups, 90% control of the ticks could be obtained by increasing the concentration for 50% kill by a factor of only 1.7 to 2.1.

The increase in susceptibility with age of ticks is obvious when pooled results for the younger larvae (2 to 4 weeks old) and the older larvae (5 to 8 weeks old) are compared. With all three compounds it required a lower concentration to kill the older larvae than younger larvae for e.g., the LC_{50} of decamethrin for 2- to 4-week old larvae was 0.000065% and that for 5- to 8-week old larvae, 0.00005%. This was investigated in more detail in further experiments with progressive incremental ages of one week and results with each of the three compounds are presented in Tables 4, 5 and 6 and Fig. 16 - 21.

Although the LC_{50} values for each age group were only slightly different, the difference between the LC_{50} for 2-week and 8-week old larvae were obvious. In the case of permethrin the value had dropped from 0.000083% to 0.000056%, a factor of 1.5; with cypermethrin the corresponding figures were 0.000103% and 0.000067%, an increased susceptibility factor of 1.5; with decamethrin the figures were 0.000066% and 0.000041%, showing that 8-week old larvae were 1.6

TABLE 4 - SUSCEPTIBILITY OF R. APPENDICULATUS UNFED LARVAE, 2 to 8 WEEKS OLD TO PERMETHRIN. RESULTS OF WEEKLY IMMERSION TESTS. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P.

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fidicial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	2	0.000083	0.000076 - 0.000093	0.000131	0.000112 - 0.000176	36	6.456 ± 1.07	1.60	>0.25
	3	0.000086	0.000080 - 0.000092	0.000165	0.000146 - 0.000196	120	4.494 ± 0.40	4.16	>0.1
	4	0.000072	0.000067 - 0.000076	0.000097	0.000089 - 0.000109	42	9.808 ± 1.26	0.19	>0.9
	5	0.000084	0.000081 - 0.000087	0.000123	0.000116 - 0.000134	179	7.766 ± 0.62	23.61	<0.001
	6	0.000067	0.000064 - 0.000070	0.000102	0.000096 - 0.000111	132	7.069 ± 0.59	2.15	>0.25
	7	0.000058	0.000053 - 0.000061	0.000128	0.000118 - 0.000145	285	3.681 ± 0.30	5.04	>0.05
	8	0.000056	0.000057 - 0.000062	0.000077	0.000073 - 0.000083	103	11.46 ± 1.04	0.73	>0.5

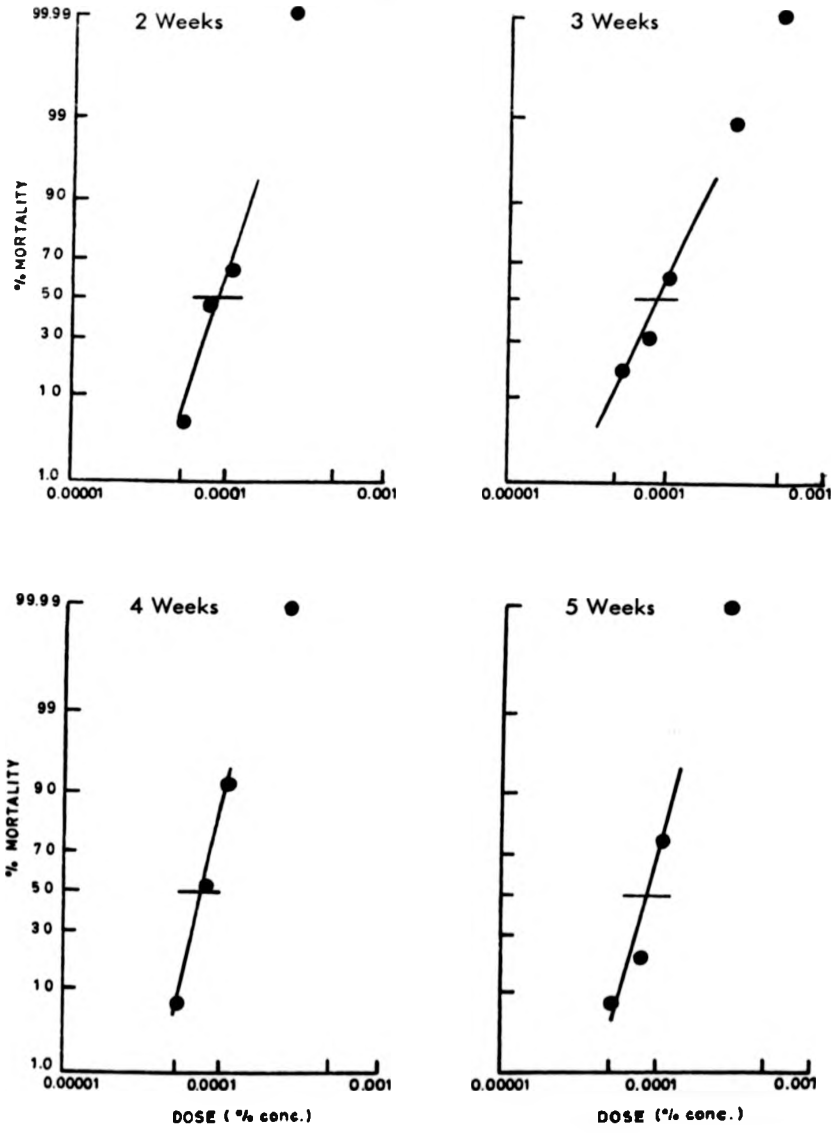


FIG. 16
RESULTS OF WEEKLY IMMERSION TESTS WITH PERMETHRIN AGAINST 2-, 3-, 4- AND 5-WEEK OLD UNFED LARVAE OF R. APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

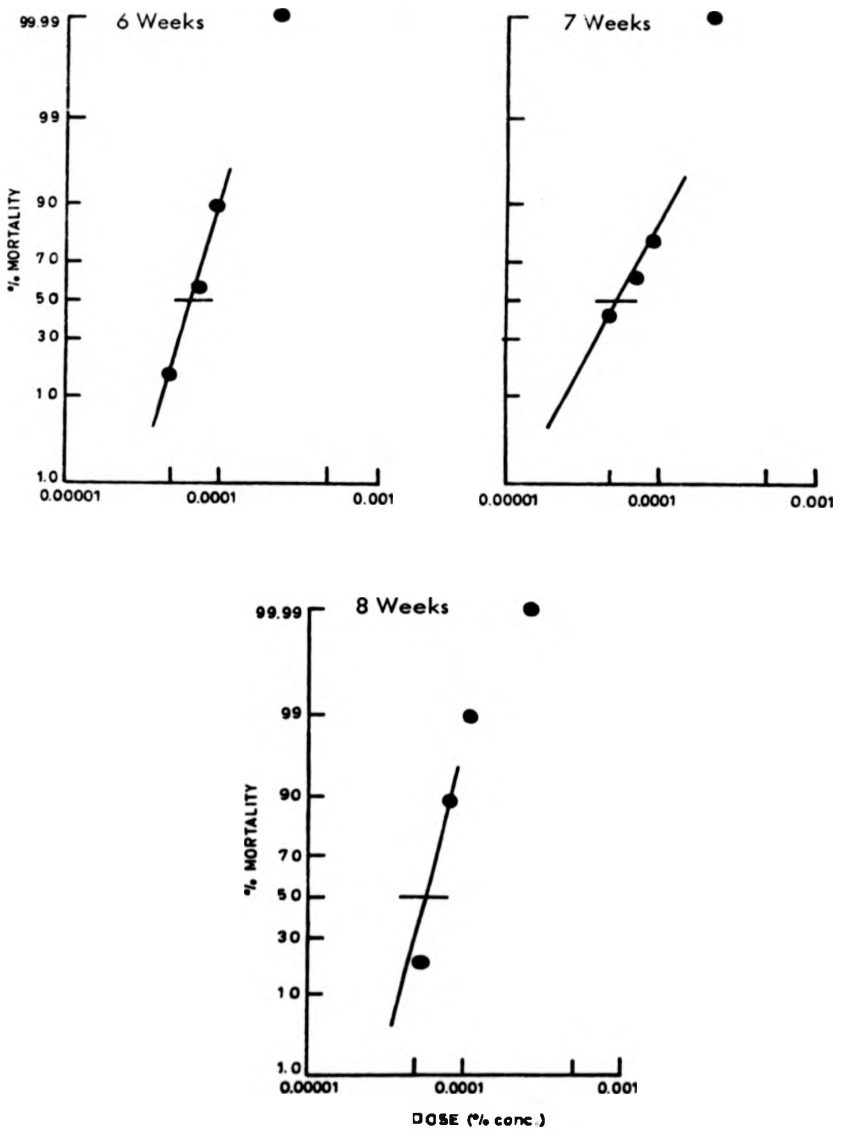


FIG.17
RESULTS OF WEEKLY IMMERSION TESTS WITH PERMETHRIN AGAINST 6-, 7- AND 8-WEEK OLD UNFED LARVAE OF R.APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

TABLE 5 - SUSCEPTIBILITY OF R. APPENDICULATUS UNFED LAVAE, 2 to 8 WEEKS OLD TO CYPERMETHRIN. RESULTS OF WEEKLY IMMERSION TESTS. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
cypermethrin	2	0.000103	0.000098 - 0.000109	0.000188	0.000174 - 0.000207	295	4.945 ± 0.25	2.75	> 0.1
	3	0.000113	0.000107 - 0.000121	0.000180	0.000163 - 0.000204	344	6.411 ± 0.47	19.98	< 0.001
	4	0.000080	0.000077 - 0.000083	0.000138	0.000128 - 0.000151	262	5.461 ± 0.35	3.001	> 0.1
	5	0.000095	0.000091 - 0.000099	0.000134	0.000123 - 0.000154	191	8.557 ± 1.09	0.07	> 0.975
	6	0.000069	0.000066 - 0.000072	0.000099	0.000093 - 0.000106	128	8.281 ± 0.68	0.35	> 0.1
	7	0.000064	0.000062 - 0.000067	0.000097	0.000091 - 0.000104	197	7.230 ± 0.52	4.84	> 0.05
	8	0.000067	0.000064 - 0.000069	0.000106	0.000098 - 0.000116	169	6.363 ± 0.49	29.56	< 0.001

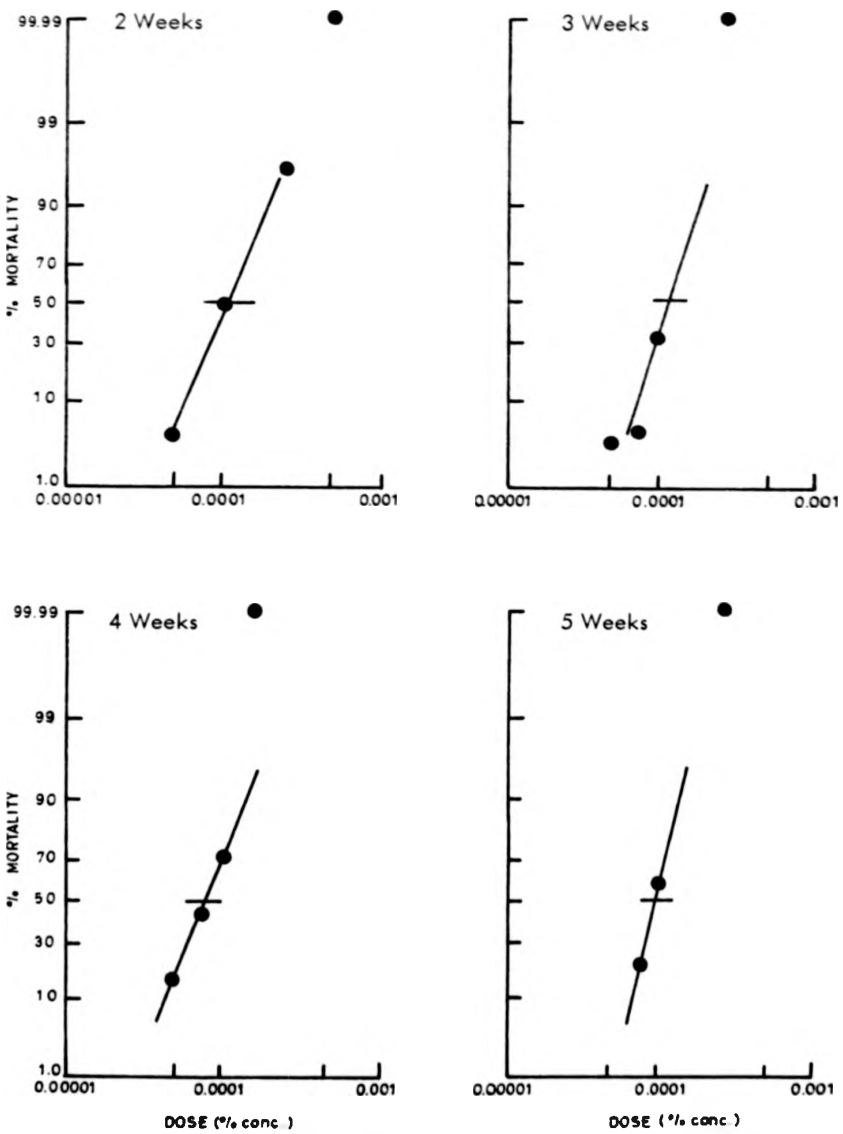


FIG. 18
RESULTS OF WEEKLY IMMERSION TESTS WITH CYPERMETHRIN AGAINST 2-, 3-, 4- AND 5-WEEK OLD UNFED LARVAE OF R. APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

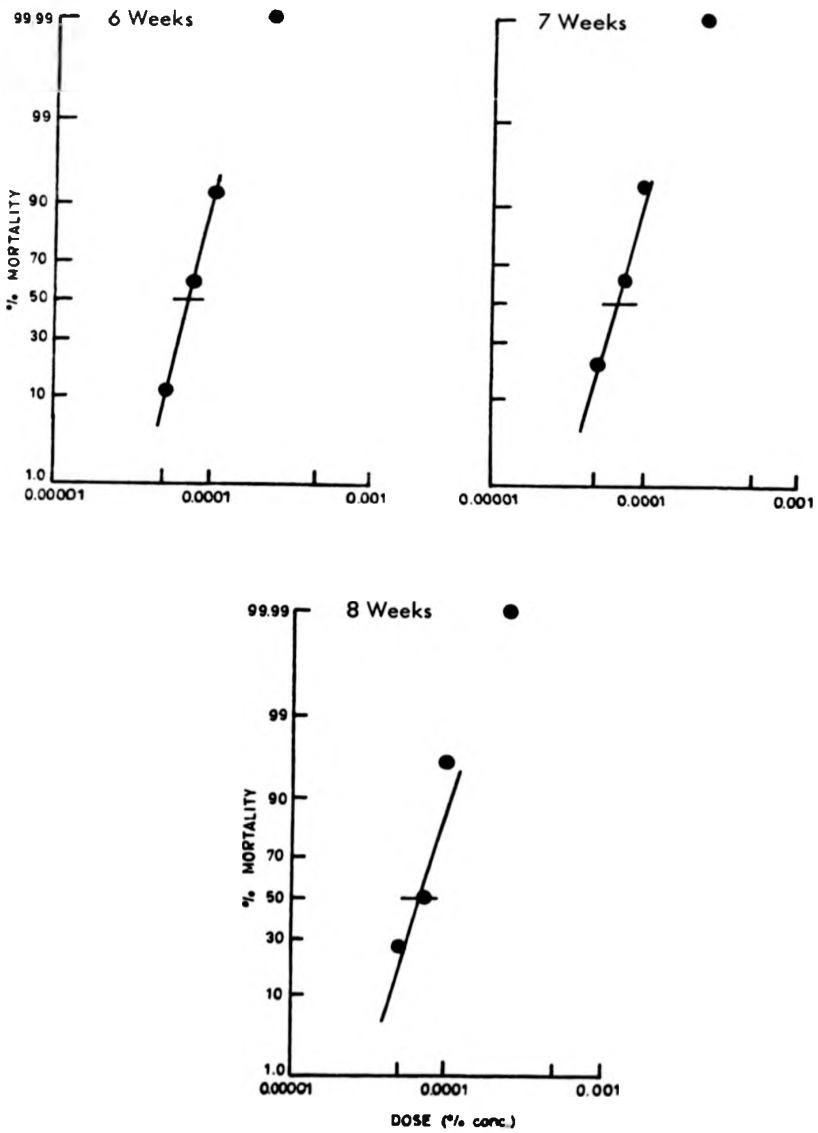


FIG. 19
RESULTS OF WEEKLY IMMERSION TESTS WITH CYPERMETHRIN AGAINST 6-, 7- AND 8 WEEK OLD UNFED LARVAE OF R. APPENDICULATUS REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

TABLE 6 - SUSCEPTIBILITY OF R. APPENDICULATUS UNFED LARVAE 2 to 8 WEEKS OLD TO DECAMETHRIN. RESULTS OF WEEKLY IMMERSION TESTS. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
decamethrin	2	0.000066	0.000063 - 0.000069	0.000122	0.000113 - 0.000136	226	4.786 ± 0.38	1.03	> 0.5
	3	0.000062	0.000060 - 0.000064	0.000113	0.000107 - 0.000120	395	4.923 ± 0.21	8.40	> 0.05
	4	0.000068	0.000066 - 0.000070	0.000102	0.000097 - 0.000108	271	7.280 ± 0.40	0.50	> 0.75
	5	0.000073	0.000069 - 0.000076	0.000116	0.000108 - 0.000127	165	6.332 ± 0.52	17.04	< 0.001
	6	0.000046	0.000042 - 0.000049	0.000102	0.000091 - 0.000117	127	3.685 ± 0.27	1.76	> 0.5
	7	0.000049	0.000044 - 0.000053	0.000107	0.000095 - 0.000124	113	3.794 ± 0.34	9.06	< 0.025
	8	0.000041	0.000039 - 0.000043	0.000064	0.000060 - 0.000068	166	6.689 ± 0.41	12.30	< 0.005

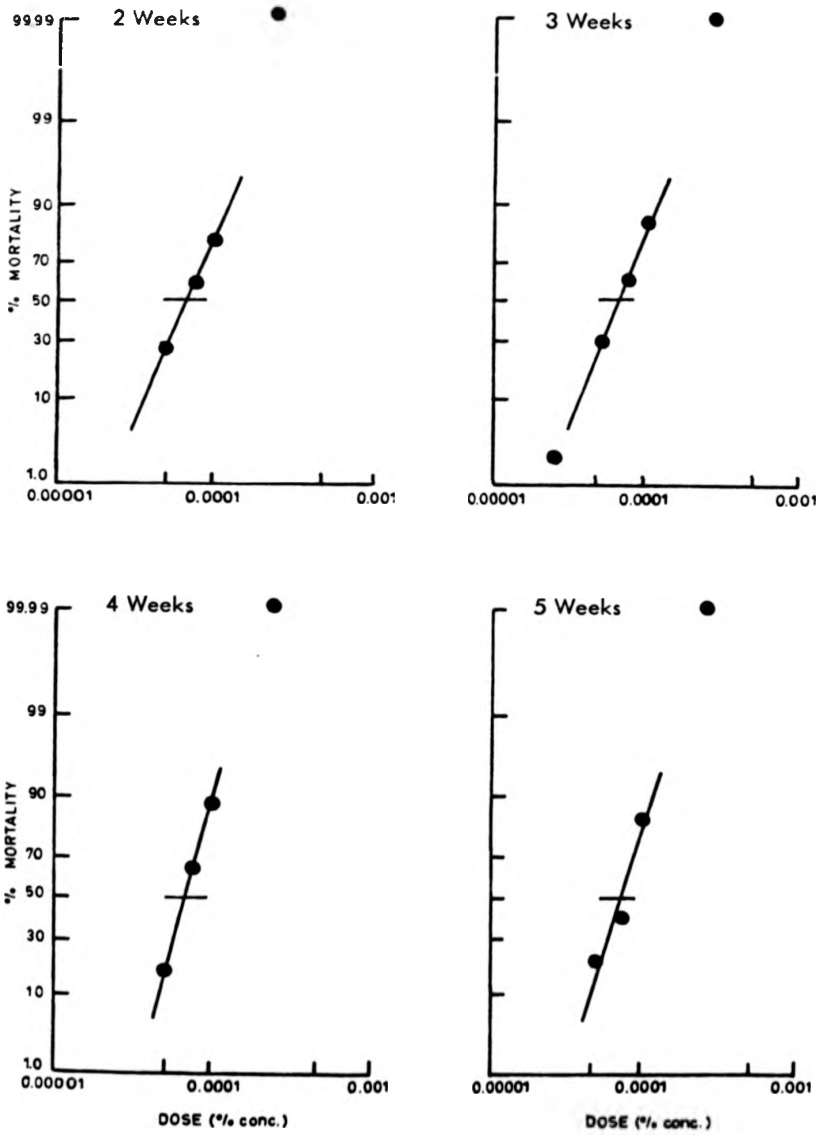


FIG.20
RESULTS OF WEEKLY IMMERSION TESTS WITH DECAMETHRIN AGAINST 2-, 3-,4- AND 5-WEEK OLD UNFED LARVAE OF R.APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

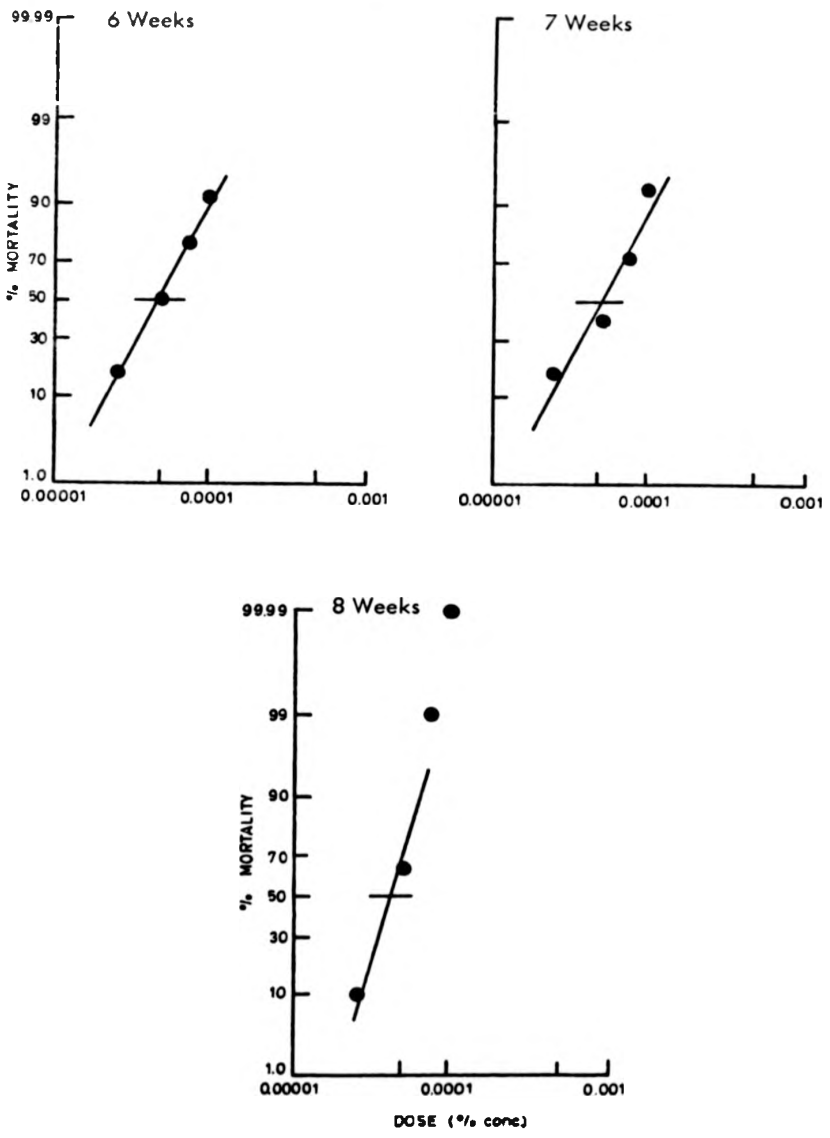


FIG.21
RESULTS OF WEEKLY IMMERSION TESTS WITH DECAMETHRIN AGAINST 6-,7- AND 8-WEEK OLD UNFED LARVAE OF R.APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

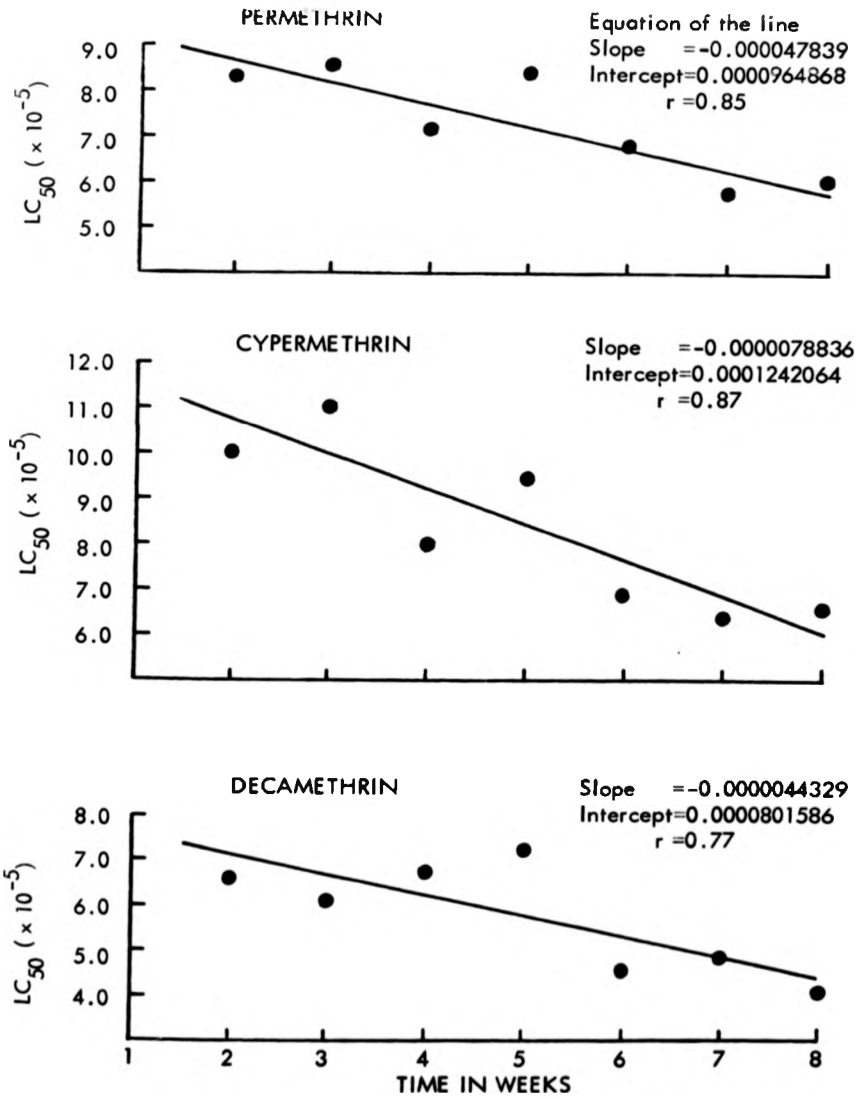


FIG.22
SUSCEPTIBILITY OF UNFED LARVAE OF R.APPENDICULATUS OF DIFFERENT AGES TO PERMETHRIN, CYPERMETHRIN AND DECAMETHRIN. RESULTS OF WEEKLY IMMERSION TESTS. REGRESSION LINES RELATING TO TIME IN WEEKS AND LC_{50} VALUES.

times more susceptible than 2-week old larvae. Comparisons of LC_{90} values also gave similar increased susceptibility factors, the oldest larvae being 1.7, 1.8 and 1.9 times more susceptible to permethrin, cypermethrin and decamethrin respectively than the youngest larvae.

To find out if there was any positive relationship between age of larvae and the concentrations necessary for 50% kill, LC_{50} values for each compound for each successive week were plotted and their regression lines drawn using the equation $y = m + c$, where y is the value of LC_{50}, x the age of the larvae, m the slope of the regression line and c (constant) the intercept on the y axis. The relationship was highly significant in each case. Fig. 22 shows the scatter points, the regression line and its equation. The correlation coefficient (r) which is the statistic most commonly used for linear correlation between two variables, was 0.85, 0.87 and 0.77, for permethrin, cypermethrin and decamethrin respectively, indicating that the correlation between the observed and estimated LC_{50} was higher than could be expected to happen by chance. The slope of the regression line in all cases was negative, indicating that increase in age of larvae results in an increase in susceptibility to the three acaricides.

3.6.2.2 The effect of three synthetic pyrethroids, amitraz and carbaryl on unfed larvae of *Amblyomma hebraeum*

An average number of 275 to 593 larvae were tested per concentration of each of the three compounds. Based on LC_{50} values decamethrin was the most effective compound against this species also in both age groups (2 to 4 and 5 to 8 weeks old larvae), followed by cypermethrin and permethrin (Table 7, Fig. 23). Decamethrin with LC_{50} values of

TABLE 7 - SUSCEPTIBILITY OF A. HEBRAEUM UNFED LARVAE, 2 to 4 WEEKS AND 5 to 8 WEEKS OLD, TO 3 SYNTHETIC PYRETHROIDS. RESULTS OF IMMERSION TESTS. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	2 - 4	0.000083	0.000079 - 0.000087	0.000213	0.000194 - 0.000238	291	3.141 ± 0.16	9.20	>0.05
	5 - 8	0.000027	0.000025 - 0.000029	0.000084	0.000075 - 0.000095	275	2.641 ± 0.12	50.91	<0.001
cypermethrin	2 - 4	0.000015	0.000014 - 0.000016	0.000047	0.000043 - 0.000053	280	2.579 ± 0.12	13.06	<0.025
	5 - 8	0.0000085	0.0000079 - 0.0000092	0.000031	0.000028 - 0.000036	304	2.260 ± 0.11	12.69	<0.025
decamethrin	2 - 4	0.000010	0.000010 - 0.000011	0.000024	0.000023 - 0.000026	335	3.503 ± 0.13	10.55	>0.05
	5 - 8	0.0000030	0.0000029 - 0.0000031	0.0000083	0.0000060 - 0.0000066	593	4.026 ± 0.15	2.42	>0.25

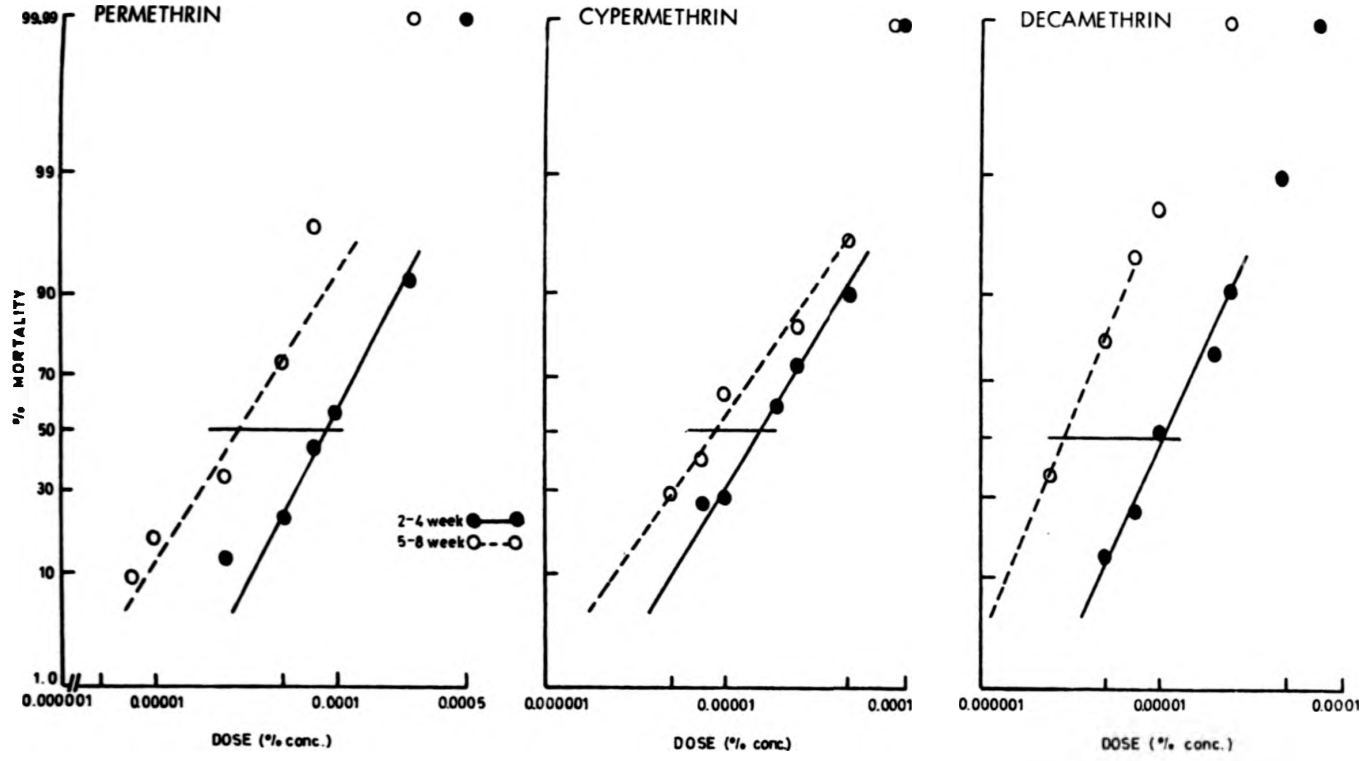


FIG.23

RESULTS OF IMMERSION TESTS WITH 3 SYNTHETIC PYRETHROIDS AGAINST 2 TO 4 WEEK AND 5 TO 8 WEEK OLD UNFED LARVAE OF A.HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

0.000010% for 2- to 4-week old larvae and 0.0000030% for 5- to 8-week old larvae was 1.5 to 2.8 times more effective than cypermethrin (LC_{50} 0.000015% and 0.0000085% respectively) and 8.3 to 9 times as effective as permethrin (LC_{50} 0.000083% and 0.000027% respectively). Cypermethrin showed a higher degree of activity against this species than permethrin, being 3.2 to 5.5 times more effective. When comparing the LC_{90} values of the three compounds, the order of increased effectiveness was similar. LC_{90} of decamethrin was 0.000024% for 2- to 4-week old larvae and 0.0000063% for 5- to 8-week old larvae, an increase in effectiveness by a factor of 1.9 to 5 compared to cypermethrin (0.000047% and 0.000031%) and by a factor of 8.9 to 13 compared to permethrin (0.000213% and 0.000084%). The slope of the regression line was steepest with decamethrin for both age groups (values of LC_{90}/LC_{50}, 2.1 to 2.4) and shallowest for cypermethrin against 5- to 8-week old larvae (LC_{90}/LC_{50}, 3.6).

An increase in susceptibility with age was seen in A. hebraeum also, older larvae (5 to 8 weeks) requiring lower concentrations to achieve 50% or 90% mortality than younger larvae (2 to 4 weeks); for instance the LC_{50} of permethrin for younger larvae was 0.000083% and that for older larvae 0.000027%, an increased susceptibility factor of 3; with cypermethrin the increased factor for the older larvae was 1.7 (0.000015% and 0.0000085% for young and old larvae respectively). Decamethrin was 3.3 times more effective against the older age group (LC_{50} 0.0000030%) than against the younger (LC_{50} 0.000010%).

When larvae were tested every week from 2 to 8 weeks after eclosion, there was no marked difference in the LC_{50} values from one week to the next; but there was an obvious difference between 2- and 8-week old larvae (Tables 8 to 10, Figs. 24 to 29). In the case of permethrin the LC_{50} value dropped from 0.000123% to 0.000014%, an

TABLE 8 - SUSCEPTIBILITY OF A. HEBRAEUM UNFED LARVAE 2 to 8 WEEKS OLD TO PERMETHRIN.
RESULTS OF WEEKLY IMMERSION TESTS. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	2	0.000123	0.000116 - 0.000131	0.000247	0.000225 - 0.000276	216	4.250 ± 0.22	9.24	< 0.05
	3	0.000061	0.000055 - 0.000067	0.000120	0.000105 - 0.000134	106	4.622 ± 0.51	2.14	> 0.25
	4	0.000036	0.000031 - 0.000040	0.000079	0.000069 - 0.000092	73	3.726 ± 0.34	17.39	< 0.001
	5	0.000039	0.000036 - 0.000042	0.000085	0.000076 - 0.000097	191	3.793 ± 0.25	9.57	< 0.01
	6	0.000034	0.000030 - 0.000038	0.000073	0.000063 - 0.000087	86	3.900 ± 0.34	2.76	> 0.5
	7	0.000020	0.000016 - 0.000023	0.000053	0.000043 - 0.000069	64	3.035 ± 0.30	2.74	> 0.25
	8	0.000014	0.000012 - 0.000015	0.000037	0.000032 - 0.000045	99	2.953 ± 0.19	6.53	> 0.1

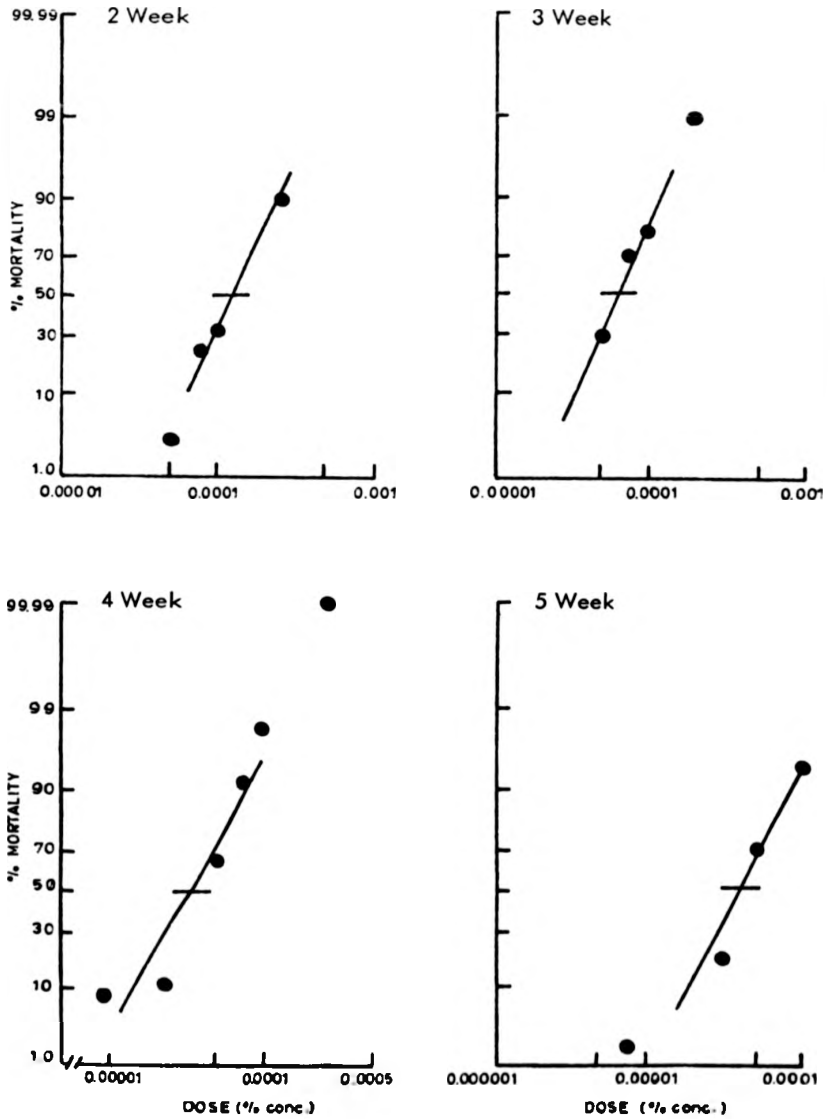


FIG. 24
RESULTS OF WEEKLY IMMERSION TESTS WITH PERMETHRIN AGAINST 2-, 3-, 4- AND 5-WEEK OLD UNFED LARVAE OF A. HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

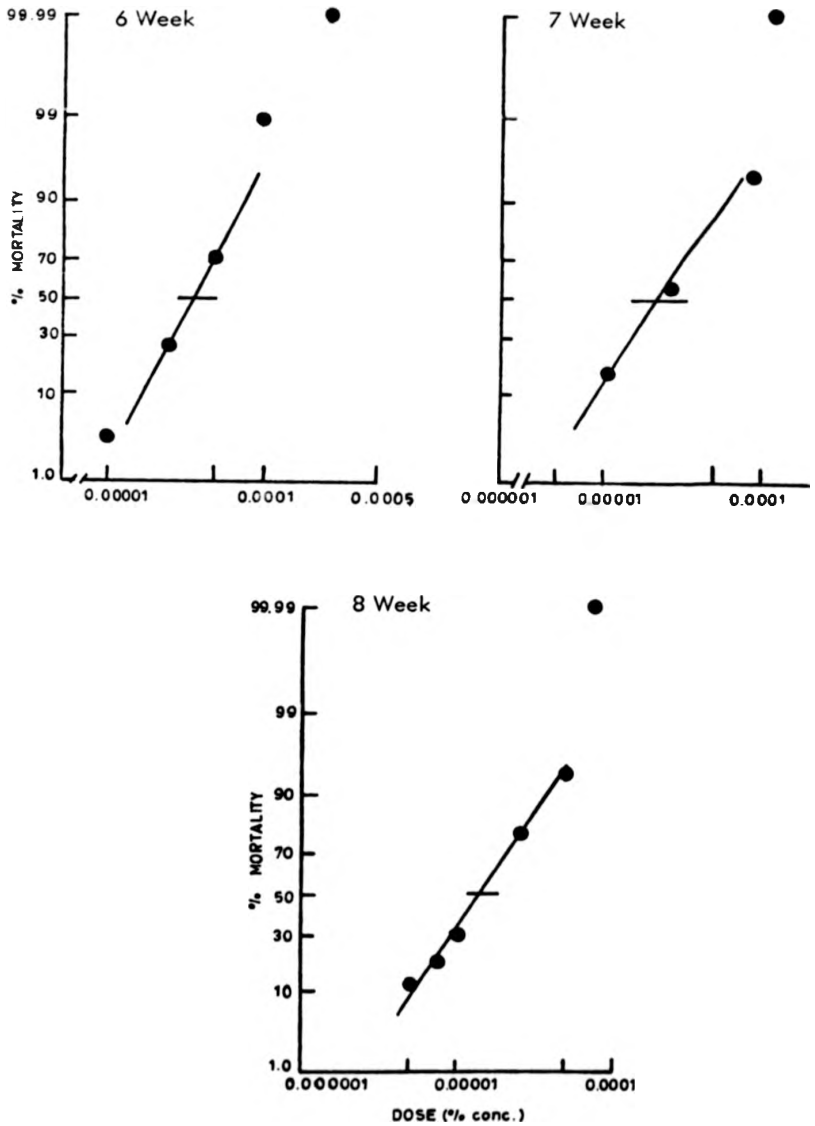


FIG.25
RESULTS OF WEEKLY IMMERSION TESTS WITH PERMETHRIN AGAINST 6-,7-, AND 8 WEEK OLD UNFED LARVAE OF A.HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

TABLE 9 - SUSCEPTIBILITY OF A. HEBRAEUM UNFED LARVAE 2 to 8 WEEKS OLD TO CYPERMETHRIN.
RESULTS OF WEEKLY IMMERSION TESTS. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
cypermethrin	2	0.000027	0.000024 - 0.000029	0.000077	0.000069 - 0.000090	173	2.825 ± 0.16	30.10	<0.001
	3	0.000016	0.000014 - 0.000017	0.000028	0.000026 - 0.000030	171	5.204 ± 0.43	10.69	<0.005
	4	0.0000087	0.0000073 - 0.000010	0.000025	0.000021 - 0.000035	71	2.733 ± 0.35	1.15	>0.75
	5	0.000012	0.000010 - 0.000013	0.000047	0.000041 - 0.000057	143	2.098 ± 0.14	19.26	<0.001
	6	0.0000089	0.0000079 - 0.0000099	0.000019	0.000016 - 0.000024	69	3.84 ± 0.40	7.08	>0.05
	7	0.0000080	0.0000073 - 0.0000091	0.000016	0.000013 - 0.000020	85	4.468 ± 0.52	0.33	>0.5
	8	0.0000060	0.0000054 - 0.0000065	0.000015	0.000012 - 0.000017	93	3.304 ± 0.82	0.33	>0.95

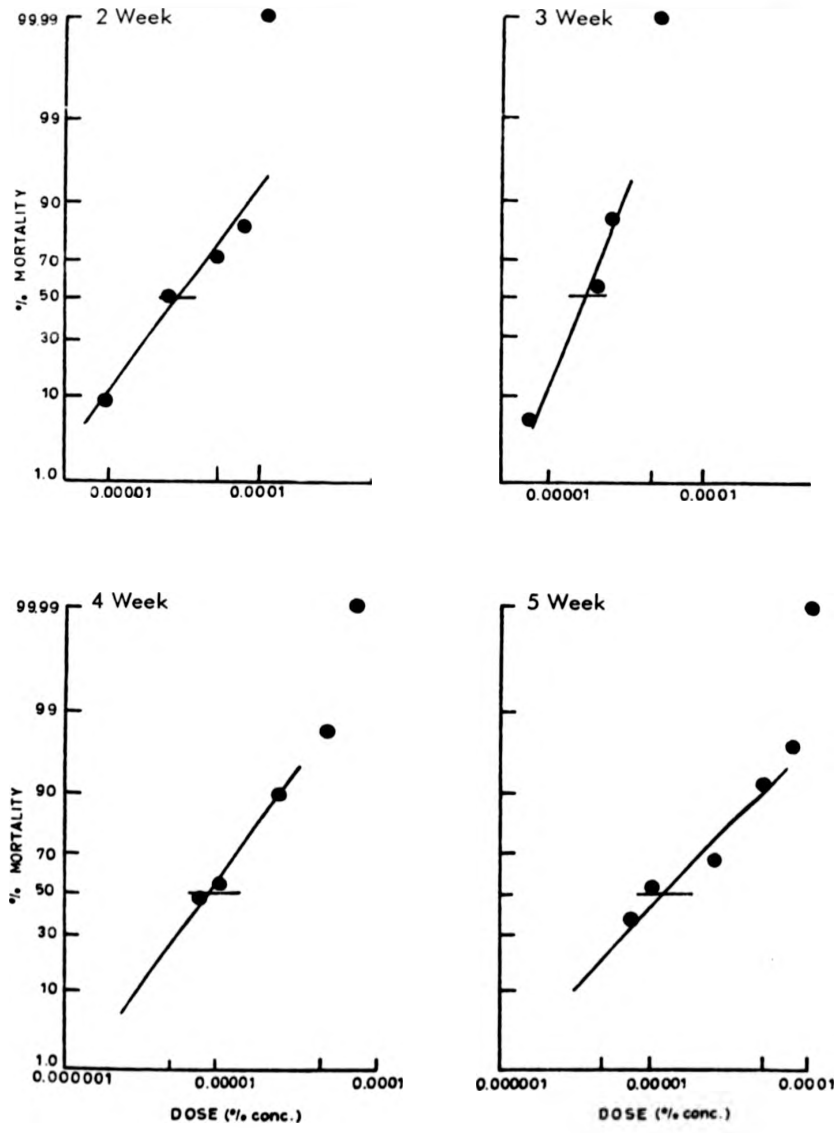


FIG.26
RESULTS OF WEEKLY IMMERSION TESTS WITH CYPERMETHRIN AGAINST 2-, 3-,4- AND 5-WEEK OLD UNFED LARVAE OF A.HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

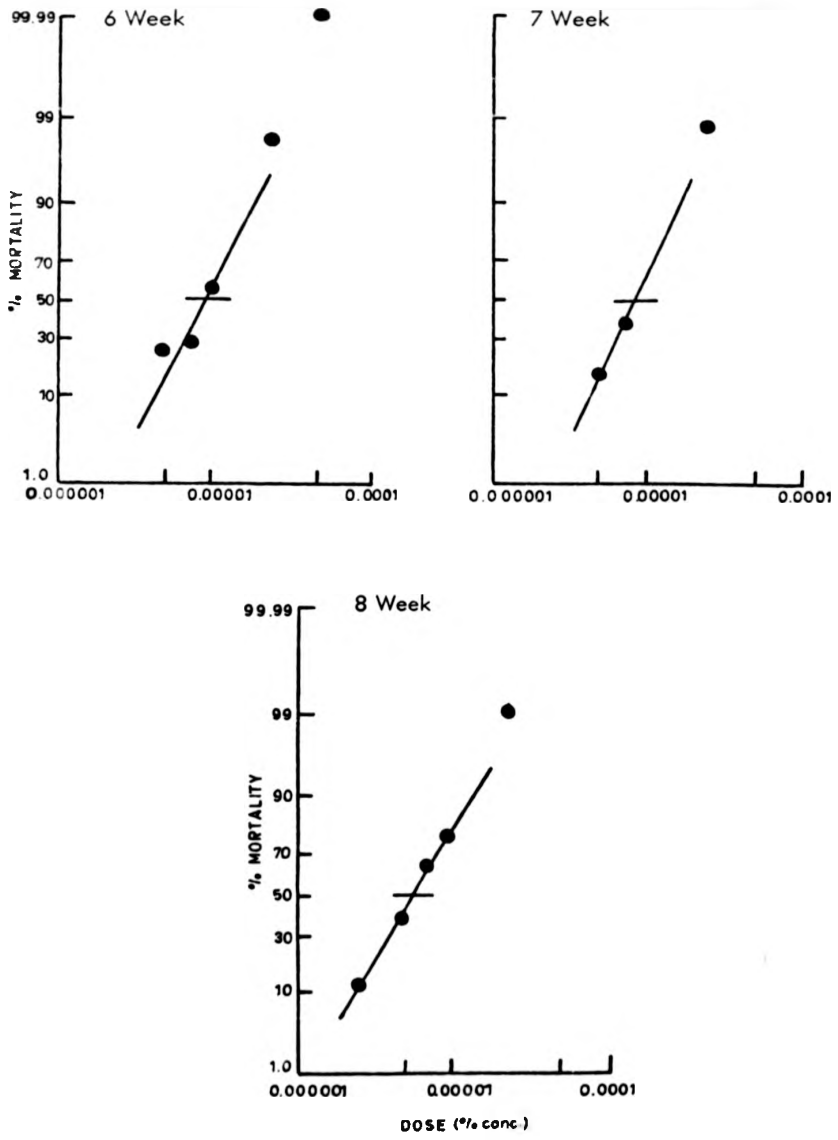


FIG.27
RESULTS OF WEEKLY IMMERSION TESTS WITH CYPERMETHRIN AGAINST 6-, 7- AND 8-WEEK OLD UNFED LARVAE OF A. HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

TABLE 10 - SUSCEPTIBILITY OF A. HEBRAEUM UNFED LARVAE, 2 to 8 WEEKS OLD TO DECAMETHRIN.
RESULTS OF WEEKLY IMMERSION TESTS. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
decamethrin	2	0.000012	0.000011 - 0.000013	0.000025	0.000022 - 0.000028	135	4.026 ± 0.22	2.78	>0.5
	3	0.000010	0.0000093 - 0.000011	0.000027	0.000024 - 0.000030	178	3.043 ± 0.17	2.34	>0.5
	4	0.0000084	0.0000078 - 0.0000090	0.000015	0.000013 - 0.000018	96	4.981 ± 0.49	18.97	<0.001
	5	0.0000047	0.0000042 - 0.0000050	0.0000077	0.0000070 - 0.0000088	72	5.829 ± 0.59	9.96	<0.025
	6	0.0000042	0.0000034 - 0.0000045	0.0000080	0.0000073 - 0.0000090	180	4.667 ± 0.33	0.20	>0.9
	7	0.0000025	0.0000023 - 0.0000027	0.0000058	0.0000051 0.0000067	165	3.517 ± 0.22	1.35	>0.5
	8	0.0000025	0.0000024 - 0.0000027	0.0000048	0.0000045 - 0.0000051	293	4.637 ± 0.23	0.90	>0.75

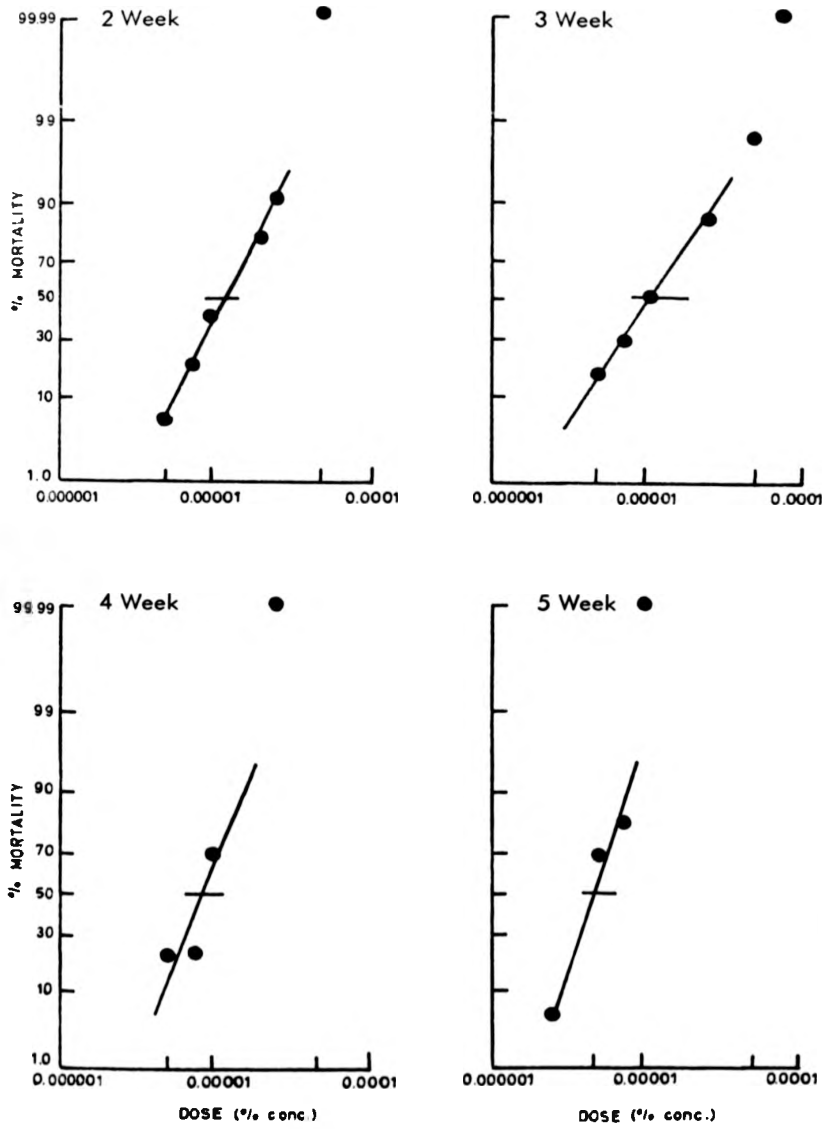


FIG. 28
RESULTS OF WEEKLY IMMERSION TESTS WITH DECAMETHRIN AGAINST 2-, 3-, 4- AND 5-WEEK OLD UNFED LARVAE OF A. HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

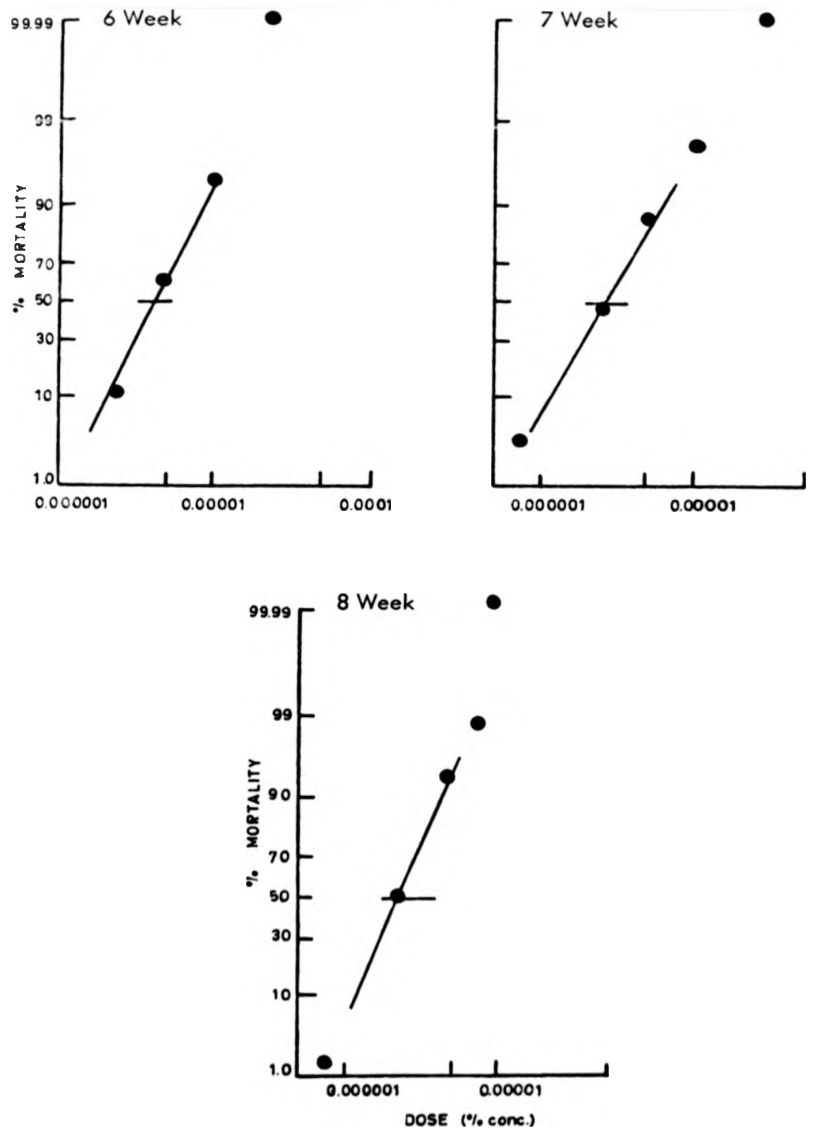


FIG. 29
RESULTS OF WEEKLY IMMERSION TESTS WITH DECAMETHRIN AGAINST 6-, 7- AND 8 WEEK OLD UNFED LARVAE OF A. HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

increased susceptibility factor of 8.8; with cypermethrin the LC_{50} decreased from 0.000027% to 0.0000060%, a factor of 4.5; a similar increased susceptibility factor (4.8) applied to decamethrin with LC_{50} values of 0.000012% for 2-week old larvae and 0.0000025% for 8-week old larvae. The LC_{50} values for each compound for each successive week, the regression line and its equation are shown in Fig. 30. The correlation coefficients were 0.86, 0.84 and 0.97 and the slope of the regression negative, indicating that as with R. appendiculatus, an increase in age of A. hebraeum larvae results in increased susceptibility to the three compounds.

The new compound amitraz has been shown to have high acaricidal activity (see Section 3.1). The effect of this compound and of the carbamate carbaryl, which is another acaricide extensively used in tick management, on unfed larvae of A. hebraeum was investigated for comparison with the three synthetic pyrethroids.

The LC_{50} of amitraz for 2- to 4-week old larvae was 0.000443% (Table 11 and Fig. 31); this was 5.3 times higher than the value for permethrin, 29.5 times higher than cypermethrin and 44.3 times higher than decamethrin. As with the synthetic pyrethroids, older larvae (LC_{50} 0.000094%) were more susceptible (4.7 times) than the younger larvae. The susceptibility of 5- to 8-week old larvae to amitraz vis-a-vis the three synthetic pyrethroids was of the same order as for 2- to 4-week old larvae.

Carbaryl was even less effective than amitraz. The LC_{50} (Table 11 and Fig. 32) for 2- to 4-week old larvae (0.00113%) was 13.6 times higher than for permethrin, 75 times higher than for cypermethrin and 113 times higher than for decamethrin. The difference in acaricidal activity was even more marked with 5- to 8-week old larvae; permethrin was 35 times, cypermethrin 112 times and decamethrin 318

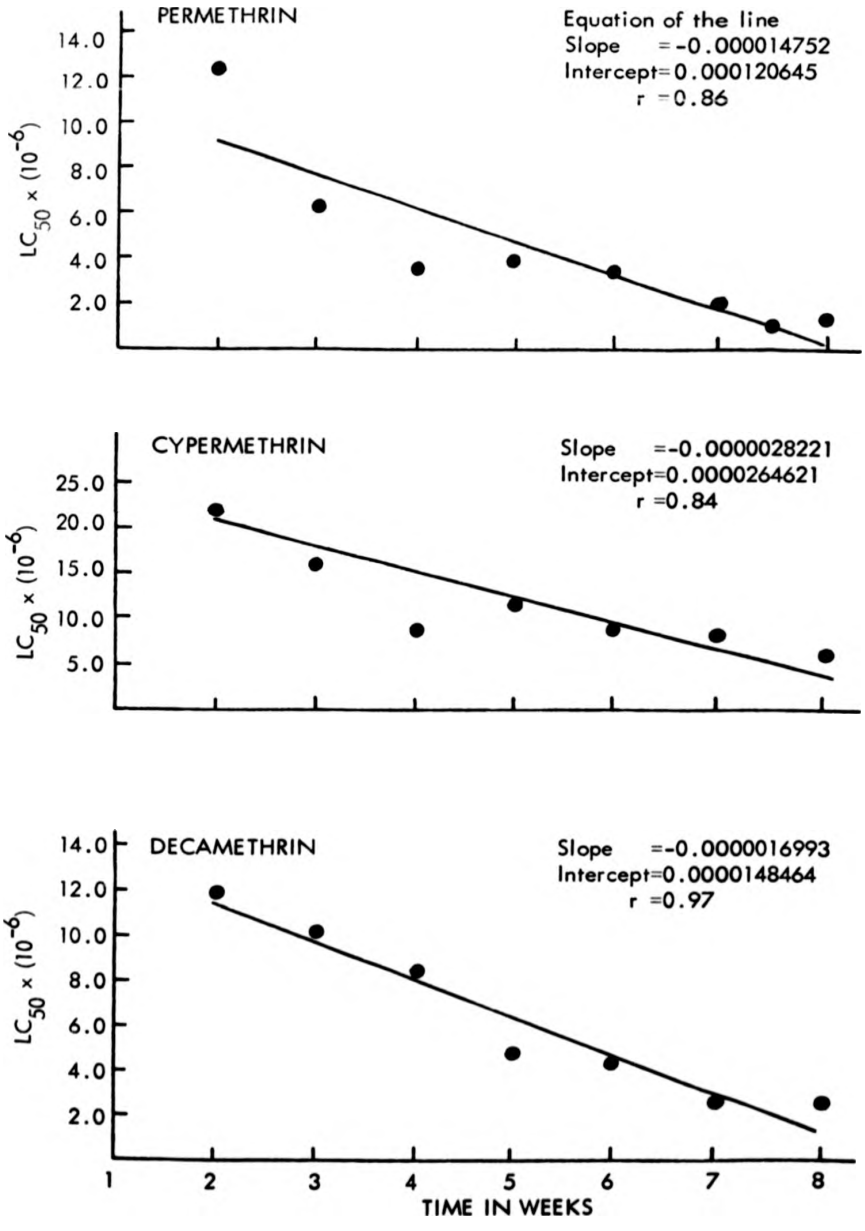


FIG.30
SUSCEPTIBILITY OF UNFED LARVAE OF A.HEBRAEUM OF DIFFERENT AGES TO PERMETHRIN, CYPERMETHRIN AND DECAMETHRIN. RESULTS OF WEEKLY IMMERSION TESTS. REGRESSION LINES RELATING TO TIME IN WEEKS AND LC_{50} VALUES.

TABLE 11 - SUSCEPTIBILITY OF A. HEBRAEUM UNFED LARVAE, 2 to 4 WEEKS AND 5 to 8 WEEKS OLD TO AMITRAZ AND CARBARYL. RESULTS OF IMMERSION TESTS. VALUES OF LC_{50} AND LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
amitraz	2 - 4	0.000443	0.000378 - 0.000515	0.03087	0.02328 - 0.04249	260	0.695 ± 0.02	231.3	<0.001
	5 - 8	0.000094	0.000076 - 0.000113	0.00175	0.00119 - 0.00290	131	1.009 ± 0.07	58.74	<0.001
carbaryl	2 - 4	0.00113	0.00103 - 0.00125	0.00294	0.00245 - 0.00379	132	3.080 ± 0.26	4.96	>0.1
	5 - 8	0.000955	0.000816 - 0.001104	0.00325	0.00269 - 0.00413	93	2.406 ± 0.19	16.33	<0.001

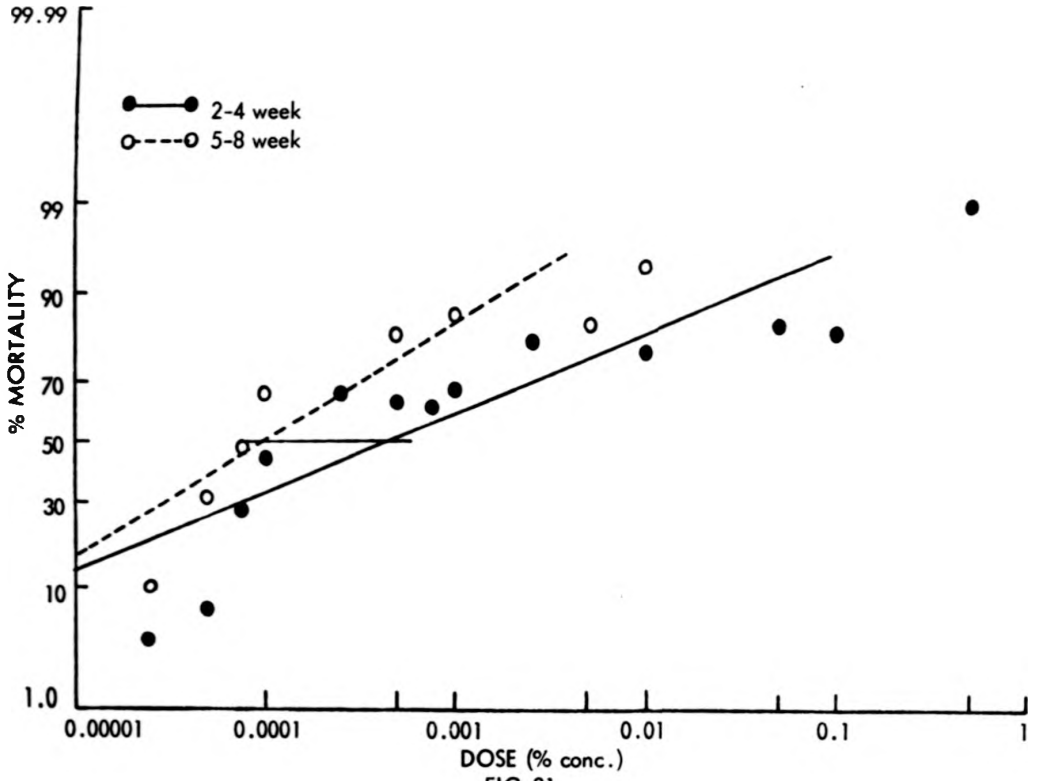


FIG.31

RESULTS OF IMMERSION TESTS WITH AMITRAZ AGAINST 2 TO 4 WEEK AND 5 TO 8 WEEK OLD UNFED LARVAE OF A.HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

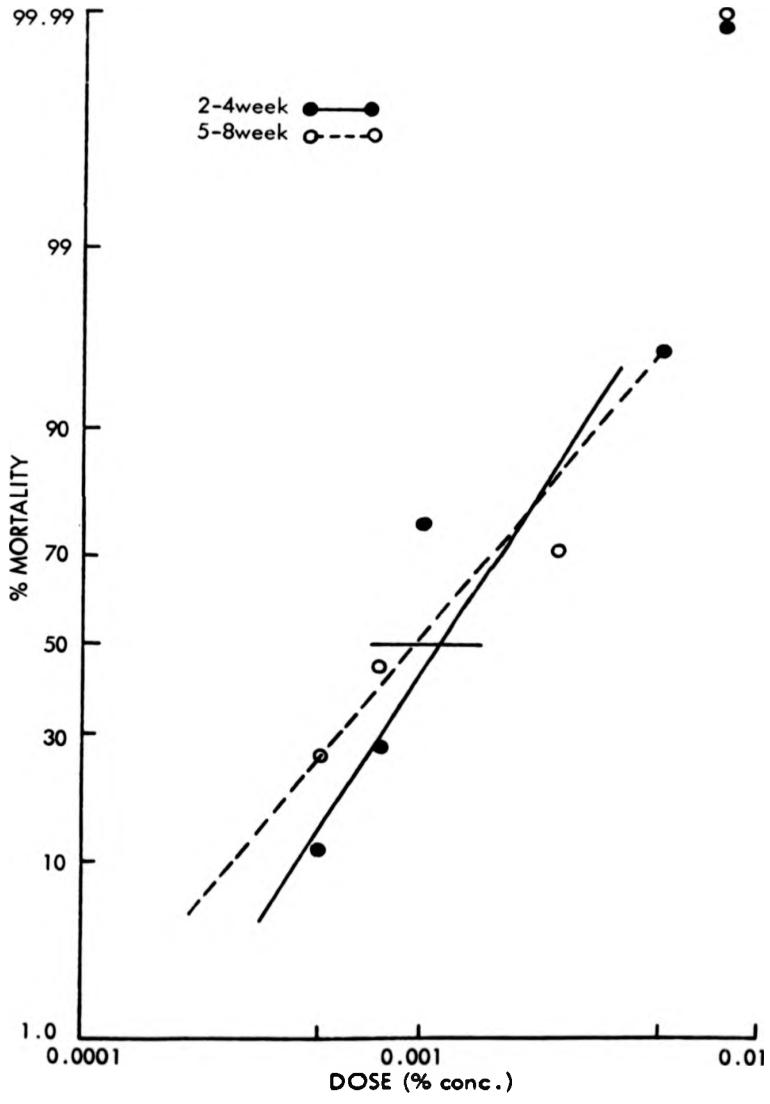


FIG.32
RESULTS OF IMMERSION TESTS WITH CARBARYL AGAINST 2 TO 4 WEEK AND 5 TO 8 WEEK OLD UNFED LARVAE OF A.HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

times more effective than carbaryl.

The regression line for amitraz was very shallow (Fig. 31) and the value of LC_{90}/LC_{50} was 70 for 2- to 4-week old larvae and 19 for 5- to 8-week old larvae, considerably shallower than for carbaryl (Fig. 32) with LC_{90}/LC_{50} values of 2.6 and 3.4 for the two age groups.

3.6.2.3 The effect of three synthetic pyrethroids, amitraz and carbaryl on unfed larvae of Amblyomma variegatum

The average number of larvae tester per concentration of each of the five compounds varied from 53 to 255, which was slightly less than the numbers used with R. appendiculatus and A. hebraeum.

Results from tests on young larvae (2 to 4 weeks) and old larvae (5 to 8 weeks) with the three compounds are presented in Table 12 and Fig. 33. As with R. appendiculatus and A. hebraeum decamethrin with LC_{50} values of 0.0000033% for 2- to 4-week old larvae and 0.0000023% for 5- to 8-week old larvae was the most effective compound. It was 11.5 and 10 times respectively as effective as permethrin (LC_{50} 0.000038% and 0.000024%) to larvae of the two age groups. Similar comparative figures for cypermethrin (LC_{50} 0.0000075% and 0.0000050%) were 2.3 and 2.1. On the basis of LC_{50} values cypermethrin was 4.8 to 5 times as effective as permethrin. LC_{90} values for decamethrin were 11 to 14 times lower than for permethrin and 2.1 to 2.2 times lower than for cypermethrin, while the values for cypermethrin compared to permethrin were 5 to 7 times lower. The LC_{90}/LC_{50} values were fairly low for all compounds and both age groups and varied from 1.8 to 2.5. From the LC_{50} values it is clear that older larvae were 1.4 times more susceptible to decamethrin, 1.5 times more susceptible to cypermethrin and 1.6 times more susceptible to permethrin. Detailed

TABLE 12 - SUSCEPTIBILITY OF A. VARIEGATUM UNFED LARVAE, 2 to 4 WEEKS AND 5 to 8 WEEKS OLD, TO 3 SYNTHETIC PYRETHROIDS. RESULTS OF IMMERSTION TESTS. VALUES OF LC_{50} AND LC_{90} (% conc.), SLOPE, Chi^2 AND P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	2 - 4	0.000038	0.000032 - 0.000043	0.000094	0.000079 - 0.000117	53	3.218 ± 0.32	3.631	>0.1
	5 - 8	0.000024	0.000021 - 0.000025	0.000055	0.000050 - 0.000061	213	3.481 ± 0.16	5.509	>0.1
cypermethrin	2 - 4	0.0000075	0.0000069 - 0.0000080	0.000014	0.000012 - 0.000016	85	5.056 ± 0.66	2.087	>0.1
	5 - 8	0.0000050	0.0000047 - 0.0000053	0.000011	0.000010 - 0.000012	255	3.686 ± 0.19	7.313	>0.05
decamethrin	2 - 4	0.0000033	0.0000029 - 0.0000035	0.0000066	0.0000060 - 0.0000073	89	4.215 ± 0.31	0.598	>0.95
	5 - 8	0.0000023	0.0000021 - 0.0000024	0.0000050	0.0000045 - 0.0000053	238	3.852 ± 0.19	0.661	>0.1

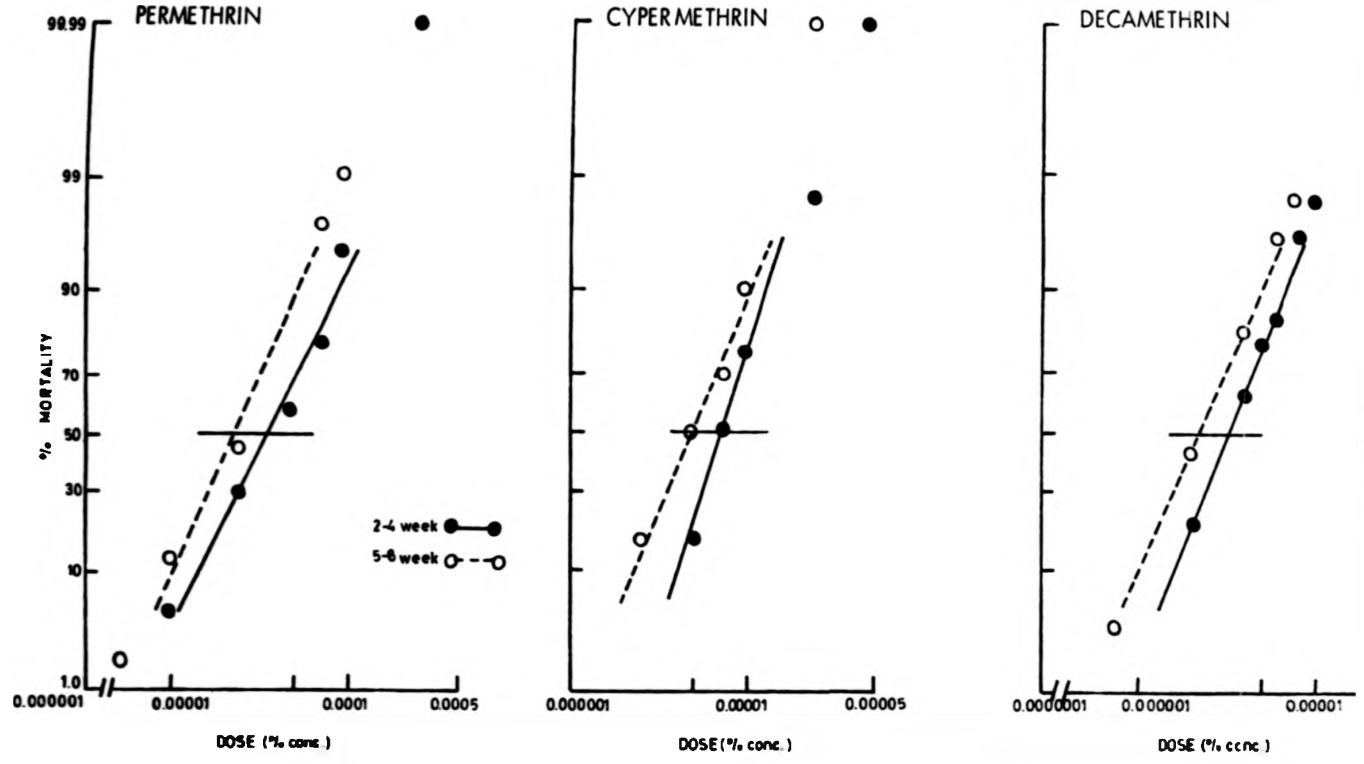


FIG.33
RESULTS OF IMMERSION TESTS WITH 3 SYNTHETIC PYRETHROIDS AGAINST 2 TO 4 WEEK AND 5 TO 8 WEEK OLD UNFED LARVAE OF A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

TABLE 13 - SUSCEPTIBILITY OF A. VARIEGATUM UNFED LARVAE, 2 to 4 WEEKS AND 5 to 8 WEEKS OLD TO AMITRAZ AND CARBARYL. RESULTS OF IMMERSION TESTS. VALUES OF LC_{50} AND LC_{90} (% conc.), SLOPE, Chi^2 AND P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
amitraz	2 - 4	0.000224	0.000191 - 0.000256	0.000570	0.000474 - 0.000741	66	3.161 ± 0.36	5.410	> 0.05
	5 - 8	0.000095	0.000085 - 0.000106	0.000286	0.000237 - 0.000367	93	2.68 ± 0.22	8.918	> 0.05
carbaryl	2 - 4	0.00534	0.00507 - 0.00560	0.0081	0.00773 - 0.00862	182	7.014 ± 0.45	20.01	< 0.001
	5 - 8	0.00237	0.00222 - 0.00252	0.00495	0.00458 - 0.00542	255	4.009 ± 0.20	2.45	> 0.25

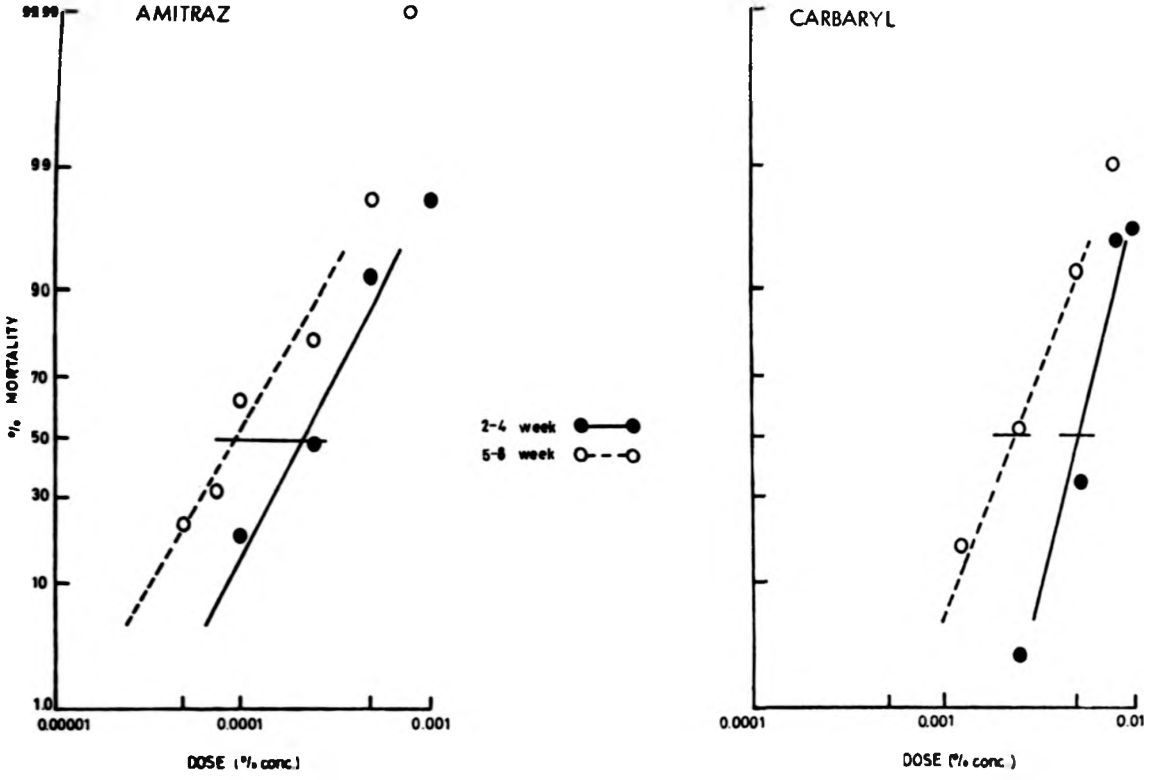


FIG. 34
RESULTS OF IMMERSION TESTS WITH AMITRAZ AND CARBARYL AGAINST 2 TO 4 WEEK AND 5 TO 8 WEEK OLD UNFED LARVAE OF A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

ageing experiments were not done with this species.

Amitraz and carbaryl were tested for their acaricidal activity against unfed larvae of A. variegatum also (Table 13 and Fig. 34). The LC_{50} of amitraz for younger larvae, 2 to 4 weeks old, was 0.000224%, and 0.000095% for older (5 to 8 weeks old) larvae, an increased susceptibility factor of 2.4 compared to 1.4 to 1.6 for the three synthetic pyrethroids. The two groups of larvae were 6 times and 4 times more susceptible to permethrin, 30 and 19 times more susceptible to cypermethrin and 68 and 41 times more susceptible to decamethrin compared to amitraz.

The LC_{50} of carbaryl were 0.00534% and 0.00237% respectively for 2- to 4-week and 5- to 8-week old larvae. Compared to the synthetic pyrethroids and amitraz, carbaryl was less effective against A. variegatum larvae than against A. hebraeum larvae. Thus permethrin was up to 140 times as effective, cypermethrin up to 712 times as effective and decamethrin up to 1618 times, and amitraz up to 25 times as effective as carbaryl against 2- to 4-week old larvae of A. variegatum.

Surprisingly the regression line for amitraz was much steeper for A. variegatum (Fig. 34) than for A. hebraeum (Fig. 31), the LC_{90}/LC_{50} of amitraz and carbaryl for the two age groups of A. variegatum ranging from 1.5 to 3.

3.6.2.4 Comparison of susceptibility of unfed larvae of R. appendiculatus, A. hebraeum and A. variegatum to three synthetic pyrethroids, amitraz and carbaryl

Of the 5 compounds tested, i.e., permethrin, cypermethrin and decamethrin, amitraz and carbaryl, decamethrin was the most effective. Of the 3 species of ticks, A. variegatum was the most susceptible to all compounds, followed by A. hebraeum and R. appendiculatus, which

was the most tolerant. This was equally true of 2- to 4-week old larvae and 5- to 8-week old larvae. The LC_{50} and LC_{90} values of the compounds for the 3 species are summarised in Tables 14 and 15. On the basis of LC_{50} A. variegatum was 1.1 to 2.2 times as susceptible A. hebraeum and 2.1 to 2.9 times as susceptible as R. appendiculatus to permethrin. Compared to R. appendiculatus, A. hebraeum was as susceptible or 2.5 times as susceptible to the same compound (Figs. 35 and 36).

The increased susceptibility of A. variegatum and A. hebraeum compared to R. appendiculatus was considerably more with cypermethrin (Figs. 37 and 38) and decamethrin (Figs. 39 and 40). Although A. variegatum larvae were only 1.3 to 3 times as susceptible as A. hebraeum to the 2 compounds, they were 12.8 to 21.7 as susceptible as R. appendiculatus. Similarly A. hebraeum larvae were 6.4 to 16.6 times as susceptible as R. appendiculatus larvae to the 2 compounds. In summary it may be stated that of the 3 synthetic pyrethroids, decamethrin was the most effective against all 3 species and that A. variegatum larvae were the most susceptible of the 3 species. The increased susceptibility of A. variegatum and A. hebraeum compared to R. appendiculatus was of a much higher order with cypermethrin and decamethrin than with permethrin.

From the limited number of experiments, A. variegatum larvae appeared to be more susceptible than A. hebraeum larvae to amitraz (Table 15 and Figs. 41 and 42); on the other hand A. hebraeum larvae were more susceptible to carbaryl than A. variegatum larvae (Table 15, Figs 43 and 44). However more experiments would be needed to confirm these observations.

TABLE 14 - COMPARISON OF SUSCEPTIBILITY OF R. APPENDICULATUS, A. HEBRAEUM AND A. VARIEGATUM UNFED LARVAE, 2 to 4 WEEKS AND 5 to 8 WEEKS OLD, TO 3 SYNTHETIC PYRETHROIDS RESULTS OF IMMERSION TESTS. VALUES OF LC_{50} and LC_{90} (% conc.)

Compound	Age in weeks	R. appendiculatus		A. hebraeum		A. variegatum	
		LC_{50} (%)	LC_{90} (%)	LC_{50} (%)	LC_{90} (%)	LC_{50} (%)	LC_{90} (%)
permethrin	2 - 4	0.000082	0.000142	0.000083	0.000213	0.000038	0.000034
	5 - 8	0.000069	0.000119	0.000027	0.000084	0.000024	0.000055
cypermethrin	2 - 4	0.000096	0.000171	0.000015	0.000047	0.0000075	0.000014
	5 - 8	0.000071	0.000120	0.0000085	0.000031	0.0000050	0.000011
decamethrin	2 - 4	0.000065	0.000108	0.000010	0.000024	0.0000033	0.0000066
	5 - 8	0.000050	0.000105	0.0000030	0.0000063	0.0000023	0.0000050

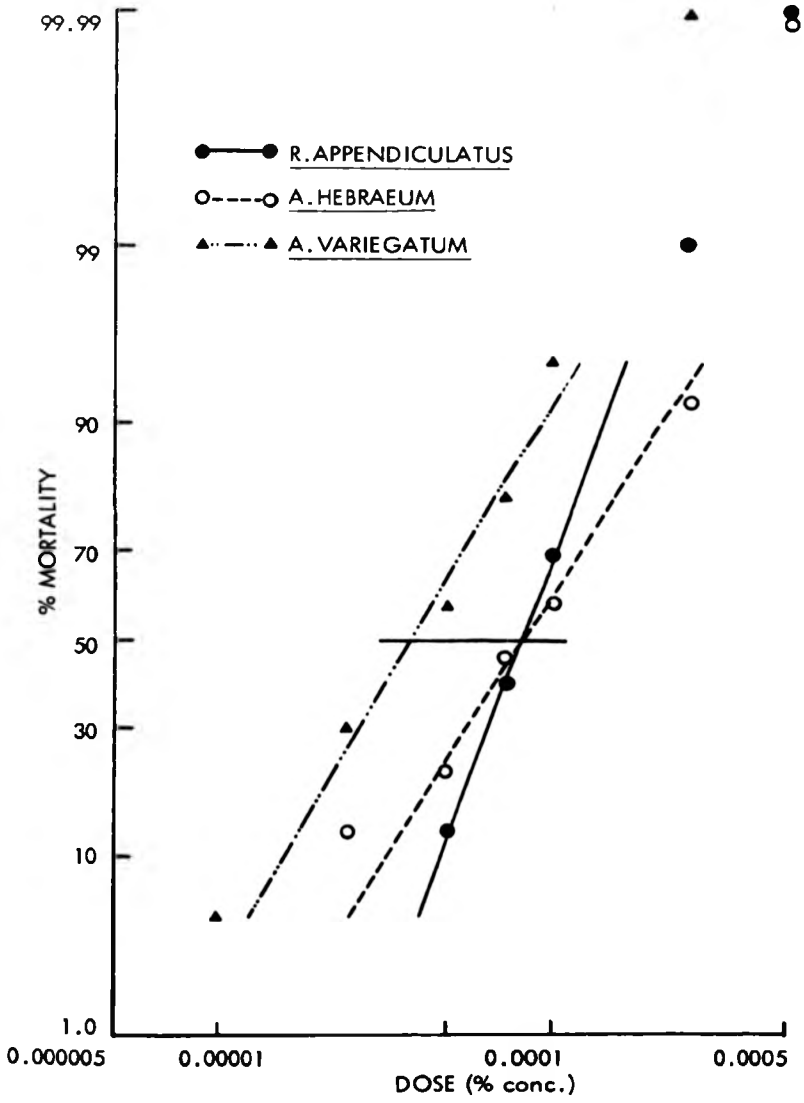


FIG. 35
RESULTS OF IMMERSION TESTS WITH PERMETHRIN AGAINST 2 TO 4 WEEK OLD UNFED LARVAE OF R. APPENDICULATUS, A. HEBRAEUM AND A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

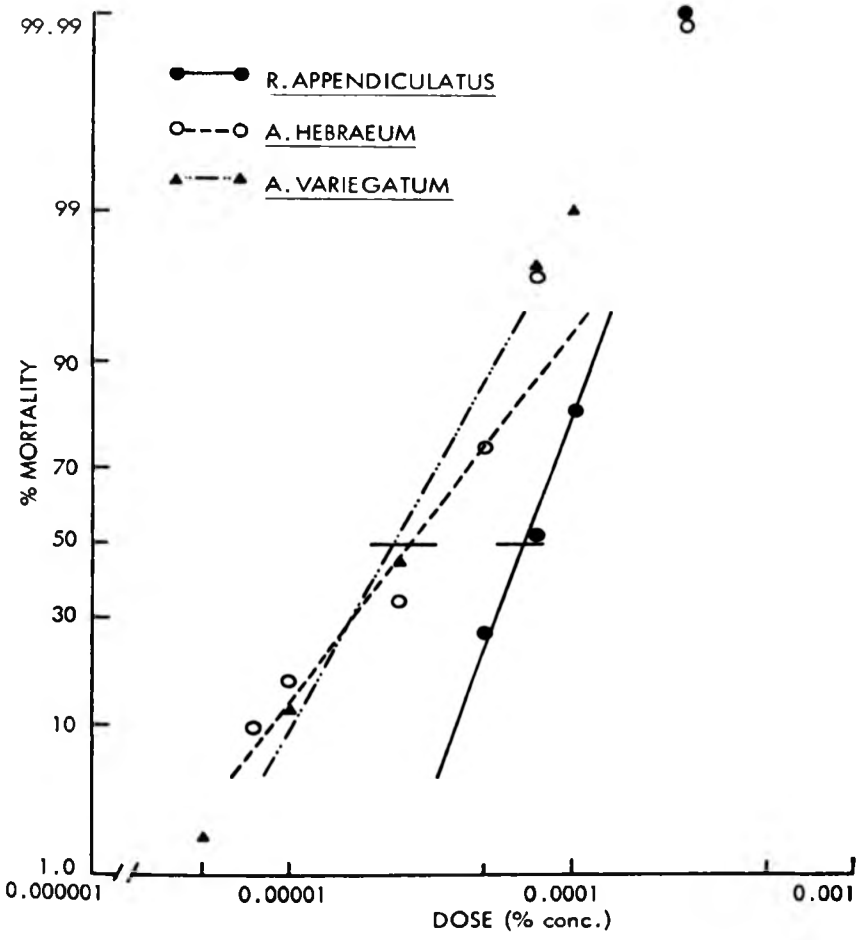


FIG. 36
RESULTS OF IMMERSION TESTS WITH PERMETHRIN AGAINST 5 TO 8 WEEK OLD UNFED LARVAE OF R. APPENDICULATUS, A. HEBRAEUM AND A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

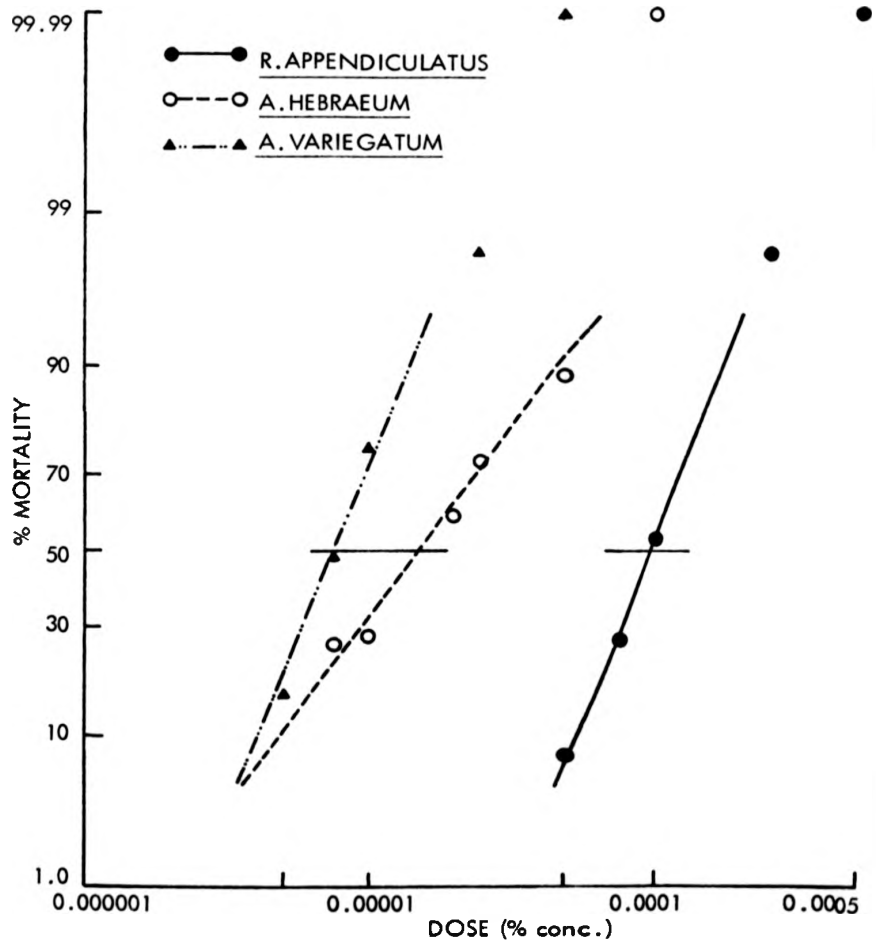


FIG. 37
RESULTS OF IMMERSION TESTS WITH CYPERMETHRIN AGAINST 2 TO 4 WEEK OLD UNFED LARVAE OF R. APPENDICULATUS, A. HEBRAEUM AND A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

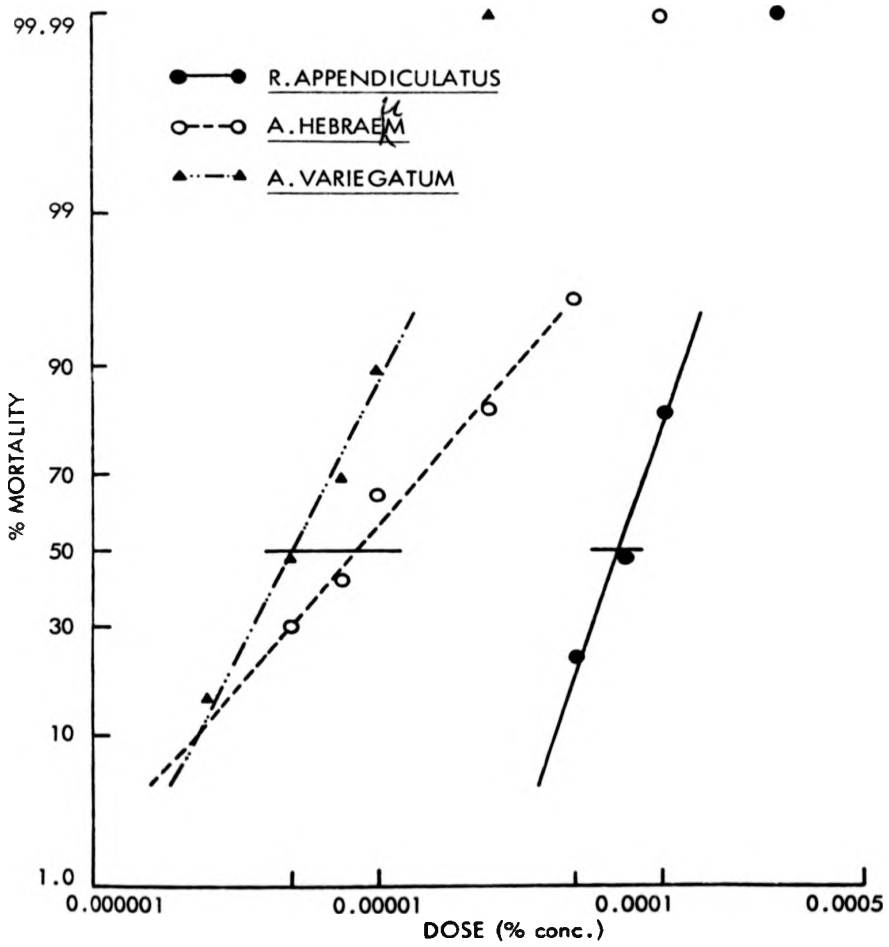


FIG. 38
RESULTS OF IMMERSION TESTS WITH CYPERMETHRIN AGAINST 5 TO 8 WEEK OLD UNFED LARVAE OF R. APPENDICULATUS, A. HEBRAEUM AND A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

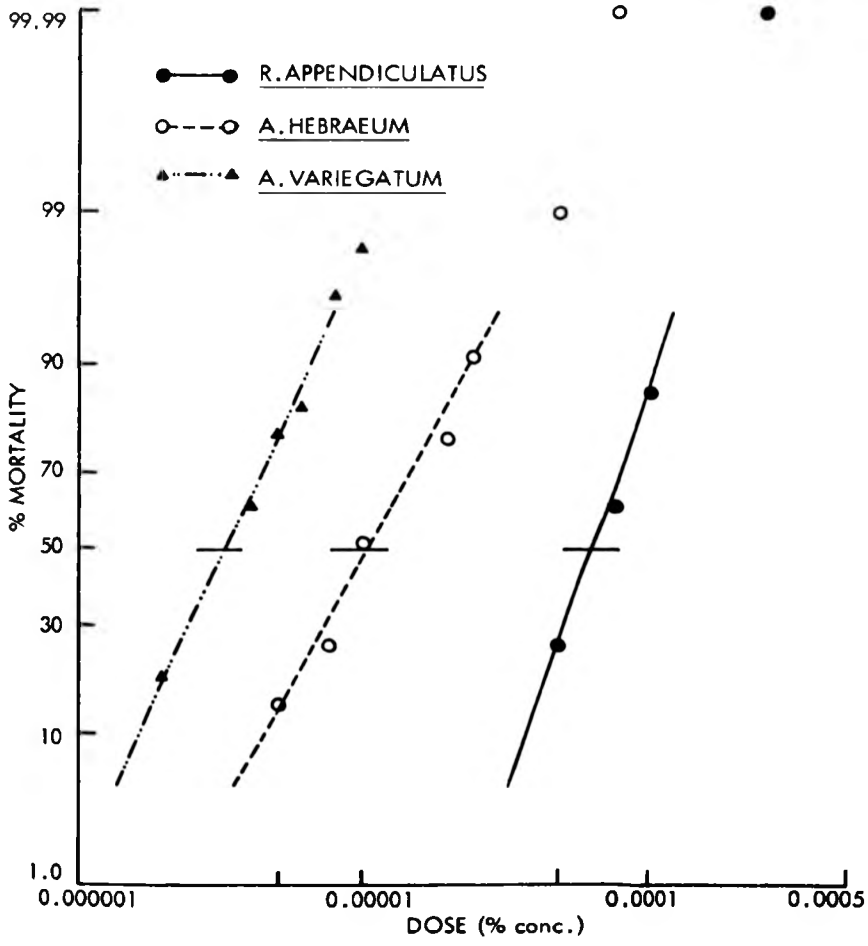


FIG. 39
RESULTS OF IMMERSION TESTS WITH DECAMETHRIN AGAINST 2 TO 4 WEEK OLD UNFED LARVAE OF R. APPENDICULATUS, A. HEBRAEUM AND A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

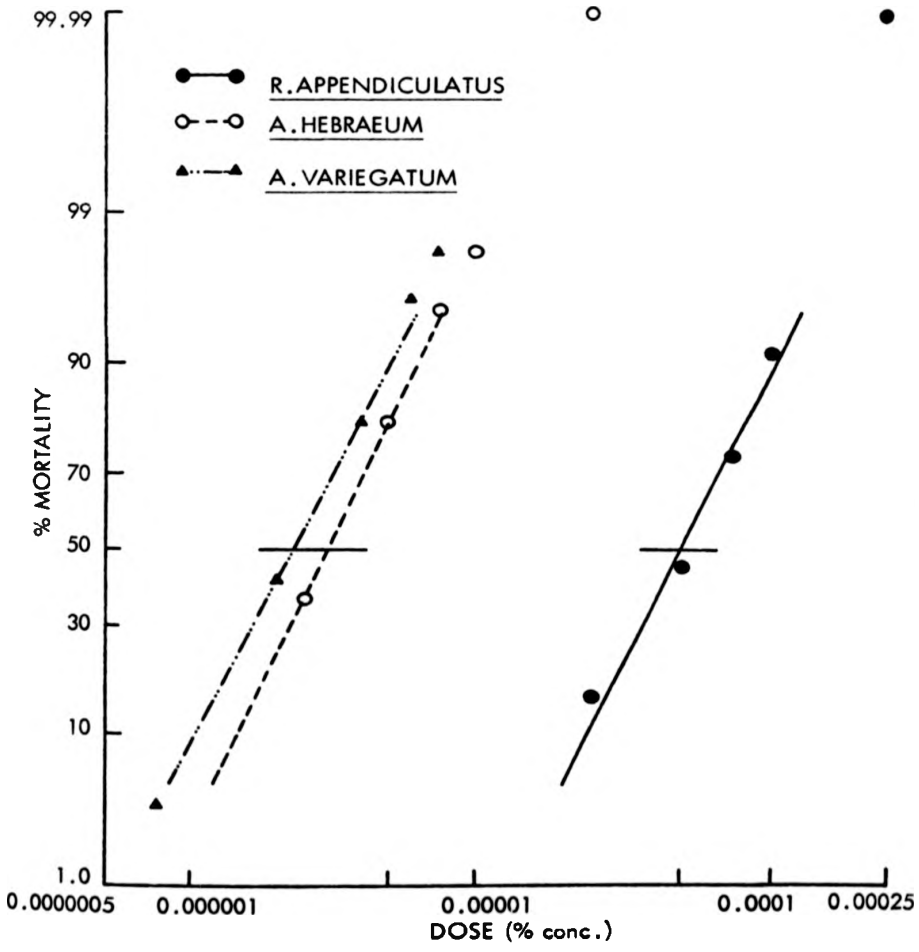


FIG.40
RESULTS OF IMMERSION TESTS WITH DECAMETHRIN AGAINST 5 TO 8 WEEK OLD UNFED LARVAE OF R.APPENDICULATUS, A.HEBRAEUM AND A.VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

TABLE 15 - COMPARISON OF SUSCEPTIBILITY OF A. HEBRAEUM AND A. VARIEGATUM UNFED LARVAE, 2 to 4 WEEKS AND 5 to 8 WEEKS OLD, TO AMITRAZ AND CARBARYL. RESULTS OF IMMERSION TESTS. VALUES OF LC_{50} and LC_{90} (% conc.)

Compound	Age in weeks	A. hebraeum		A. variegatum	
		LC_{50} (%)	LC_{90} (%)	LC_{50} (%)	LC_{90} (%)
Amitraz	2 - 4	0.000443	0.03087	0.000224	0.000570
	5 - 8	0.000094	0.00175	0.000095	0.000286
Carbaryl	2 - 4	0.00113	0.00294	0.00534	0.0081
	5 - 8	0.000955	0.00325	0.00237	0.00495

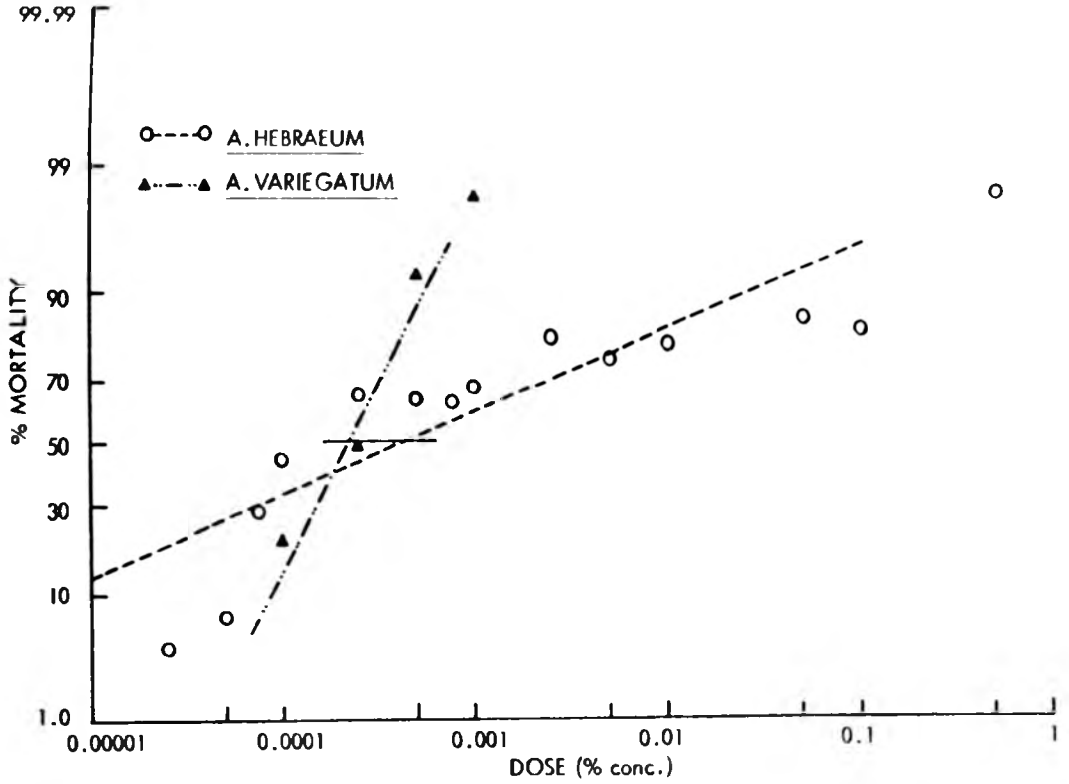


FIG. 41

RESULTS OF IMMERSION TESTS WITH AMITRAZ AGAINST 2 TO 4 WEEK OLD UNFED LARVAE OF A. HEBRAEUM AND A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

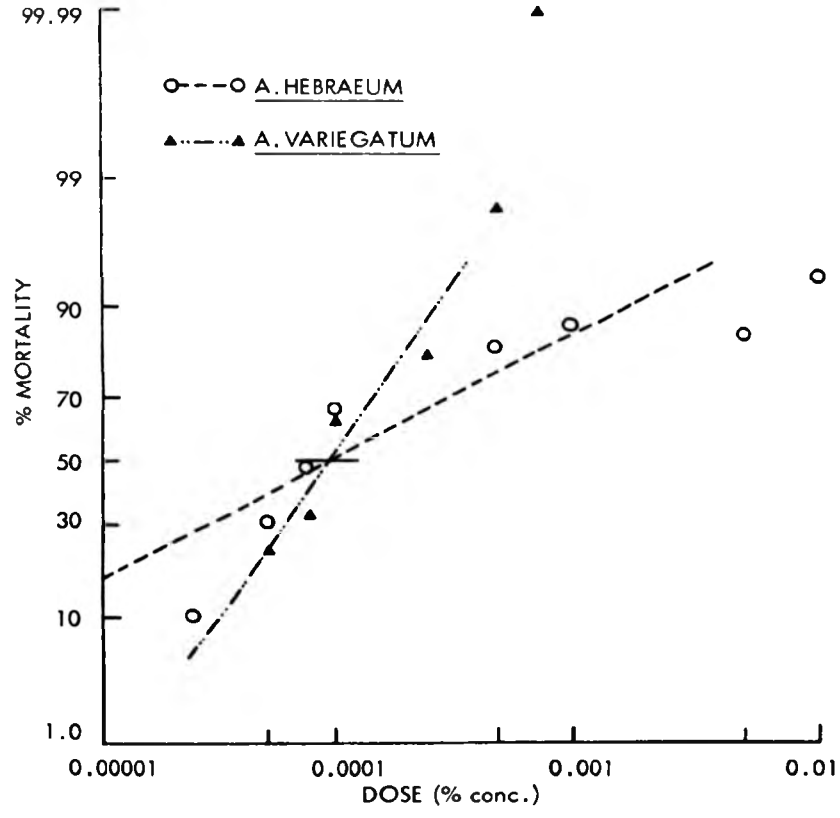


FIG. 42
RESULTS OF IMMERSION TESTS WITH AMITRAZ AGAINST 5 TO 8 WEEK OLD UNFED LARVAE OF A. HEBRAEUM AND A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

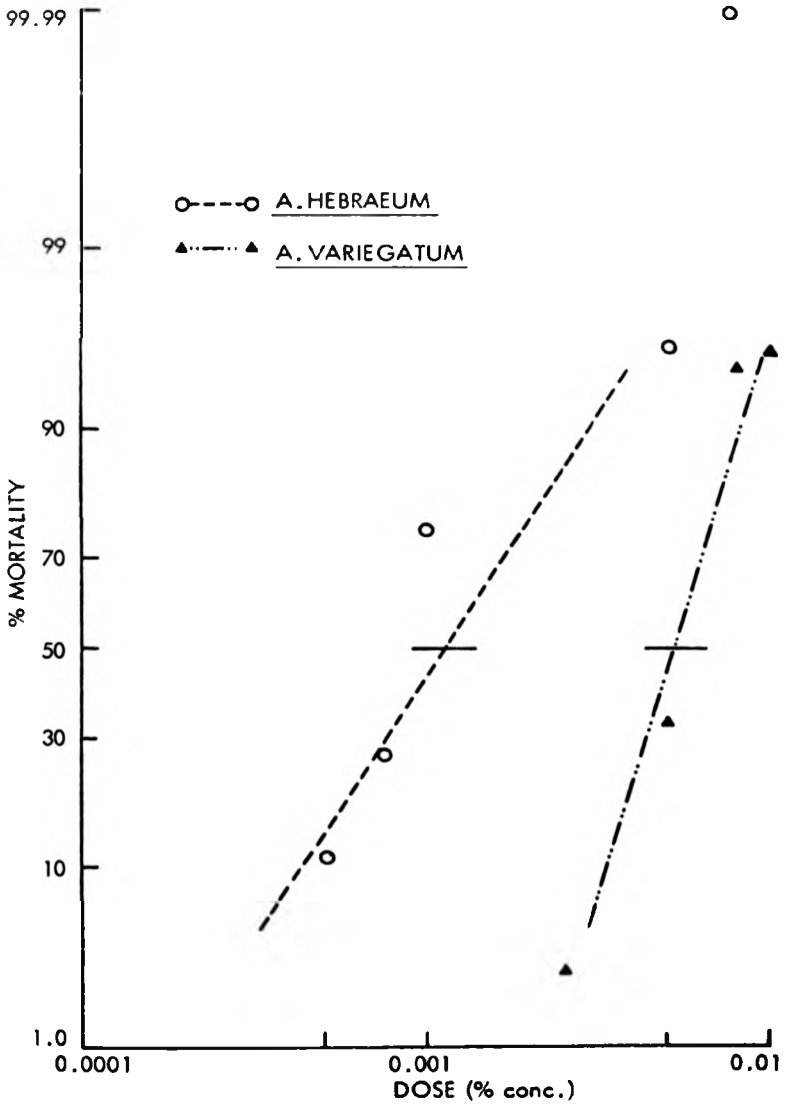


FIG.43
RESULTS OF IMMERSION TESTS WITH CARBARYL AGAINST 2 TO 4 WEEK OLD UNFED LARVAE OF A. HEBRAEUM AND A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

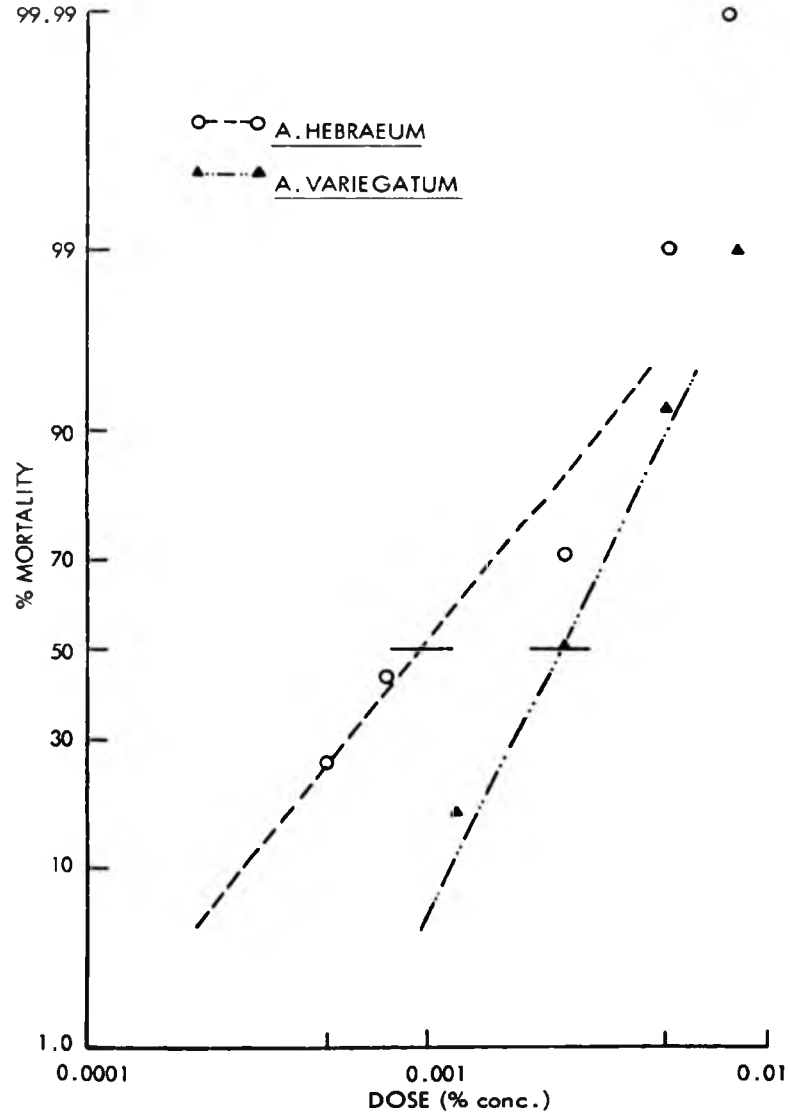


FIG. 44
RESULTS OF IMMERSION TESTS WITH CARBARYL AGAINST 5 TO 8 WEEK OLD UNFED LARVAE OF A. HEBRAEUM AND A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

3.6.3 Results of tests with nymphal ticks

3.6.3.1 The effect of permethrin and decamethrin on unfed nymphs of Rhipicephalus appendiculatus and Amblyomma hebraeum tested by the impregnated packet technique

A limited series of experiments were done with packets impregnated with permethrin and decamethrin and unfed nymphs of R. appendiculatus and A. hebraeum.

Different combinations of exposure times and holding times were used to assess the LD_{50} and LD_{90} of the 2 compounds. With exposure periods of 24, 48 and 72 hours and a constant holding time of 24 hours, decamethrin was the more effective compound and there was a decrease in the value of LD_{50} and LD_{90} of both permethrin and decamethrin for R. appendiculatus nymphs with an increase in the exposure period (Table 16). The LD_{50} for permethrin dropped from 71.26 mg/m^2 after 24 hours exposure to 50.45 mg/m^2 after 72 hours exposure, a factor of 1.4, and for decamethrin from 61.85 mg/m^2 to 19.17 mg/m^2, a factor of 3.2, suggesting that prolonged exposure to decamethrin was more effective than prolonged exposure to permethrin. The same trend was seen when the LD_{90} values were compared, a factor of 1.6 for permethrin and 4.2 for decamethrin.

A similar but less marked drop was also seen with a constant exposure period of 24, 48 or 72 hours but with different holding times i.e., 24, 48 and 72 hours (Table 17). In these experiments the most effective combination tested was decamethrin with an exposure period of 72 hours and a holding time of 48 hours, when the LD_{50} for R. appendiculatus was as low as 14.27 mg/m^2.

Unfed nymphs of A. hebraeum were tested only in decamethrin impregnated packets with a 24 hour exposure period, but with holding periods of 24, 48 and 72 hours (Table 18). The drop in LD_{50} from

TABLE 16 - SUSCEPTIBILITY OF R. APPENDICULATUS UNFED NYMPHS TO PERMETHRIN AND DECAMETHRIN. RESULTS OF TESTS USING IMPREGNATED PACKET TECHNIQUE WITH DIFFERENT EXPOSURE TIMES AND CONSTANT HOLDING TIME. VALUES OF LD_{50} and LD_{90} (mg/m^2), SLOPE, Chi^2 and P

Compound	Exposure time/ Holding time	LD_{50} (mg/m^2)	95% fiducial limits to LD_{50}	LD_{90} (mg/m^2)	95% fiducial limits to LD_{90}	Average number of ticks per dose	Slope ± SE	Chi^2	P
permethrin	24h/24h	71.26	69.62 - 72.93	112.99	108.13 - 118.98	450	6.40 ± 0.29	11.25	< 0.005
	48h/24h	59.61	56.02 - 63.00	76.53	71.19 - 86.46	32	11.80 ± 1.91	16.55	< 0.001
	72h/24h	50.45	46.47 - 54.05	71.06	65.41 - 80.30	34	8.61 ± 1.17	3.57	> 0.25
decamethrin	24h/24h	61.85	53.44 - 73.51	119.54	95.96 - 169.59	33	4.47 ± 0.61	0.84	> 0.25
	48h/24h	31.33	28.52 - 34.46	43.93	39.19 - 52.84	33	8.72 ± 1.35	5.88	< 0.025
	72h/24h	19.17	17.06 - 21.13	28.60	25.68 - 3.29	43	7.37 ± 1.02	6.33	< 0.05

TABLE 17 - SUSCEPTIBILITY OF R. APPENDICULATUS UNFED NYMPHS TO PERMETHRIN AND DECAMETHRIN. RESULTS OF TESTS USING IMPREGNATED PACKET TECHNIQUE WITH DIFFERENT COMBINATIONS OF EXPOSURE TIMES AND HOLDING TIMES. VALUES OF LD_{50} and LD_{90} (mg/m^2), SLOPE, Chi^2 and P

Compound	Exposure time/ Holding time	LD_{50} (mg/m^2)	95% fiducial limits to LD_{50}	LD_{90} (mg/m^2)	95% fiducial limits to LD_{90}	Average number of ticks per dose	Slope ± SE	Chi^2	P
permethrin	24h/24h	71.26	69.62 - 72.93	112.99	108.13 - 118.98	450	6.40 ± 0.29	11.25	< 0.005
	24h/48h	67.78	64.35 - 71.24	86.49	80.85 - 96.41	38	12.11 ± 1.77	1.08	> 0.75
	48h/24h	59.61	56.02 - 63.00	76.53	71.19 86.46	32	11.80 ± 1.91	16.55	< 0.001
	48h/48h	58.63	54.85 - 62.09	76.19	70.73 - 86.49	32	11.27 ± 1.87	16.97	< 0.001
decamethrin	24h/24h	61.85	54.44 - 73.51	119.54	95.96 - 169.59	33	4.47 ± 0.61	0.84	> 0.25
	24h/48h	53.92	47.49 61.34	109.89	91.70 - 144.09	35	4.14 ± 0.51	4.37	> 0.25
	24h/72h	20.85	18.55 - 23.24	33.55	29.30 - 41.41	36	6.20 ± 0.90	3.46	> 0.10
	48h/24h	31.33	28.52 - 34.46	43.93	39.19 - 52.84	33	8.72 ± 1.35	5.88	< 0.025
	48h/48h	16.57	13.99 - 19.11	32.08	26.97 - 41.93	33	4.46 ± 0.66	9.82	< 0.01
	48h/72h	16.47	13.83 - 19.18	32.38	26.40 - 46.90	33	4.36 ± 0.77	13.24	< 0.001
	72h/24h	19.17	17.06 - 21.13	28.60	25.68 - 33.29	43	7.37 ± 1.02	6.33	< 0.05
	72h/48h	14.27	12.44 - 16.08	23.97	20.82 - 29.42	43	5.69 ± 0.78	4.68	< 0.05

TABLE 18 - SUSCEPTIBILITY OF A. HEBRAEUM UNFED NYMPHS TO DECAMETHRIN.
RESULTS OF TESTS USING IMPREGNATED PACKET TECHNIQUE WITH CONSTANT EXPOSURE TIME AND DIFFERENT HOLDING TIMES. VALUES OF LD_{50} and LD_{90} (mg/m^2), SLOPE, Chi^2 and P

Compound	Exposure time/ Holding time	LD_{50} (mg/m^2)	95% fiducial limits to LD_{50}	LD_{90} (mg/m^2)	95% fiducial limits to LD_{90}	Average number of ticks per dose	Slope ± SE	Chi^2	P
decamethrin	24h/24h	26.85	22.35 - 30.83	43.90	36.73 - 68.24	20	6.00 ± 1.49	1.13	>0.75
	24h/48h	27.79	22.29 - 32.98	50.52	39.90 - 107.76	20	4.94 ± 1.43	0.11	>0.5
	24h/72h	25.43	19.68 - 29.71	45.19	36.71 - 83.10	20	5.13 ± 1.44	0.02	>0.75

26.85 mg/m^2 after 24 hours holding time to 25.43 mg/m^2 after 72 hours holding time was much less than with R. appendiculatus under similar conditions. However unfed nymphs of A. hebraeum were more susceptible than unfed nymphs of R. appendiculatus to decamethrin.

3.6.3.2 The effect of 3 synthetic pyrethroids, amitraz and carbaryl on unfed nymphs tested by the tea bag technique

3.6.3.2.1 Results of tests with Rhipicephalus appendiculatus

The 3 synthetic pyrethroids were tested against 2-week, 5-week and 8-week old nymphs by the tea bag technique. Amitraz and carbaryl were tested only on 2-week old nymphs.

The average number of nymphs tested per concentration of permethrin, cypermethrin and decamethrin varied from 38 to 248. LC_{50} and LC_{90} values with their 95% fiducial limits, slope of the regression lines, Chi^2 and P are given in Table 19 and the regression lines are shown in Figs. 45 to 47. Decamethrin was the most effective compound with LC_{50} values of 0.00027%, 0.00007% and 0.000028% for 2-, 5- and 8-week old nymphs respectively. LC_{50} values of cypermethrin (0.00037%, 0.00011%, and 0.00008%) were only slightly lower than the LC_{50} values for permethrin, (0.00040%, 0.00018% and 0.00009%). The slope of the regression lines was steep with all 3 compounds, suggesting that as with larvae, the concentration required to effect 90% kill was close to the concentration required to obtain 50% kill. Comparing the values for all 3 compounds and the 3 age groups, 90% control of the nymphal ticks could be obtained by increasing the concentration for 50% kill by a factor of only 1.7 to 2.6, very close to the factor of 1.7 to 2.1 for unfed larvae of the same species.

There was an increase in susceptibility to all 3 compounds with increase in age of the nymphs (Table 19). Thus, 8-week old nymphs

TABLE 19 - SUSCEPTIBILITY OF R. APPENDICULATUS UNFED NYMPHS, 2, 5 and 8 WEEKS OLD TO 3 SYNTHETIC PYRETHROIDS. RESULTS OF TESTS WITH TEA BAG TECHNIQUE. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	2	0.00040	0.00039 - 0.00042	0.00082	0.00077 - 0.00088	248	4.195 ± 0.19	28.2	<0.001
	5	0.00018	0.00016 - 0.00020	0.00034	0.00029 - 0.00040	38	4.700 ± 0.49	4.06	>0.25
	8	0.00009	0.00008 - 0.00010	0.00015	0.00015 - 0.00019	39	5.038 ± 0.60	1.46	>0.75
cypermethrin	2	0.00037	0.00035 - 0.00039	0.00082	0.00076 - 0.00088	214	3.744 ± 0.16	51.67	<0.001
	5	0.00011	0.00009 - 0.00012	0.00027	0.00023 - 0.00034	41	3.219 ± 0.37	7.77	>0.5
	8	0.00008	0.000078 - 0.00010	0.00021	0.000164 - 0.00030	41	3.791 ± 0.42	9.16	<0.025
decamethrin	2	0.00027	0.00025 - 0.00028	0.00058	0.00054 - 0.00064	178	3.775 ± 0.18	25.2	<0.001
	5	0.00007	0.00006 - 0.00008	0.00013	0.00011 - 0.00018	40	4.554 ± 0.86	3.85	>0.1
	8	0.000028	0.000024 - 0.000032	0.000063	0.000053 - 0.000082	41	3.684 ± 0.52	5.43	>0.1

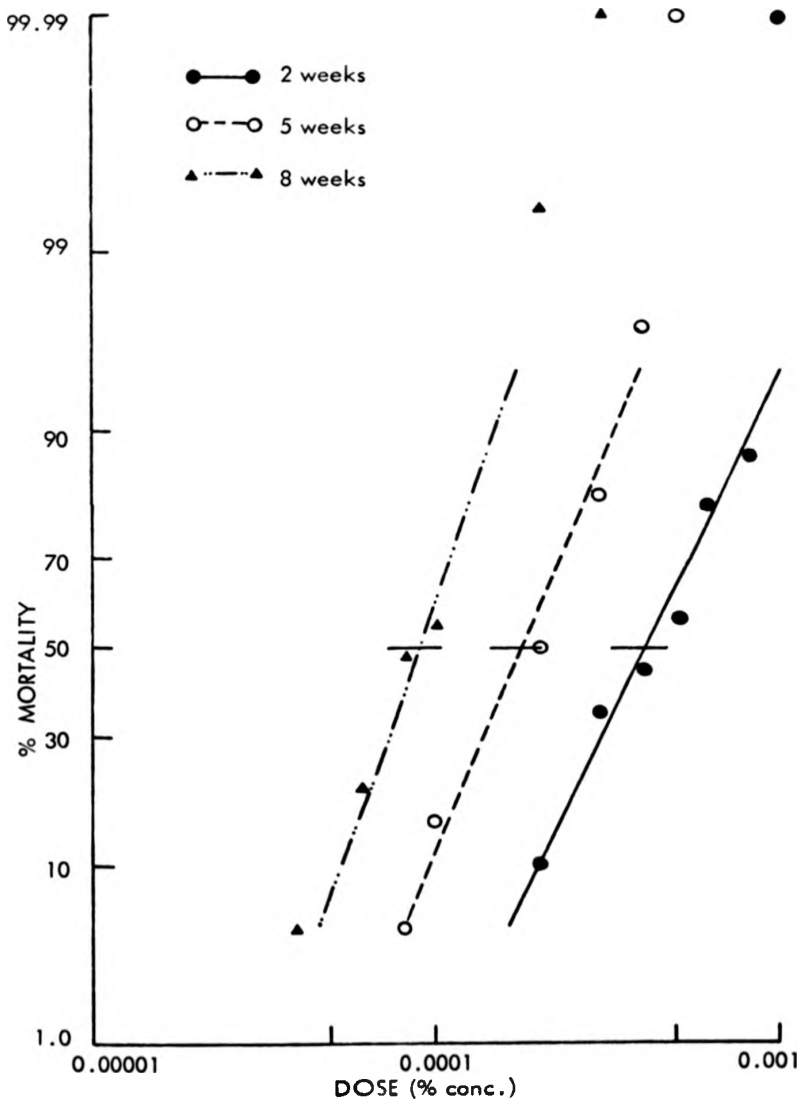


FIG.45
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH PERMETHRIN AGAINST 2-,5- AND 8-WEEK OLD UNFED NYMPHS OF R.APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

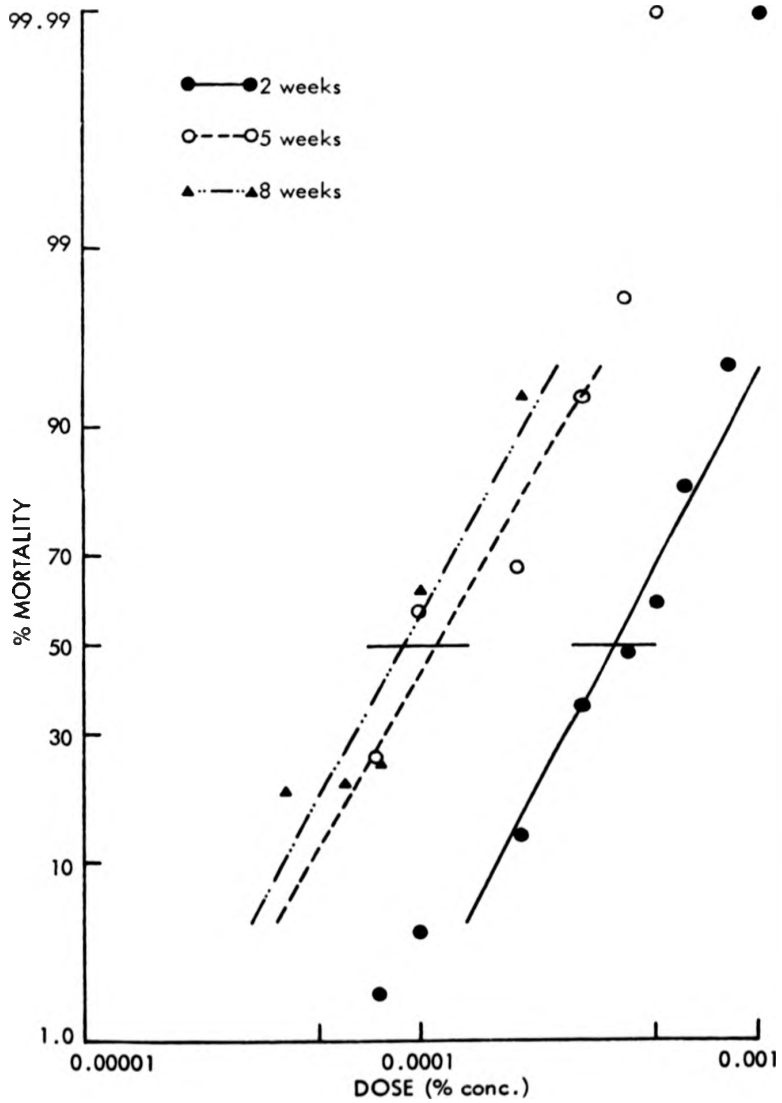


FIG. 46
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH CYPERMETHRIN AGAINST 2-, 5- AND 8-WEEK OLD UNFED NYMPHS OF R. APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

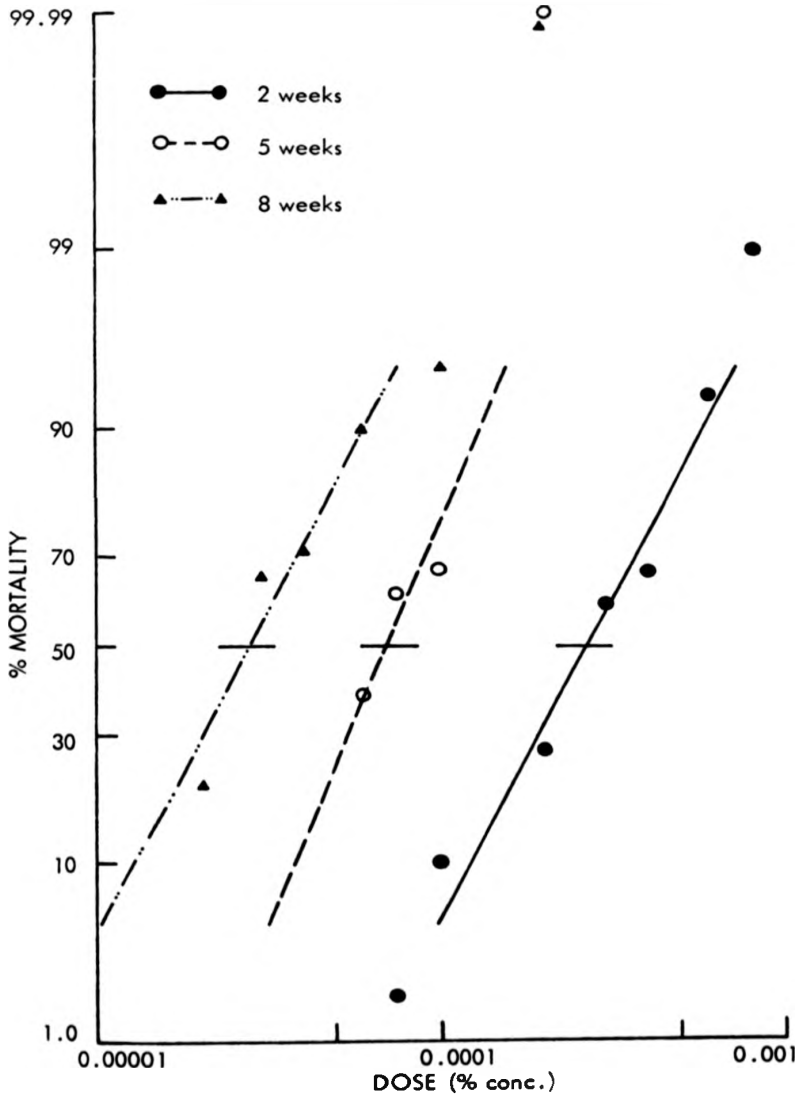


FIG.47
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH DECAMETHRIN AGAINST 2-, 5- AND 8-WEEK OLD UNFED NYMPHS OF R. APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

were 4.4 times more susceptible to permethrin, 4.6 times more susceptible to cypermethrin and 0.8 times more susceptible to decamethrin than 2-week old nymphs.

The effect of ageing on susceptibility to permethrin was investigated in detail by testing nymphs every week up to 8 weeks after moulting and then at 10 weeks and 12 weeks. The results are presented in Table 20 and Fig. 48. There was a slight but consistent drop in the LC_{50} and LC_{90} values over the test period. 12-week old nymphs (LC_{50} 0.000052%) were 8.3 times more susceptible than 1-week old nymphs (LC_{50} 0.000432%). The relationship between age of nymphs and LC_{50} values was investigated by plotting the LC_{50} values against age in weeks and drawing a regression line using the formula $y = mx + c$ (see Section 3.6.2.1). The relationship was highly significant. Fig. 49 shows the scatter points, the regression line and its equation. The correlation coefficient was 0.9 indicating that the correlation between the observed and estimated LC_{50} was higher than could be expected to happen by chance. The negative slope of the regression line indicates that increase in age of the nymphs results in increased susceptibility to permethrin.

Amitraz had a LC_{50} value of 0.0017% and carbaryl a LC_{50} value of 0.0046% when tested against 2-week old nymphs. (Table 21 and Fig. 50). Both acaricides were much less effective than the synthetic pyrethroids, carbaryl less so than amitraz. Permethrin, cypermethrin and decamethrin were 4.2, 4.6 and 6.3 times respectively as effective as amitraz and 11.5, 12.4 and 17 times respectively as effective as carbaryl.

3.6.3.2.2 Results of tests with Amblyomma hebraeum

The average number of nymphs tested per concentration of the 3

TABLE 20 - SUSCEPTIBILITY OF R. APPENDICULATUS UNFED NYMPHS, 1 to 10 WEEKS AND 12 WEEKS OLD, TO PERMETHRIN. RESULTS OF WEEKLY TESTS WITH TEA BAG TECHNIQUE. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	1	0.000432	0.000389 - 0.000472	0.000792	0.000705 - 0.000940	43	4.875 ± 0.60	6.83	>0.1
	2	0.000271	0.000234 - 0.000302	0.000534	0.000471 - 0.000640	41	4.349 ± 0.55	6.12	>0.1
	3	0.000234	0.000208 - 0.000258	0.000423	0.000376 - 0.000495	40	4.973 ± 0.54	3.28	>0.5
	4	0.000187	0.000164 - 0.000212	0.000406	0.000350 - 0.000491	38	3.819 ± 0.36	4.60	>0.025
	5	0.000180	0.000160 - 0.000201	0.000337	0.000294 - 0.000403	38	4.700 ± 0.46	4.06	>0.25
	6	0.000142	0.000127 - 0.000159	0.000276	0.000237 - 0.000336	39	4.426 ± 0.43	7.62	>0.1
	7	0.000112	0.000099 - 0.000128	0.000237	0.000195 - 0.000312	39	3.940 ± 0.43	1.27	>0.75
	8	0.000086	0.000078 - 0.000095	0.000154	0.000133 - 0.000193	39	5.038 ± 0.60	1.46	>0.75
	10	0.000072	0.000063 - 0.000083	0.000180	0.000145 - 0.000251	39	3.226 ± 0.39	2.37	>0.5
	12	0.000052	0.000045 - 0.000058	0.000110	0.000094 - 0.000139	37	3.923 ± 0.52	1.83	>0.75

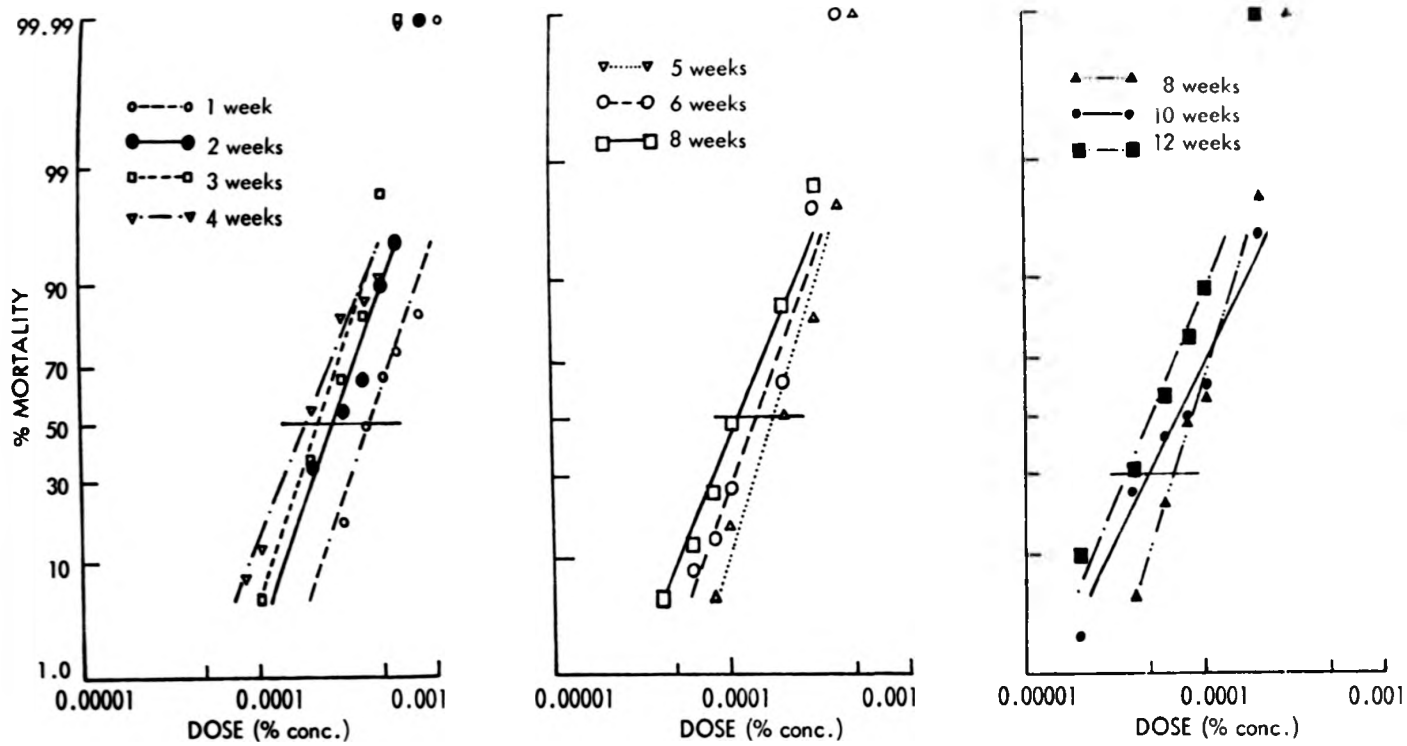


FIG.48

RESULTS OF WEEKLY TESTS BY THE TEA BAG TECHNIQUE WITH PERMETHRIN AGAINST 1-TO 10- AND 12-WEEK OLD UNFED NYMPHS OF R.APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

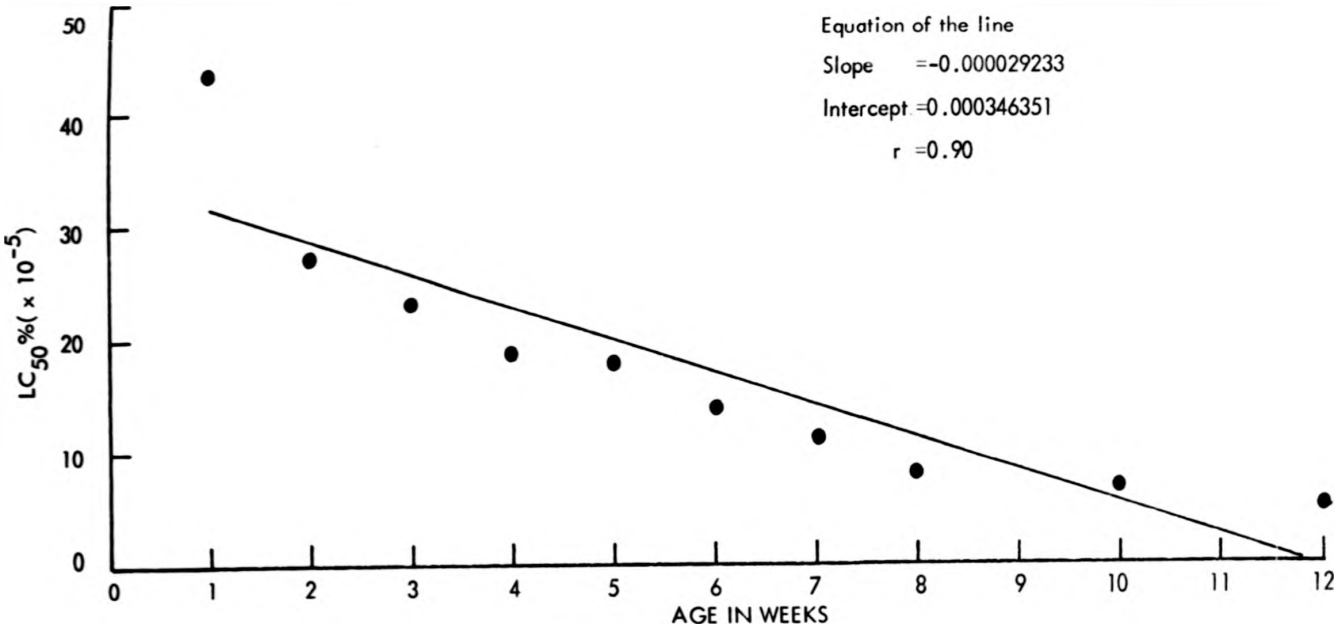


FIG.49

SUSCEPTIBILITY OF UNFED NYMPHS OF R.APPENDICULATUS OF DIFFERENT AGES TO PERMETHRIN. RESULTS OF WEEKLY TESTS OF THE TEA BAG TECHNIQUE. REGRESSION LINE RELATING TO TIME IN WEEKS AND LC_{50} VALUES.

TABLE 21 - SUSCEPTIBILITY OF R. APPENDICULATUS UNFED NYMPHS, 2 WEEKS OLD, TO AMITRAZ AND CARBARYL. RESULTS OF TESTS WITH TEA BAG TECHNIQUE. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
Amitraz	2	0.0017	0.0014 - 0.0021	0.01	0.0077 - 0.0141	73	1.681 ± 0.13	11.16	<0.05
Carbaryl	2	0.0046	0.0044 - 0.0048	0.0077	0.0072 - 0.0083	131	5.828 ± 0.41	4.44	>0.25

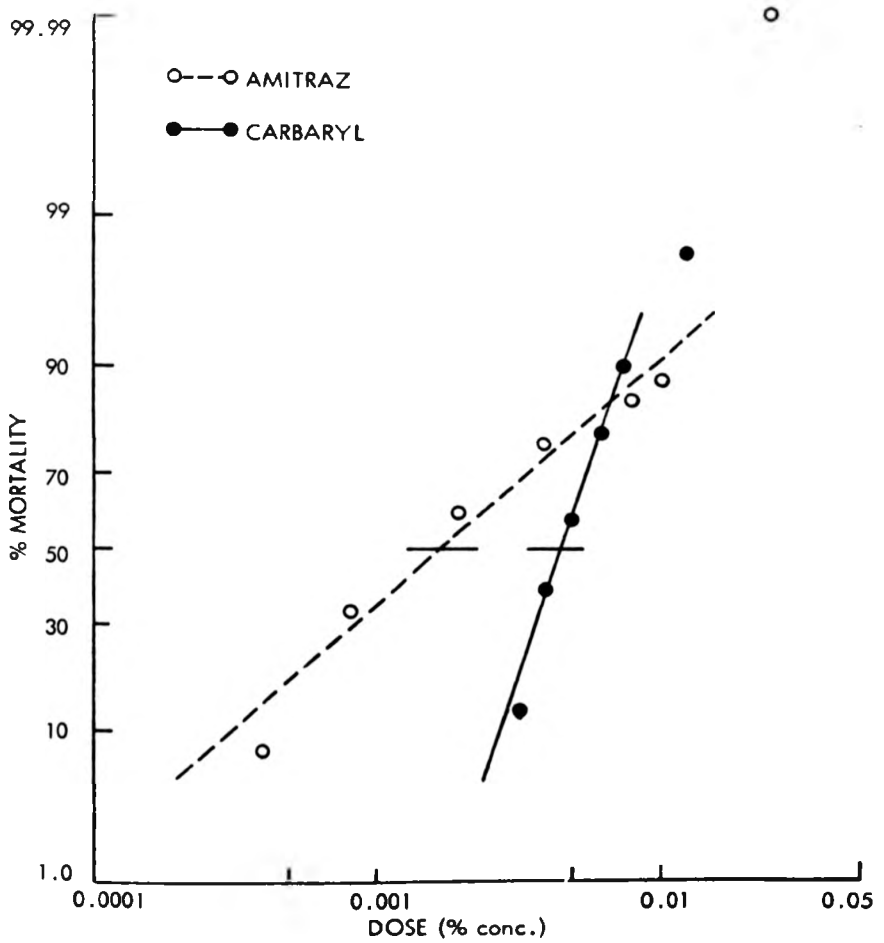


FIG.50
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH AMITRAZ AND CARBARYL AGAINST 2-WEEK OLD UNFED NYMPHS OF R.APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION SND PERCENTAGE KILLS.

synthetic pyrethroids varied from 32 to 132. LC_{50} and LC_{90} values with their 95% fiducial limits, slope of the regression lines, Chi^2 and P are given in Table 22 and the regression lines are shown in Figs. 51 to 53.

Decamethrin again was the most effective compound with LC_{50} values of 0.00009%, 0.000036% and 0.000016% for 2-, 5- and 8-week old nymphs respectively. The LC_{50} values of cypermethrin were 0.00030%, 0.00017% and 0.000075% and those for permethrin, 0.00033%, 0.00021% and 0.000075% respectively for the 3 age groups. Thus on the basis of LC_{50} values, decamethrin was 3.3 to 4.7 times as effective as cypermethrin and 3.6 to 5.8 times as effective as permethrin. Permethrin was nearly as effective as cypermethrin for all 3 age groups.

The LC_{50} values would suggest an increase in susceptibility to all 3 compounds with increasing age of the nymphs; thus 8-week old nymphs were 4.4 times more susceptible to permethrin, 4 times more susceptible to cypermethrin and 5.6 times more susceptible to decamethrin than 2-week old nymphs. As the age of the nymphs increased, the LC_{90} values of the 3 compounds tended to be closer together as shown by the convergence of the regression lines, in contrast to R. appendiculatus, the regression lines became shallower as the age of the nymphs increased. The LC_{90}/LC_{50} values of the 3 compounds for 2-week old nymphs were 1.6 to 1.8; for 5-week old nymphs this had increased to 1.9 to 4.2; ratios as high as 3.7 to 8.5 were observed with 8-week old nymphs. The LC_{90}/LC_{50} values with nymphs of R. appendiculatus on the other hand varied from 1.7 to 2.6 only for the 3 compounds and the 3 age groups.

Amitraz and carbaryl were tested against 2-week old nymphs only. Amitraz with LC_{50} of 0.0017% and carbaryl with a LC_{50} of 0.0047% (Table 23 and Fig. 54) were both less effective than the synthetic

TABLE 22 - SUSCEPTIBILITY OF A. HEBRAEUM UNFED NYMPHS, 2, 5 and 8 WEEKS OLD, TO 3 SYNTHETIC PYRETHROIDS. RESULTS OF TESTS WITH TEA BAG TECHNIQUE.
VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	2	0.00033	0.00030 - 0.00035	0.00053	0.00048 - 0.00062	62	6.035 ± 0.58	1.39	>0.5
	5	0.00021	0.00018 - 0.00023	0.00041	0.00035 - 0.00053	38	4.246 ± 0.53	2.16	>0.5
	8	0.000075	0.000056 - 0.000094	0.00028	0.00021 - 0.00040	36	2.271 ± 0.30	6.18	>0.1
cypermethrin	2	0.00030	0.00023 - 0.00032	0.00055	0.00049 - 0.00064	130	4.794 ± 0.39	4.20	>0.1
	5	0.00017	0.00014 - 0.00022	0.00068	0.00048 - 0.0012	38	2.150 ± 0.27	9.29	<0.05
	8	0.000075	0.000052 - 0.000101	0.00064	0.00041 - 0.00127	40	1.375 ± 0.18	2.90	>0.5
decamethrin	2	0.00009	0.00008 - 0.00010	0.00015	0.00014 - 0.00018	65	5.510 ± 0.49	3.34	>0.5
	5	0.000036	0.000027 - 0.000043	0.00015	0.00012 - 0.00021	132	2.058 ± 0.28	6.25	>0.1
	8	0.000016	0.000011 - 0.00002	0.000093	0.000064 - 0.000171	32	1.653 ± 0.23	4.46	>0.25

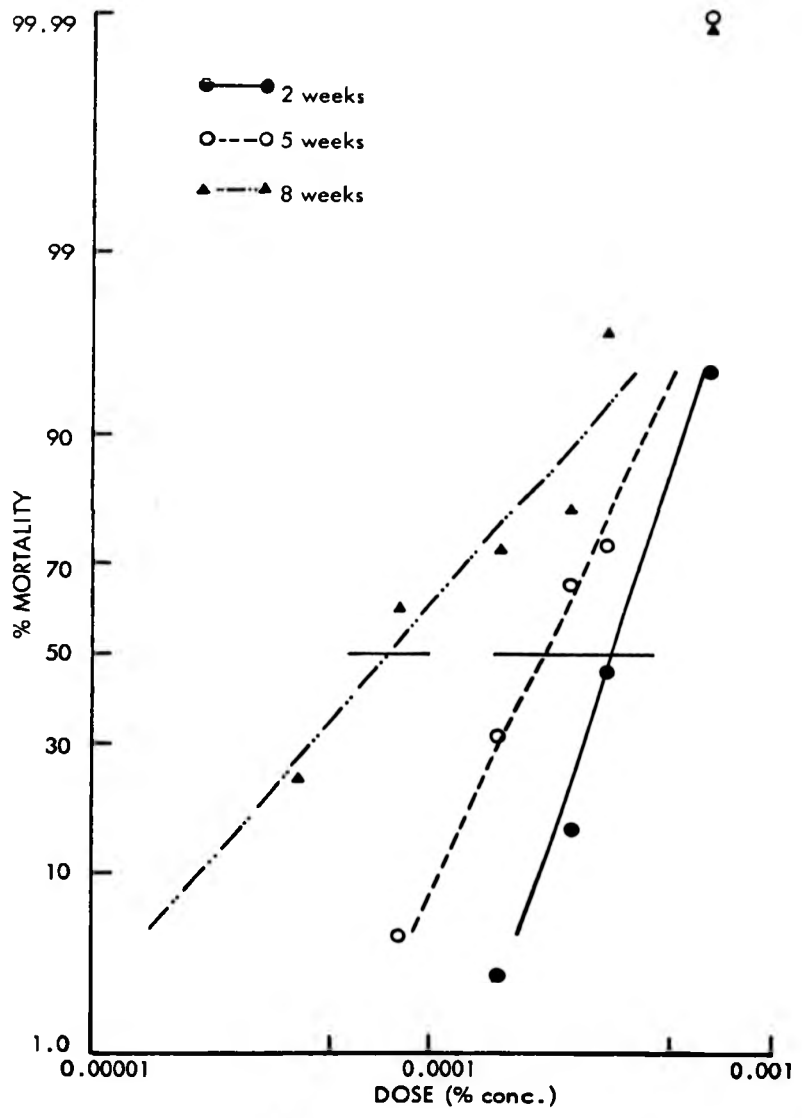


FIG.51
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH PERMETHRIN AGAINST 2-,5- AND 8-WEEK OLD UNFED NYMPHS OF A.HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

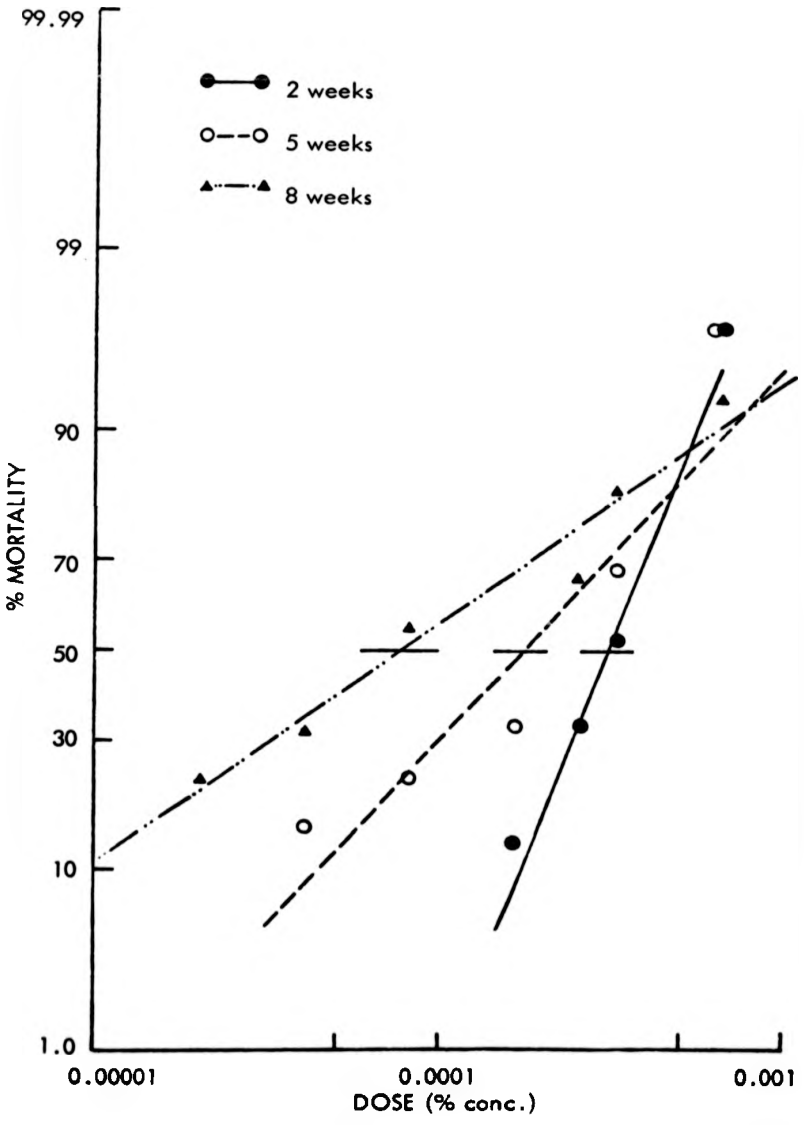


FIG.52
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH CYPERMETHRIN AGAINST 2-,5- AND 8-WEEK OLD UNFED NYMPHS OF A.HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

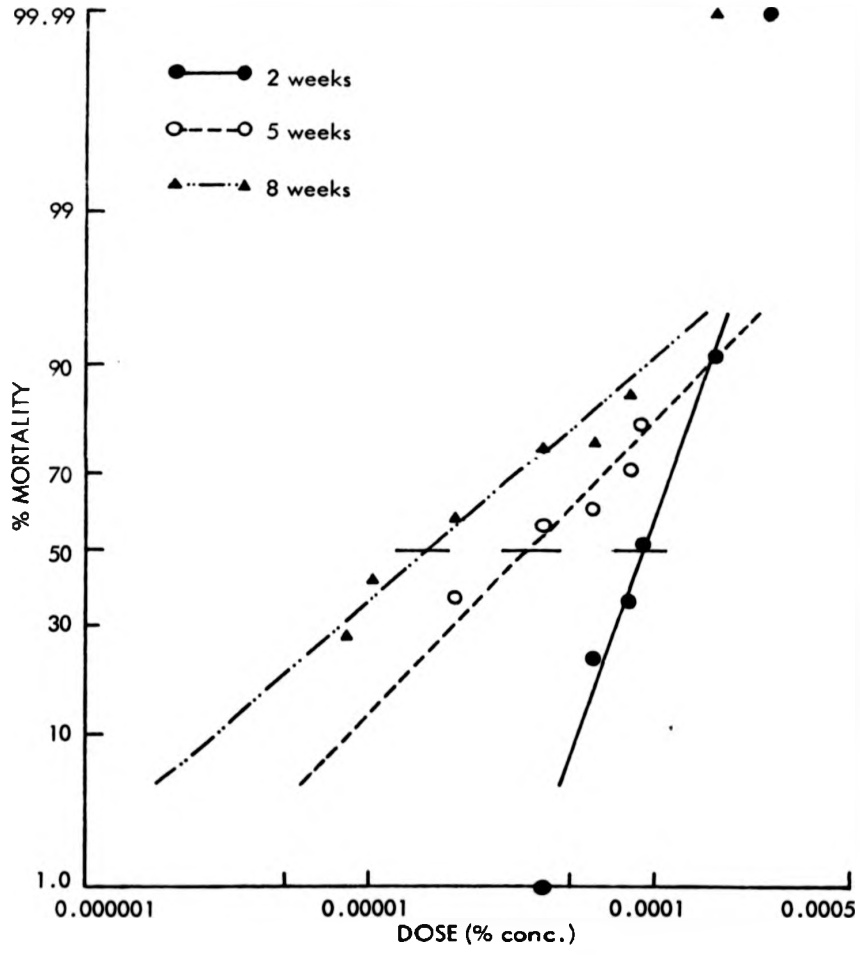


FIG. 53
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH DECAMETHRIN AGAINST 2-, 5- AND 8-WEEK OLD UNFED NYMPHS OF A. HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

TABLE 23 - SUSCEPTIBILITY OF A. HEBRAEUM UNFED NYMPHS, 2 WEEKS OLD, TO AMITRAZ AND CARBARYL. RESULTS OF TESTS WITH TEA BAG TECHNIQUE. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
Amitraz	2	0.0017	0.0014 - 0.0022	0.0054	0.0040 - 0.0088	29	2.593 ± 0.37	4.20	>0.1
Carbaryl	2	0.0047	0.0043 - 0.0051	0.0090	0.0078 - 0.010	65	4.648 ± 0.43	16.18	<0.005

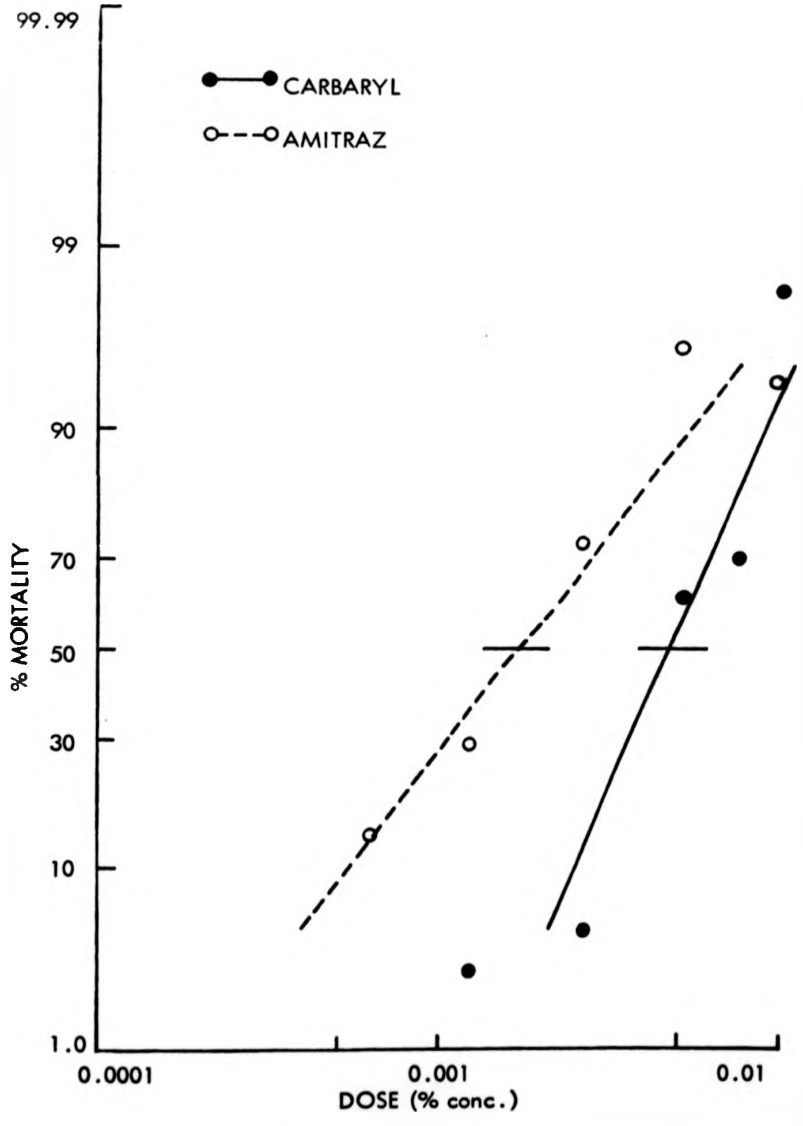


FIG. 54
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH AMITRAZ AND CARBARYL AGAINST 2-WEEK OLD UNFED NYMPHS OF A. HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

pyrethroids. Permethrin, cypermethrin and decamethrin were 5, 6 and 19 times respectively as effective as amitraz, and 14, 16 and 52 times as effective as carbaryl. Since the LC_{50} values of amitraz (0.0017%) and carbaryl (0.0047%) for A. hebraeum were the same as for R. appendiculatus (0.0017% and 0.0046% respectively), the higher relative efficiency of the 3 pyrethroids vis-a-vis amitraz and carbaryl would appear to be due to the higher susceptibility of A. hebraeum to the pyrethroids.

3.6.3.2.3 Results of tests with Amblyomma variegatum

Permethrin, cypermethrin, decamethrin, amitraz and carbaryl were tested only against 2-week old nymphs of this species. Results are presented in Tables 24 and 25 and Figs. 55 and 56.

Decamethrin with LC_{50} value of 0.000039% was 2.2 times as effective as cypermethrin (LC_{50} 0.000087%) and 4.6 times as effective as permethrin (LC_{50} 0.00018%). Cypermethrin was twice as effective as permethrin for nymphs of this species. The slope of the regression lines was fairly steep for all 3 compounds, the LC_{90}/LC_{50} values ranging from 1.7 to 2.3, very similar to the values obtained for A. hebraeum nymphs of the same age and for R. appendiculatus nymphs.

Amitraz and carbaryl with LC_{50} values of 0.0016% and 0.0026% were both considerably less effective than the pyrethroids, which were 8.8 to 41 times as effective as amitraz and 14.4 to 67 times as effective as carbaryl.

3.6.3.2.4 Comparison of susceptibility of unfed nymphs of R. appendiculatus, A. hebraeum and A. variegatum to 3 synthetic pyrethroids, amitraz and carbaryl

The LC_{50} and LC_{90} values of permethrin, cypermethrin and decame-

TABLE 24 - SUSCEPTIBILITY OF A. VARIEGATUM UNFED NYMPHS, 2 WEEKS OLD, TO 3 SYNTHETIC PYRETHROIDS. RESULTS OF TESTS WITH TEA BAG TECHNIQUE. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	2	0.00018	0.00016 - 0.0002	0.00041	0.00035 - 0.00049	106	3.610 ± 0.29	19.76	<0.001
cypermethrin	2	0.000087	0.000081 0.000094	0.00015	0.00013 - 0.000017	108	5.560 ± 0.47	0.42	>0.75
decamethrin	2	0.000039	0.000036 - 0.000042	0.000069	0.000062 - 0.000078	118	5.224 ± 0.42	0.17	>0.9

TABLE 25 - SUSCEPTIBILITY OF A. VARIEGATUM UNFED NYMPHS, 2 WEEKS OLD, TO AMITRAZ AND CARBARYL. RESULTS OF TESTS WITH TEA BAG TECHNIQUE. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Age in weeks	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
Amitraz	2	0.0016	0.0012 - 0.0021	0.0052	0.0040 - 0.0081	39	2.602 ± 0.43	14.09	<0.001
Carbaryl	2	0.0026	0.0020 - 0.0033	0.0071	0.0054 - 0.0104	25	2.981 ± 0.39	7.91	>0.05

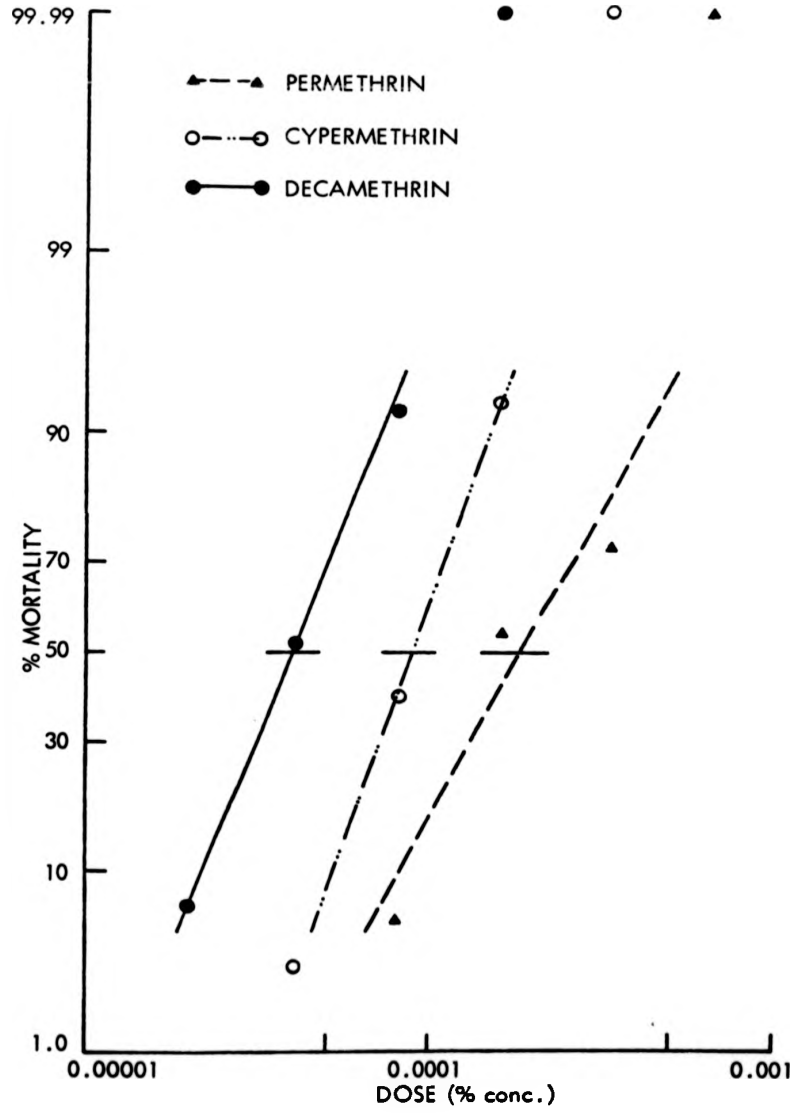


FIG.55
RESULTS OF TEST BY THE TEA BAG TECHNIQUE WITH 3 SYNTHETIC PYRETHROIDS AGAINST 2-WEEK OLD UNFED NYMPHS OF A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

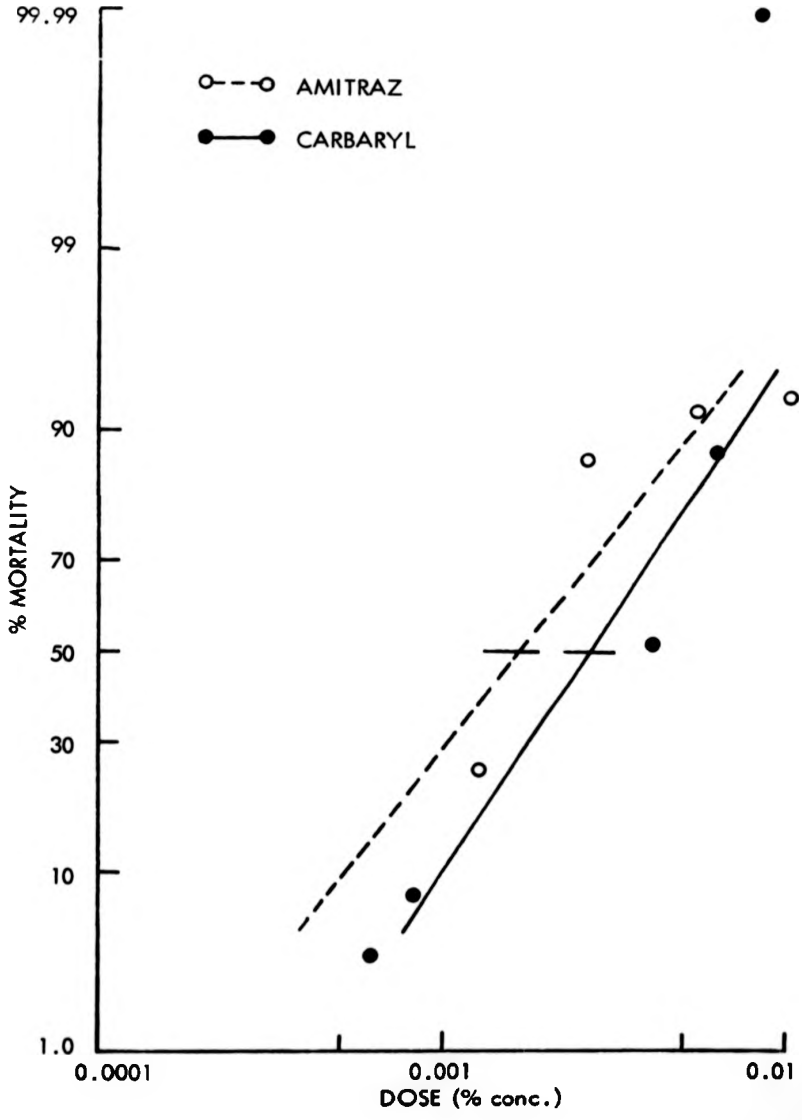


FIG.56
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH AMITRAZ AND CARBARYL AGAINST 2-WEEK OLD UNFED NYMPHS OF A.VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

thrin for 2-, 5- and 8-week old nymphs of R. appendiculatus and A. hebraeum and 2-week old nymphs of A. variegatum, and of amitraz and carbaryl for 2-week old nymphs of all 3 species are given in Table 26 and compared in Figs. 57 to 60.

On the basis of LC_{50} and LC_{90} values of the pyrethroids for 2-week old nymphs, A. variegatum was the most susceptible and decamethrin the most effective compound. 2-week old nymphs of A. variegatum (LC_{50} 0.00018%) were 1.8 times as susceptible as A. hebraeum (LC_{50} 0.00033%) and 2.2 times as susceptible as R. appendiculatus (LC_{50} 0.00040%) to permethrin. The increased susceptibility factor of A. variegatum, compared to A. hebraeum and R. appendiculatus was more with cypermethrin (3.5 and 4.2) and decamethrin (2.3 and 6.9). Thus as with unfed larvae, A. variegatum nymphs are most susceptible to the synthetic pyrethroids followed by A. hebraeum and R. appendiculatus.

However, there did not appear to be any marked difference in susceptibility between the 3 species to amitraz and carbaryl. The LC_{50} values of amitraz for 2-week old nymphs were the same for all 3 species (Table 26). The LC_{90} values for A. hebraeum and A. variegatum were also very close and the LC_{90} value for R. appendiculatus was nearly twice the value for A. hebraeum and A. variegatum. On the other hand R. appendiculatus and A. hebraeum appear to be equally susceptible to carbaryl on the basis of LC_{50} and LC_{90} values and A. variegatum nearly twice as susceptible as R. appendiculatus and A. hebraeum.

3.6.3.3 Susceptibility of fed nymphs of R. appendiculatus and A. hebraeum to permethrin tested by the dipping technique and of R. appendiculatus, A. hebraeum and A. variegatum to decamethrin tested by tea bag technique

During a dip wash or spray wash, attached engorging ticks on

TABLE 26 - COMPARISON OF SUSCEPTIBILITY OF R. APPENDICULATUS AND A. HEBRAEUM UNFED NYMPHS, 2, 5 and 8 WEEKS OLD, AND OF A. VARIEGATUM UNFED NYMPHS, 2 WEEKS OLD, TO 3 SYNTHETIC PYRETHROIDS, AMITRAZ AND CARBARYL. RESULTS OF TESTS WITH TEA BAG TECHNIQUE. VALUES OF LC_{50} and LC_{90} (% conc.)

Compound	Age in weeks	R. appendiculatus		A. hebraeum		A. variegatum	
		LC_{50} (%)	LC_{90} (%)	LC_{50} (%)	LC_{90} (%)	LC_{50} (%)	LC_{90} (%)
permethrin	2	0.00040	0.00082	0.00033	0.00053	0.00018	0.00041
	5	0.00018	0.00034	0.00021	0.00041	-	-
	8	0.00009	0.00015	0.000075	0.00028	-	-
cypermethrin	2	0.00037	0.00082	0.00030	0.00055	0.000087	0.00015
	5	0.00011	0.00027	0.00017	0.00068	-	-
	8	0.00008	0.00021	0.000075	0.00064	-	-
decamethrin	2	0.00027	0.00058	0.00009	0.00015	0.000039	0.000069
	5	0.00007	0.00013	0.000036	0.00015	-	-
	8	0.000028	0.000063	0.000016	0.000093	-	-
Amitraz	2	0.0017	0.01	0.0017	0.0054	0.0016	0.0052
Carbaryl	2	0.0046	0.0077	0.0047	0.0090	0.0026	0.0071

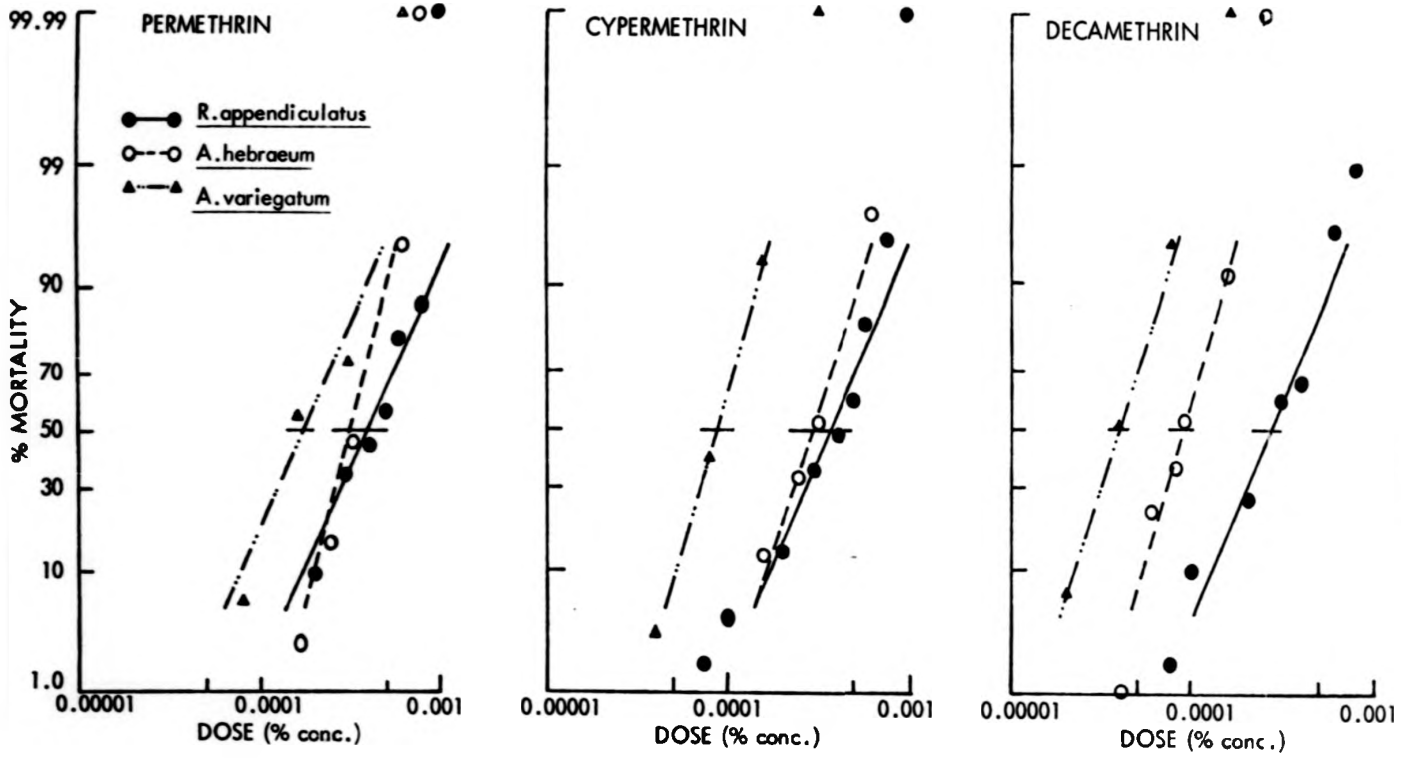


FIG.57

RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH 3 SYNTHETIC PYRETHROIDS AGAINST 2-WEEK OLD UNFED NYMPHS OF R.APPENDICULATUS, A.HEBRAEUM AND A.VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

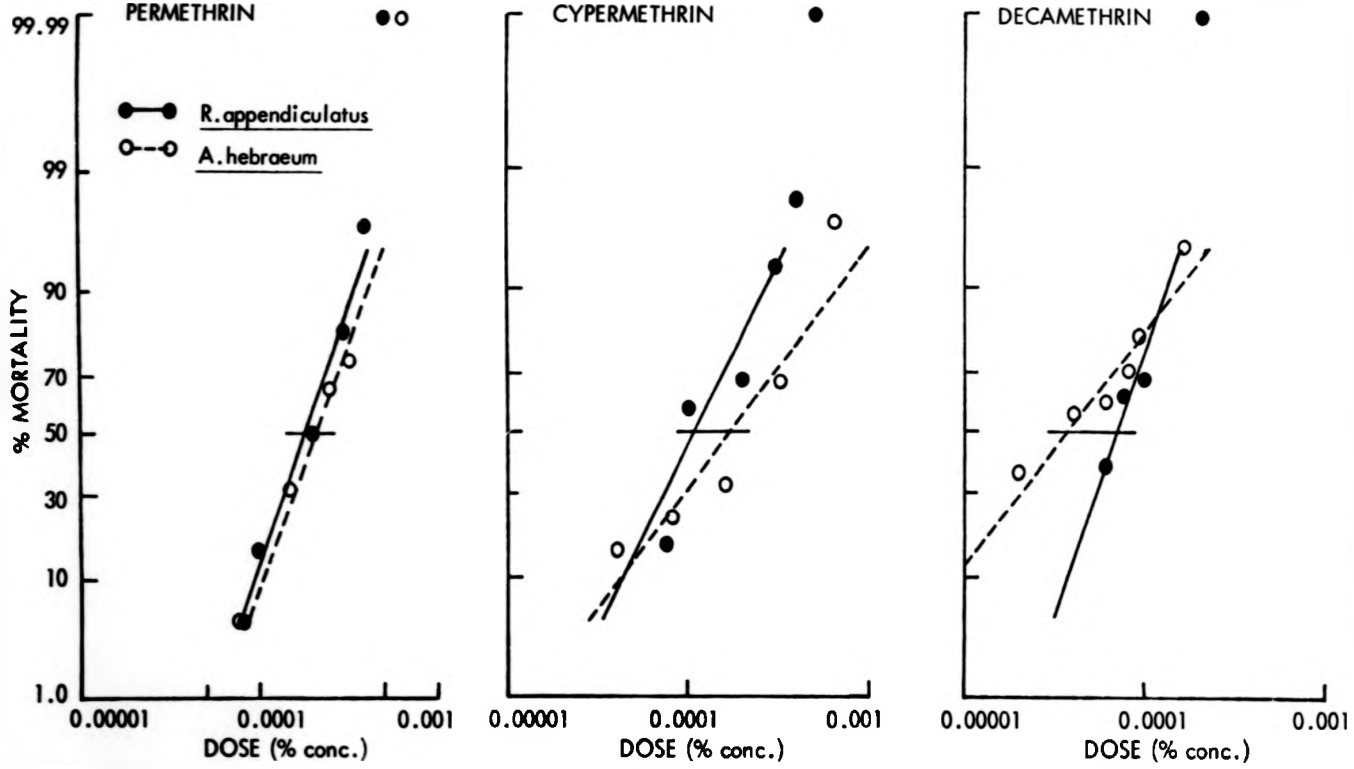


FIG.58

RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH 3 SYNTHETIC PYRETHROIDS AGAINST 5-WEEK OLD NYMPHS OF R.APPENDICULATUS AND A.HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

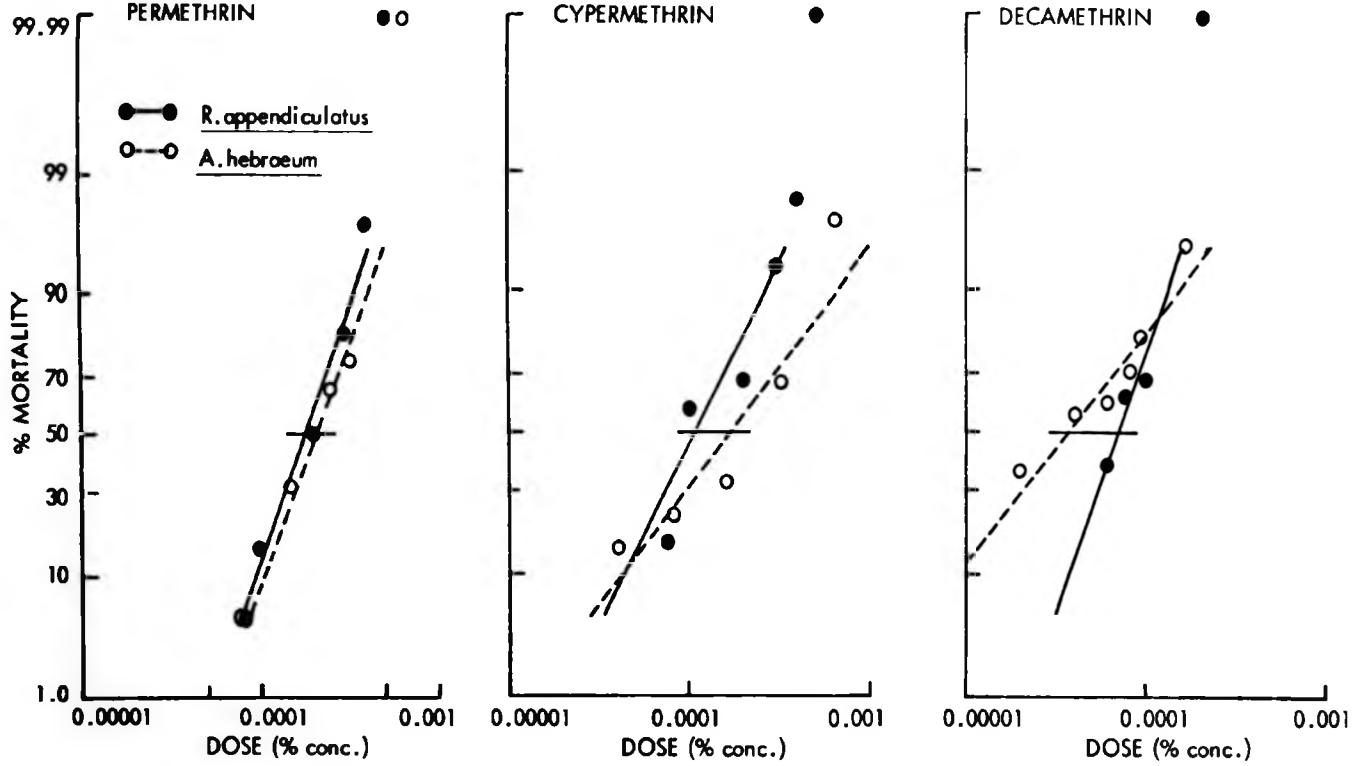


FIG. 58

RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH 3 SYNTHETIC PYRETHROIDS AGAINST 5-WEEK OLD NYMPHS OF R. APPENDICULATUS AND A. HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

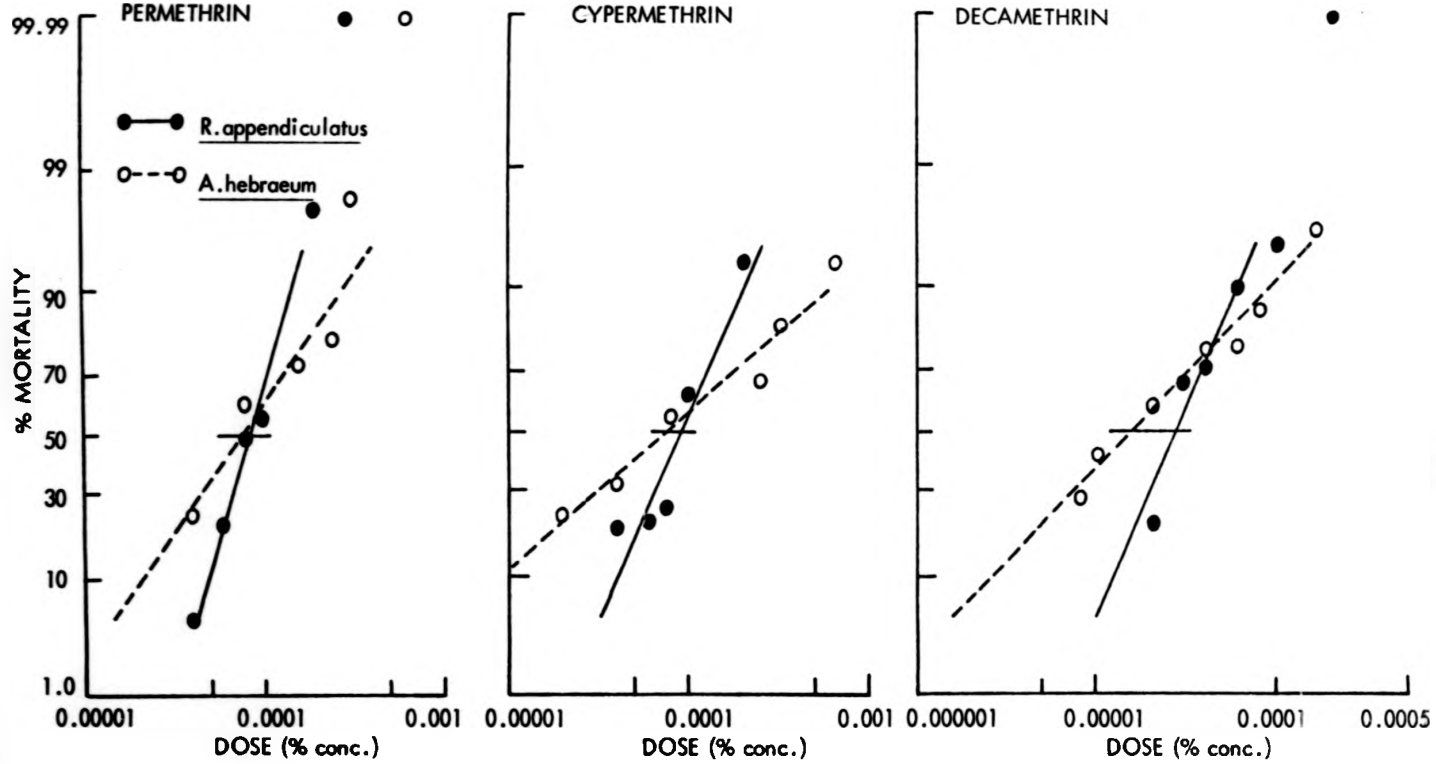


FIG.59

RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH 3 SYNTHETIC PYRETHROIDS AGAINST 8-WEEK OLD UNFED NYMPHS OF R.APPENDICULATUS AND A.HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

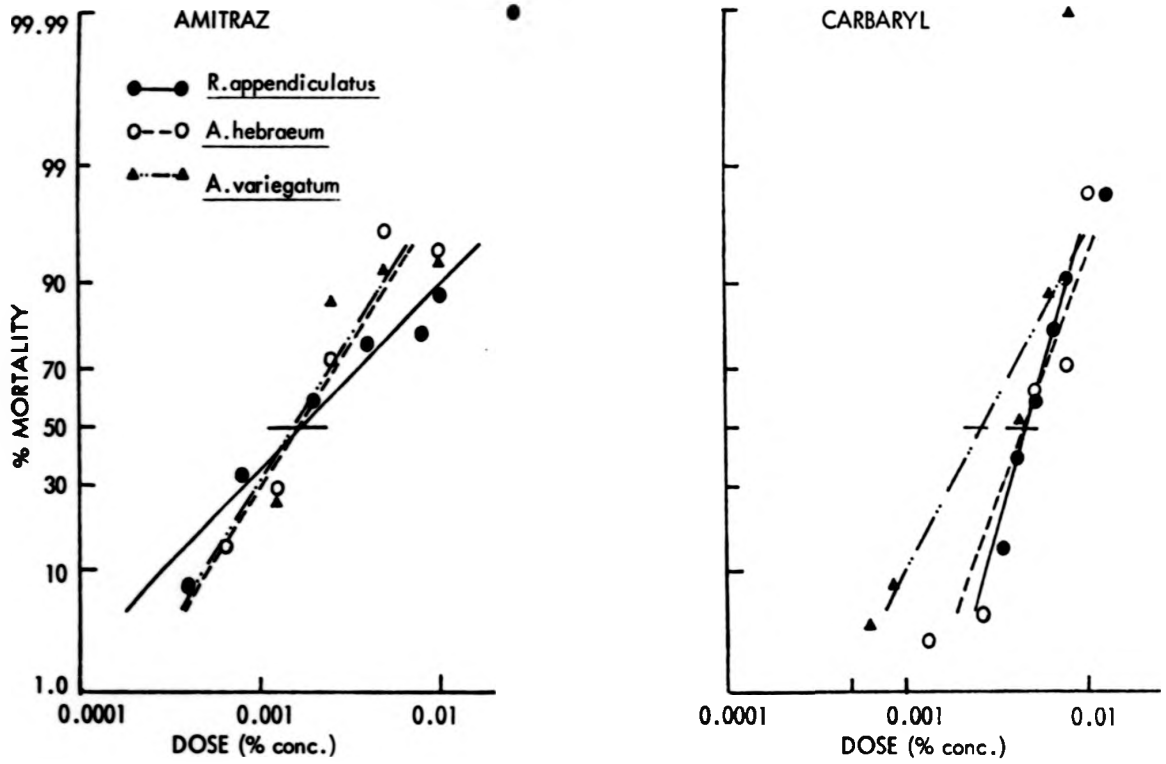


FIG.60

RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH AMITRAZ AND CARBARYL AGAINST 2-WEEK OLD NYMPHS OF R.APPENDICULATUS, A.HEBRAEUM AND A.VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS.

animals come into contact with acaricidal solutions. The effect of acaricides on such ticks may not be the same as on unfed ticks. To investigate this, ticks in different stages of engorgment should be tested. However, in exploratory experiments I tested only fully engorged nymphs of R. appendiculatus, A. hebraeum and A. variegatum, immediately after detachment. The acaricides tested were permethrin and decamethrin and testing was by dipping and by the tea bag technique.

Acaricidal treatment affected the engorged ticks in different ways. At high concentrations the ticks died without going into the premoult phase. With increasingly lower concentrations the nymphs proceeded to the premoult phase, but died without moulting, or moulted but with the adults dying in situ without being able to emerge from the nymphal exuvium. Absence of acaricidal activity was shown by emergence of active live adult ticks. The mortality in these experiments is therefore the cumulative total of the different sequential mortalities.

Another effect of acaridical treatment was seen in the duration of the premoult period.

Only a limited number of experiments were done with fed nymphs. Fed nymphs of R. appendiculatus and A. hebraeum immediately after detachment were tested with permethrin by the dipping technique. This method was later abandoned, when the tea bag technique was adopted as standard procedure for unfed nymphs and adults. Decamethrin was tested against all 3 species by the tea bag technique. In the experiments by dipping or tea bag technique, the treated ticks were observed for up to 48 days and scoring was initially done only for cumulative mortality. In a later series of experiments using the tea bag technique the effect of decamethrin on prolongation of the premoult period of fed nymphs of all 3 species was also investigated.

In these tests also the ticks were examined for up to 48 days, by which time all the nymphs in the premoult phase which were alive had moulted.

In the dipping experiments with permethrin, an average number of 31 nymphs of R. appendiculatus and 18 nymphs of A. hebraeum per concentration was used. The LC_{50} for R. appendiculatus based on cumulative mortality was 0.126% and for A. hebraeum 0.088% (Table 27), confirming the difference in susceptiblity of the two species. The concentration for 90% kill, obtained by extrapolation, was very high for both species, 1.335% for R. appendiculatus (10.6 times as high as LC_{50}) and 3.505% for A. hebraeum (40 times as high as LC_{50}), which may be beyond the levels acceptable for field use. However, the data are too few for any definite conclusions to be drawn. What this experiment did suggest was that fed nymphs are far more tolerant to permethrin than unfed nymphs which were tested by the tea bag technique, 315 times in the case of R. appendiculatus and 267 times in the case of A. hebraeum.

The average number of nymphs per concentration in the tea bag technique with decamethrin was 20 for R. appendiculatus, 18 for A. hebraeum, and 20 for A. variegatum. LC_{50} and LC_{90} values with their fiducial limits, slope, Chi^2 and P are given in Table 28. The LC_{50} for R. appendiculatus was 0.065%, for A. hebraeum 0.022% and for A. variegatum 0.025%. R. appendiculatus was again the most tolerant of the 3 species. A. hebraeum and A. variegatum appeared to be equally susceptible. The LC_{90}/LC_{50} values were very high with R. appendiculatus and A. hebraeum (9.9 and 21.6 respectively) but low (2.9) with A. variegatum. Results from these experiments also suggest an increased tolerance of fed nymphs, compared to unfed nymphs, tested by the tea bag technique, R. appendiculatus, A. hebraeum and

TABLE 27 - SUSCEPTIBILITY OF R. APPENDICULATUS AND A. HEBRAEUM FED NYMPHS TO PERMETHRIN. RESULTS OF TESTS BY DIPPING TECHNIQUE. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Species	LC_{50} (%)	95% fiducial limits of LC_{50}	LC_{90} (%)	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	R. appendiculatus	0.126	0.084 - 0.214	1.335	31	1.250 ± 0.17	1.17	>0.75
	A. hebraeum	0.088	0.036 - 0.447	3.505	18	0.801 ± 0.18	0.16	>0.97

TABLE 28 - SUSCEPTIBILITY OF R. APPENDICULATUS, A. HEBRAEUM AND A. VARIEGATUM TO FED NYMPHS TO DECAMETHRIN. RESULTS OF TESTS WITH TEA BAG TECHNIQUE.
VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Species	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
R. appendiculatus	0.065	0.041 - 0.108	0.643	0.280 - 4.996	20	1.287 ± 0.29	0.99	> 0.90
A. hebraeum	0.022	0.010 - 0.045	0.476	0.169 - 5.113	18	0.965 ± 0.21	11.19	< 0.025
A. variegatum	0.025	0.012 - 0.034	0.072	0.054 - 0.129	20	2.789 ± 0.71	0.12	> 0.50

TABLE 29 - CUMULATIVE MORTALITY AND AVERAGE PREMOULT PERIOD OF R. APPENDICULATUS, A. HEBRAEUM AND A. VARIEGATUM FED NYMPHS, TREATED WITH DECAMETHRIN. RESULTS OBTAINED BY THE TEA BAG TECHNIQUE

Concentration	R. appendiculatus					A. hebraeum					A. variegatum				
	Total number tested	Dead without moulting	Moulted but dead	Cumulative mortality (%)	Average pre-moulting period (days)	Total number tested	Dead without moulting	Moulted but dead	Cumulative mortality (%)	Average pre-moulting period (days)	Total number tested	Dead without moulting	Moulted but dead	Cumulative mortality (%)	Average pre-moulting period (days)
0.01	40	0	0	0	21.35	20	2	0	10	34.33	20	9	1	23*	35.09
0.05	60	4	17	35	25.69	20	5	2	35	34.93	20	10	6	69	36.09
0.1	68	6	35	60	27.54	20	8	3	55	36.26	20	9	10	92	35.63
0.25	36	14	22	100	33.34	20	19	1	100	38.0	20	10	10	100	46.0
Contr.	60	0	0	0	20.33	20	0	0	0	28.6	20	6	1	35	33

* Mortalities corrected by Abbot's formula

A. varietagum fed nymphs being 240 times, 244 times and 641 times respectively as tolerant as unfed nymphs. It is interesting to note that the low LC_{90}/LC_{50} values of 1.7 to 2.6 for unfed nymphs were not obtained with fed nymphs of R. appendiculatus or A. hebraeum. With A. variegatum the value appeared to change little with fed nymphs.

The effect of decamethrin on the premoult period of fed nymphs of R. appendiculatus, A. hebraeum and A. variegatum were compared by the tea bag technique. The numbers tested per concentration was 20 for A. hebraeum and A. variegatum and 36 to 68 for R. appendiculatus. Corrections for control mortality in A. variegatum nymphs were made by Abbott's formula. The results are presented in Table 29. There was a clear increase in the duration of the average premoult period as well as of cumulative mortality of all 3 species, with increasing concentrations of the acaricide. The average premoult period of the control batch of R. appendiculatus which had no acaricidal treatment was 20.33 days; this had increased to 33.34 days at 0.25% concentration, i.e., by a factor of 1.6. Corresponding figures for A. hebraeum were 28.6 days and 38 days, an increase in the premoult period by a factor of 1.3 and 33 days and 46 days for A. variegatum an increase in premoult period by a factor of 1.4. At the highest concentration tested (0.25%) 14 out of 36 (39%) R. appendiculatus, 19 out of 20 (95%) A. hebraeum and 10 out of 20 (50%) A. variegatum fed nymphs were dead before entering the premoult phase.

3.6.4 Results of tests with unfed adult ticks

3.6.4.1 The effect of 3 synthetic pyrethroids on adult ticks tested by topical application

All 3 synthetic pyrethroids were tested for activity against adults of R. appendiculatus and A. hebraeum. No tests were done on

adults of A. variegatum by this method, since this species was in short supply.

3.6.4.1.1 Results of tests with Rhipicephalus appendiculatus

The age of males and females used in the tests usually varied from 2 to 4 weeks, although results from a small number of younger and older adults were also included. Results from all the experiments were pooled. Mortality was read at 7 days post-treatment.

Since a measured quantity, i.e., 1 µl was applied to each tick, LD_{50} and LD_{90} values rather than LC_{50} and LC_{90}, have been calculated on the basis of 1 µl of the different concentrations, and are expressed as ng per tick. The average number of males and females tested per dose was 12 to 94. The average weight of a female tick was 5 mg, males of this species were slightly heavier and averaged 6.7 mg. LD_{50} and LD_{90} values with 95% fiducial limits, slope of the regression lines, Chi^2 and P based on 7 days mortality are given in Table 30 and the regression lines shown in Fig. 61. On the basis of data for 7 days post-treatment, decamethrin was the most effective compound with LD_{50} of 16.89 ng per female and 6.77 ng per male. The values of cypermethrin were 80.19 ng per female and 78.78 ng per male. Only females were tested with permethrin, which was the least effective compound with a LD_{50} of 114.66 ng. The relative potency of cypermethrin : decamethrin for males was 1 : 12, and that of permethrin : cypermethrin : decamethrin for females, 1 : 1.4 : 6.8. The slope of the regression lines was steep in all cases, the LD_{90}/LD_{50} values varying from 1.8 to 4.

3.6.4.1.2 Results of tests with Amblyomma hebraeum

The age of males and females in the tests usually ranged from

TABLE 30 - SUSCEPTIBILITY OF UNFED FEMALES AND MALES OF R. APPENDICULATUS TO PERMETHRIN, CYPERMETHRIN AND DECAMETHRIN. RESULTS OF TESTS BY TOPICAL APPLICATION. VALUES OF LD_{50} and LD_{90} (ng/tick), SLOPE, Chi^2 and P

Compound	Sex	LD_{50} (ng/tick)	95% fiducial limits to LD_{50}	LD_{90} (ng/tick)	95% ficucial limits to LD_{90}	Average number of ticks per dose	Slope ± SE	Chi^2	P
permethrin	F	114.66	101.30 - 129.64	457.52	375.88 - 584.76	94	2.132 ± 0.14	7.55	>0.1
	M	-	-	-	-	-	- -	-	-
cypermethrin	F	80.19	60.27 - 103.45	208.45	152.90 - 356.59	18	3.089 ± 0.54	7.69	>0.05
	M	78.78	52.02 - 103.73	169.99	122.53 - 169.97	12	3.837 ± 1.11	0.92	>0.5
decamethrin	F	16.89	14.82 - 19.31	36.90	30.71 - 47.72	48	±	2.80	>0.25
	M	6.77	5.55 - 7.95	12	9.94 - 17.40	20	5.109 ± 1.02	1.48	>0.25

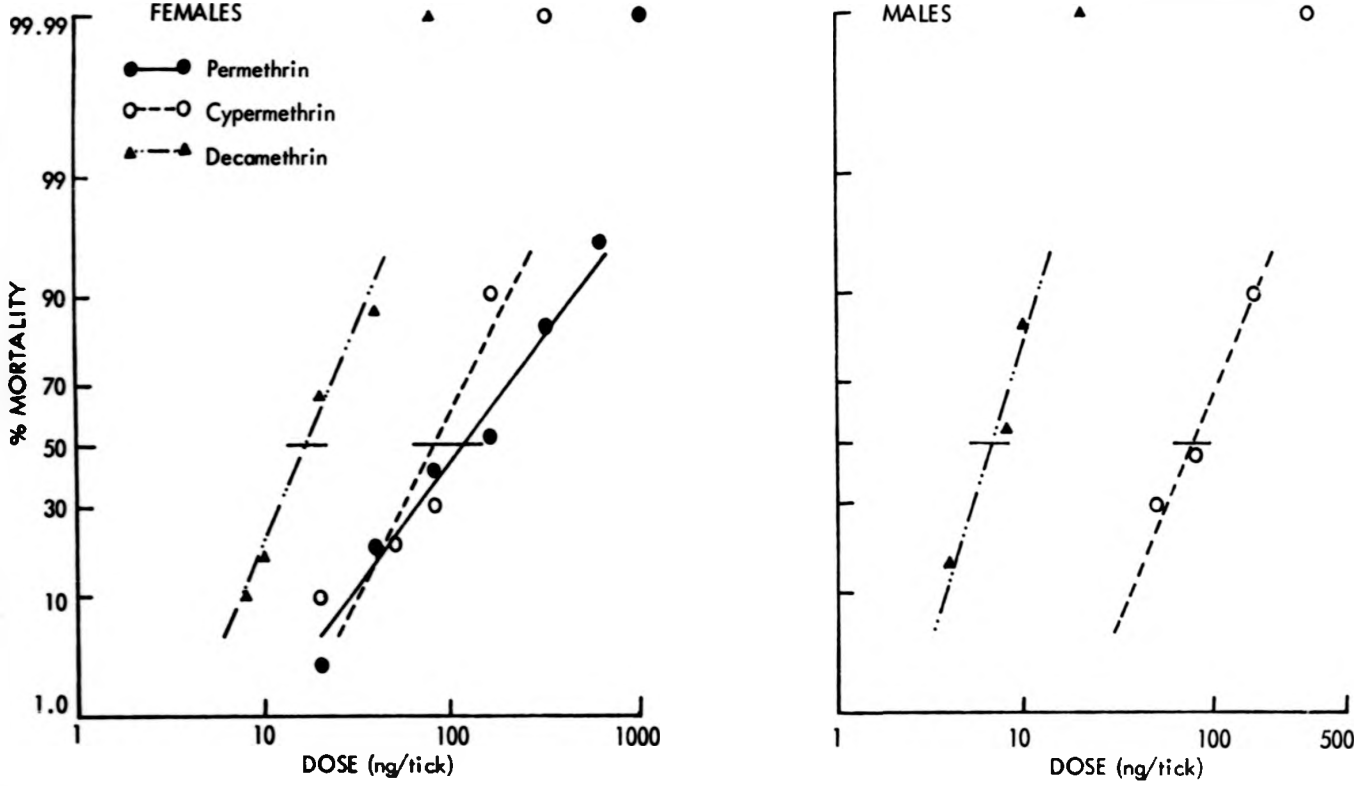


FIG.61
RESULTS OF TESTS BY TOPICAL APPLICATION WITH PERMETHRIN, CYPERMETHRIN AND DECAMETHRIN AGAINST UNFED FEMALES AND WITH CYPERMETHRIN AND DECAMETHRIN AGAINST UNFED MALES OF R.APPENDICULATUS. REGRESSION LINES RELATING TO DOSE (ng/tick) AND PERCENTAGE KILLS.

TABLE 31 - SUSCEPTIBILITY OF UNFED FEMALES AND MALES OF A. HEBRAEUM TO PERMETHRIN, CYPERMETHRIN AND DECAMETHRIN. RESULTS OF TESTS BY TOPICAL APPLICATION. VALUES OF LD_{50} and LD_{90} (ng/tick), SLOPE, Chi^2 and P

Compound	Sex	LD_{50} (ng/tick)	95% fiducial limits to LC_{50}	LD_{90} (ng/tick)	95% fiducial limits to LD_{90}	Average number of ticks per dose	Slope ± SE	Chi^2	P
permethrin	F	333.10	246.09 - 452.25	573.48	429.11 - 1271.83	10	5.431 ± 1.55	2.98	> 0.05
	M	155.73	96.91 - 244.66	390.81	261.41 - 1116.40	10	3.207 ± 0.88	0.66	> 0.50
cypermethrin	F	177.36	131.28 - 241.85	305.00	227.84 - 689.47	10	5.443 ± 1.57	4.65	< 0.05
	M	63.94	44.08 - 90.29	143.33	98.91 - 384.75	10	3.655 ± 0.99	3.58	> 0.10
decamethrin	F	36.17	29.29 - 45.70	67.03	51.21 - 132.60	17	4.783 ± 1.19	4.50	< 0.05
	M	17.02	11.72 - 22.87	51.48	35.36 - 113.45	17	2.667 ± 0.58	3.18	> 0.10

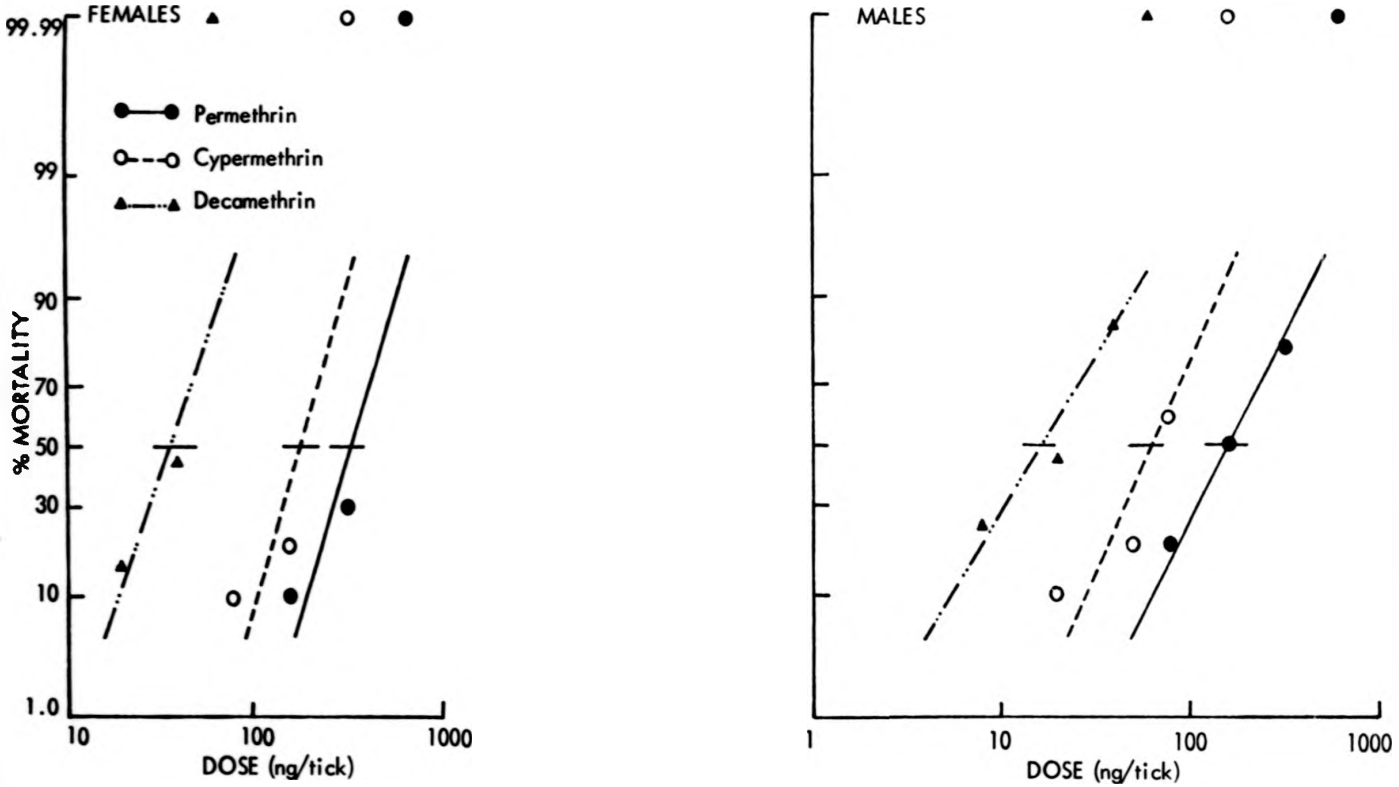


FIG.62

RESULTS OF TESTS BY TOPICAL APPLICATION WITH PERMETHRIN, CYPERMETHRIN AND DECAMETHRIN AGAINST UNFED FEMALES AND MALES OF A. HEBRAEUM. REGRESSION LINES RELATING TO DOSE (ng/tick) AND PERCENTAGE KILLS.

2 to 3 weeks. The average number tested per dose, 10 to 17, was much smaller than the number of R. appendiculatus tested. The average weight of a female of this species was 31 mg and males (average weight 24 mg) were lighter in contrast to R. appendiculatus where the males were heavier than females. The LD_{50} and LD_{90} values expressed as ng per tick with 95% fiducial limits, slope of the regression line, Chi^2 and P based on 7 days mortality are presented in Table 31. The regression lines are shown in Fig. 62. Decamethrin with LD_{50} values of 36.17 ng and 17.02 ng for females and males respectively was again the most potent compound, followed by cypermethrin (LD_{50} 177.36 ng per female, 63.94 ng per male) and permethrin (LD_{50} 333.10 ng per female, 155.73 ng per male). The relative potencies of permethrin : cypermethrin : decamethrin based on LD_{50} value were 1 : 1.9 : 9.2 for females and 1 : 2.4 : 9.1 for males. The slope of the regression lines was steep with all 3 compounds and the LD_{90} / LD_{50} values ranged from 1.7 to 3, very similar to the values obtained for R. appendiculatus.

3.6.4.1.3 Comparison of susceptibility of unfed adults of R. appendiculatus and A. hebraeum to 3 synthetic pyrethroids

The topical application method has the advantage that the exact dose could be determined with some accuracy. Since the ticks are treated individually, corrections could be made for difference in the weight of the ticks. This is a matter of some importance when one has to compare the relative susceptibility of males and females of the same species, whose weights may be different or of different species with different weights. It is therefore important to convert the dose in ng per tick and express these as ng per mg of tick body weight

(tbw) so that the results could be standardised by making the necessary adjustments for differences in weight, in much the same way as LD_{50} doses of toxic compounds for higher animals are expressed on the basis of mg per kg body weight. This makes comparison of doses more valid and gives a more realistic picture of the comparative susceptibility of males and females and of different species of ticks to acaricidal compounds.

On the basis of 50% and 90% lethal doses expressed as ng per tick, R. appendiculatus adults would appear to be more susceptible than A. hebraeum, an apparent departure from results reported so far for unfed larvae, unfed nymphs and unfed adults tested by immersion or tea bag technique, where A. hebraeum is the more susceptible of the two species. However, A. hebraeum isthe heavier of the two species and if corrections are made for the weight of the ticks, A. hebraeum adults are seen to be more susceptible than R. appendiculatus adults, thus fitting the general pattern of susceptibility of the two species. By standardising the dose per mg of tick body weight the LD_{50s} of permethrin for A. hebraeum were 10.7 ng for females and 6.5 ng for males (Table 32). For R. appendiculatus females, the LD_{50} was 23.0 ng (no experiments were done with males). R. appendiculatus males and females were more tolerant to cypermethrin and decamethrin also than males and females of A. hebraeum (Table 32). Based on ng per mg tick body weight, female A. hebraeum were 2.15 times as susceptible as female R. appendiculatus to permethrin, and 2.8 times as susceptible to cypermethrin and decamethrin. Males of R. appendiculatus were 4.4 times as tolerant to cypermethrin and 1.4 times as tolerant to decamethrin as males of A. hebraeum. Topical application clearly showed that males of R. appendiculatus and A. hebraeum were more susceptible than females. This was clear whether the comparison was

TABLE 32 - COMPARISON OF SUSCEPTIBILITY OF UNFED FEMALES AND MALES OF R. APPENDICULATUS AND A. HEBRAEUM TO 3 SYNTHETIC PYRETHROIDS. RESULTS OF TESTS BY TOPICAL APPLICATION BASED ON LD_{50} and LD_{90} VALUES EXPRESSED AS ng PER TICK AND AS ng PER mg OF TICK BODY WEIGHT (tbw)

Compound	Sex	R. appendiculatus				A. hebraeum			
		LD_{50} (ng/tick)	LD_{50} (ng/mg tbw)	LD_{90} (ng/tick)	LD_{90} (ng/mg tbw)	LD_{50} (ng/tick)	LD_{50} (ng/mg tbw)	LD_{90} (ng/tick)	LD_{90} (ng/mg tbw)
permethrin	F	114.66	23.0	457.52	91.5	333.10	10.7	573.48	18.5
	M	-	-	-	-	155.73	6.5	390.81	16.3
cypermethrin	F	80.19	16.0	208.45	41.7	177.36	5.7	305.00	9.8
	M	78.78	12.0	169.99	25.4	63.94	2.7	143.33	6.0
decamethrin	F	16.89	3.4	36.90	7.4	36.17	1.2	67.03	2.2
	M	6.77	1.0	12.00	1.8	17.02	0.7	51.48	2.1

made on the basis of ng per tick or ng per mg tbw. Based on ng per mg tbw LD_{50} values, males of R. appendiculatus were 1.3 times as susceptible as females to cypermethrin and 3.4 times as susceptible as females to decamethrin. Males of A. hebraeum were 1.6 times, 2.1 times and 1.7 times as susceptible as females to permethrin, cypermethrin and decamethrin respectively.

3.6.4.2 The effect of 3 synthetic pyrethroids on unfed adult ticks tested by the tea bag technique

3.6.4.2.1 Results of tests with Rhipicephalus appendiculatus

The age of ticks tested varied from 2 to 8 weeks, but since the number tested at each concentration never exceeded 27, results for all age groups from all the experiments were pooled. Mortality was read at 7 days and 14 days post-treatment and the LC_{50} and LC_{90} values calculated for both days.

The average number of males or females tested per concentration of each compound varied from 10 to 27. The LC_{50} and LC_{90} values with 95% fiducial limits, slope of the regression lines, Chi^2 and P based on 7 day mortality and 14 day mortality are given in Tables 33 and 34. Figs. 63 and 64 show the regression lines.

On the basis of data for 7 days post-treatment, decamethrin proved to be the most effective compound against both sexes (LC_{50} 0.0006%), followed by cypermethrin (LC_{50} for males 0.0023%, for females 0.0026%). Permethrin with LC_{50} of 0.0053% for males and 0.0066% for females was the least effective of the 3 compounds. Thus, decamethrin was 3.8 to 4.4 times as effective as cypermethrin and 8.8 to 11 times as effective as permethrin. Cypermethrin was only just over twice as effective as permethrin. While males and females were equally susceptible to decamethrin, males were slightly more susceptible to the other 2 compounds. The pooled results comprising tests

TABLE 33 - SUSCEPTIBILITY OF UNFED FEMALES AND MALES OF R. APPENDICULATUS TO PERMTETHRIN, CYPERMETHRIN AND DECAMETHRIN. RESULTS OF TESTS WITH TEA BAG TECHNIQUE. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P, BASED ON MORTALITY AT 7 DAYS POST-TREATMENT

Compound	Sex	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
permethrin	F	0.0066	0.0059 - 0.0075	0.0105	0.0089 - 0.0145	15	6.403 ± 1.19	2.353	>0.5
	M	0.0053	0.0046 - 0.0059	0.0088	0.0075 - 0.0114	17	5.795 ± 0.96	3 745	>0.25
cypermethrin	F	0.0026	0.0020 - 0.0031	0.0058	0.0045 - 0.0091	25	3.637 ± 0.68	7.034	<0.05
	M	0.0023	0.0019 - 0.0028	0.0062	0.0047 - 0.0087	27	2.922 ± 0.37	8.453	>0.05
decamethrin	F	0.0006	0.0005 - 0.0006	0.00100	0.0009 - 0.0012	27	5.543 ± 0.86	0.945	>0.9
	M	0.0006	0.0005 - 0.0006	0.00085	0.0007 - 0.0012	18	8.419 ± 2.24	1.074	>0.5

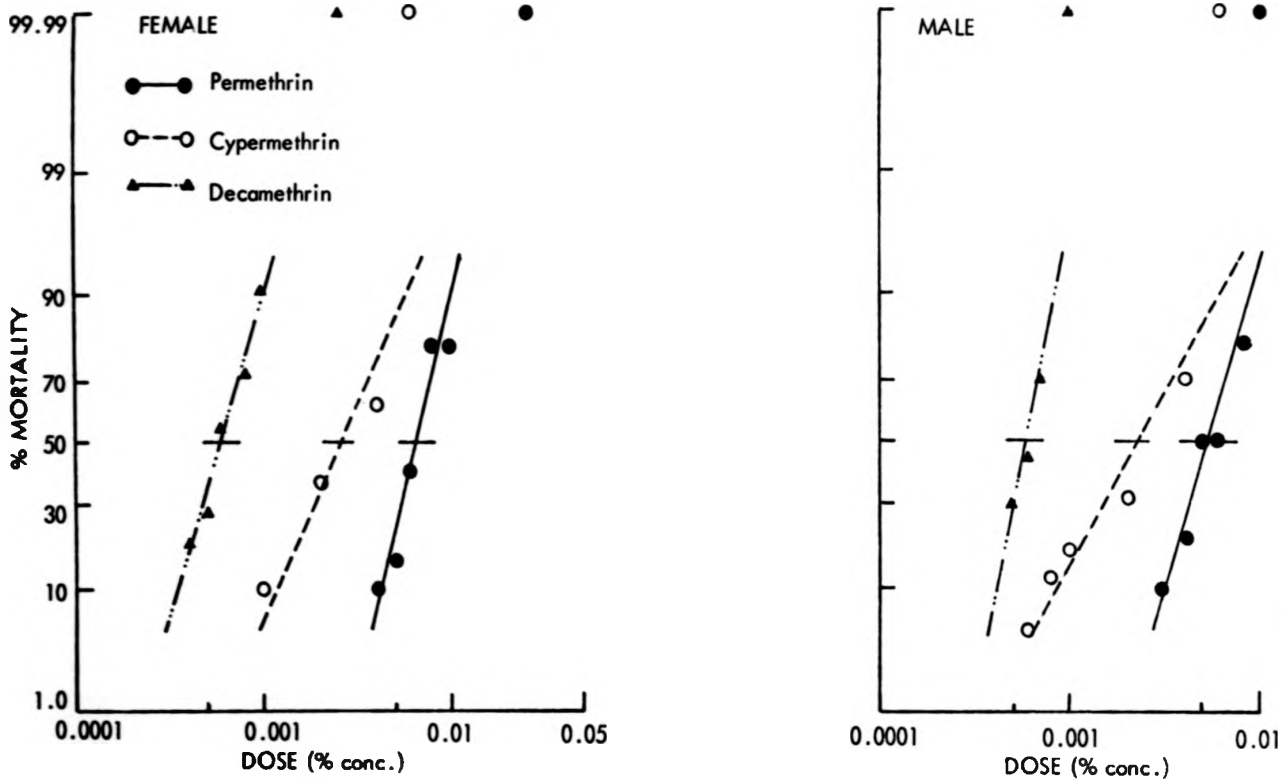


FIG. 63

RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH PERMETHRIN, CYPERMETHRIN AND DECAMETHRIN AGAINST UNFED FEMALES AND MALES OF R. APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS AT 7 DAYS POST - TREATMENT.

TABLE 34 - SUSCEPTIBILITY OF UNFED FEMALES AND MALES OF R. APPENDICULATUS TO CYPERMETHRIN AND DECAMETHRIN. RESULTS OF TESTS WITH TEA BAG TECHNIQUE. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P BASED ON MORTALITY AT 14 DAYS POST-TREATMENT

Compound	Sex	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
cypermethrin	F	0.0020	0.0014 - 0.0026	0.0038	0.0028 - 0.0073	10	4.545 ± 1.14	0.342	>0.95
	M	0.0019	0.0013 - 0.0026	0.0048	0.0032 - 0.0113	10	3.103 ± 0.70	2.285	>0.5
decamethrin	F	0.0006	0.0005 - 0.0007	0.0010	0.0008 - 0.0018	10	5.537 ± 1.43	0.973	>0.9
	M	0.0006	0.0005 - 0.0007	0.0008	0.0007 - 0.0013	10	8.416 ± 2.05	1.336	>0.5

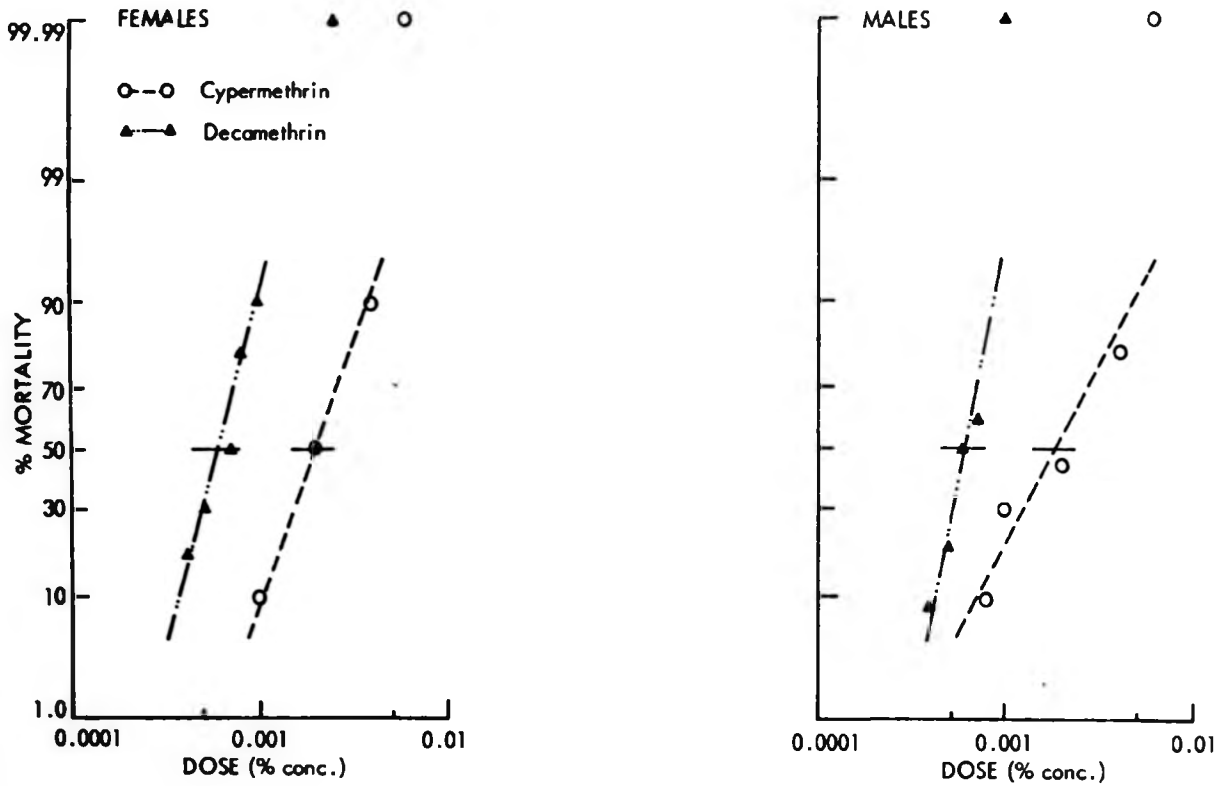


FIG.64
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH CYPERMETHRIN AND DECAMETHRIN AGAINST UNFED FEMALES AND MALES OF R.APPENDICULATUS. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS AT 14 DAYS POST-TREATMENT.

with ticks of different ages makes it impossible to draw any definite conclusions about relative susceptibility of the sexes.

The slope of the regression line was steep with all 3 compounds, (Fig. 63) suggesting that as with larvae and nymphs, the concentration required for 90% mortality was close to the concentration for 50% kill, an increase of the LC_{50} by a factor of 1.4 to 2.7 giving 90% control. These values are very close to the corresponding figures of 1.7 to 2.6 for unfed nymphs and 1.7 to 2.1 for unfed larvae.

Mortality at 14 days post-treatment was scored only in tests with cypermethrin and decamethrin (Table 34 and Fig. 64). Since the LC_{50} and LC_{90} values were the same or only slightly lower than those obtained at 7 days, it would appear that at least for this species mortality stabilises at 7 days following treatment.

3.6.4.2.2 Results of tests with Amblyomma hebraeum

Only one compound, decamethrin, was tested against adults of this species. In 3 tests the age of ticks used varied from 3 to 16 weeks. The pooled results are presented in Table 35 and Fig. 65. Based on 7-day mortality, the LC_{50} for females was 0.00025% and for males 0.00021%. There was not a great deal of difference between these figures and the LC_{50} values of 0.00022% for females and 0.00025% for males based on 14-day mortality. The regression lines were shallower than for R. appendiculatus and the LC_{90}/LC_{50} values based on 7-day mortality ranged from 3 to 5.2. There did not appear to be any marked difference in susceptibility of the two sexes.

3.6.4.2.3 Results of tests with Amblyomma variegatum

Only decamethrin was tested against A. variegatum and the pooled results from 3 tests on 2- to 8-week old males and females are

TABLE 35 - SUSCEPTIBILITY OF UNFED FEMALES AND MALES OF A. HEBRAEUM TO DECAMETHRIN. RESULTS OF TESTS WITH TEA BAG TECHNIQUE*. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Days post-treatment	Sex	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
decamethrin	7	F	0.00025	0.00017 - 0.00035	0.0013	0.0008 - 0.0032	19	1.822 ± 0.34	0.607	>0.95
	14	F	0.00022	0.00007 0.00037	0.0026	0.0011 - 0.0614	19	1.205 ± 0.37	1.125	>0.75
	7	M	0.00021	0.00015 - 0.00028	0.00065	0.00048 - 0.00113	19	2.665 ± 0.48	3.120	>0.25
	14	M	0.00025	0.00015 - 0.00034	0.0011	0.0007 - 0.0028	19	1.966 ± 0.41	6.809	>0.05

* Mortality scored at 7 and 14 days after treatment

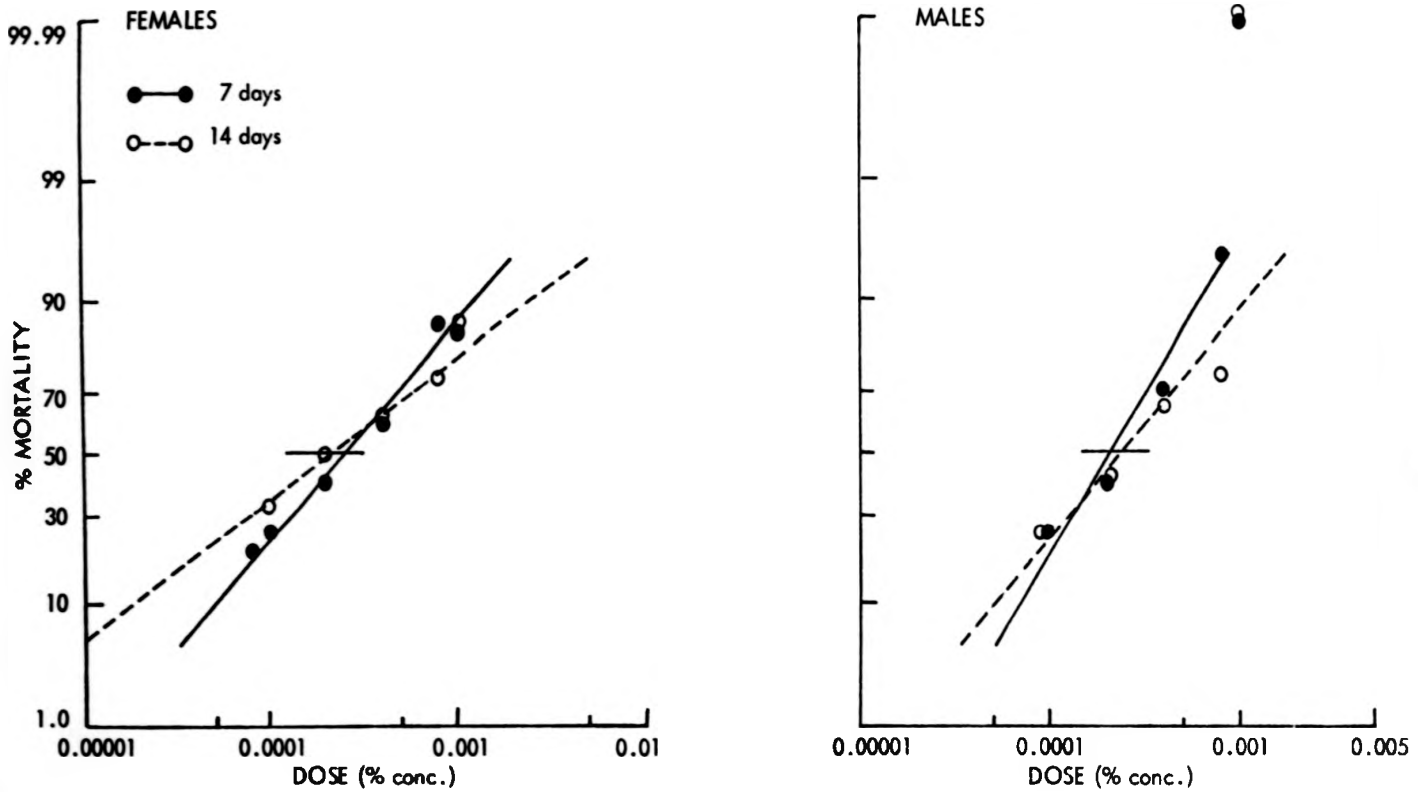


FIG.65

RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH DECAMETHRIN AGAINST UNFED FEMALES AND MALES OF A.HEBRAEUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS AT 7 AND 14 DAYS POST-TREATMENT.

TABLE 36 - SUSCEPTIBILITY OF UNFED FEMALES AND MALES OF A. VARIEGATUM TO DECAMETHRIN. RESULTS OF TESTS WITH TEA BAG TECHNIQUE*. VALUES OF LC_{50} and LC_{90} (% conc.), SLOPE, Chi^2 and P

Compound	Days post-treatment	Sex	LC_{50} (%)	95% fiducial limits to LC_{50}	LC_{90} (%)	95% fiducial limits to LC_{90}	Average number of ticks per conc.	Slope ± SE	Chi^2	P
decamethrin	7	F	0.00019	0.00016 - 0.00024	0.00036	0.00028 - 0.00052	16	4.835 ± 0.83	1.925	>0.5
	14	F	0.00026	0.00021 - 0.00032	0.00053	0.00041 - 0.00083	18	4.091 ± 0.69	1.814	>0.5
	7	M	0.00013	0.0001 - 0.00017	0.00035	0.00024 - 0.00075	15	2.880 ± 0.53	2.104	>0.5
	14	M	0.00014	0.00011 - 0.00019	0.00037	0.00026 - 0.00078	15	2.978 ± 0.54	2.501	>0.5

* Mortality scored at 7 and 14 days after treatment

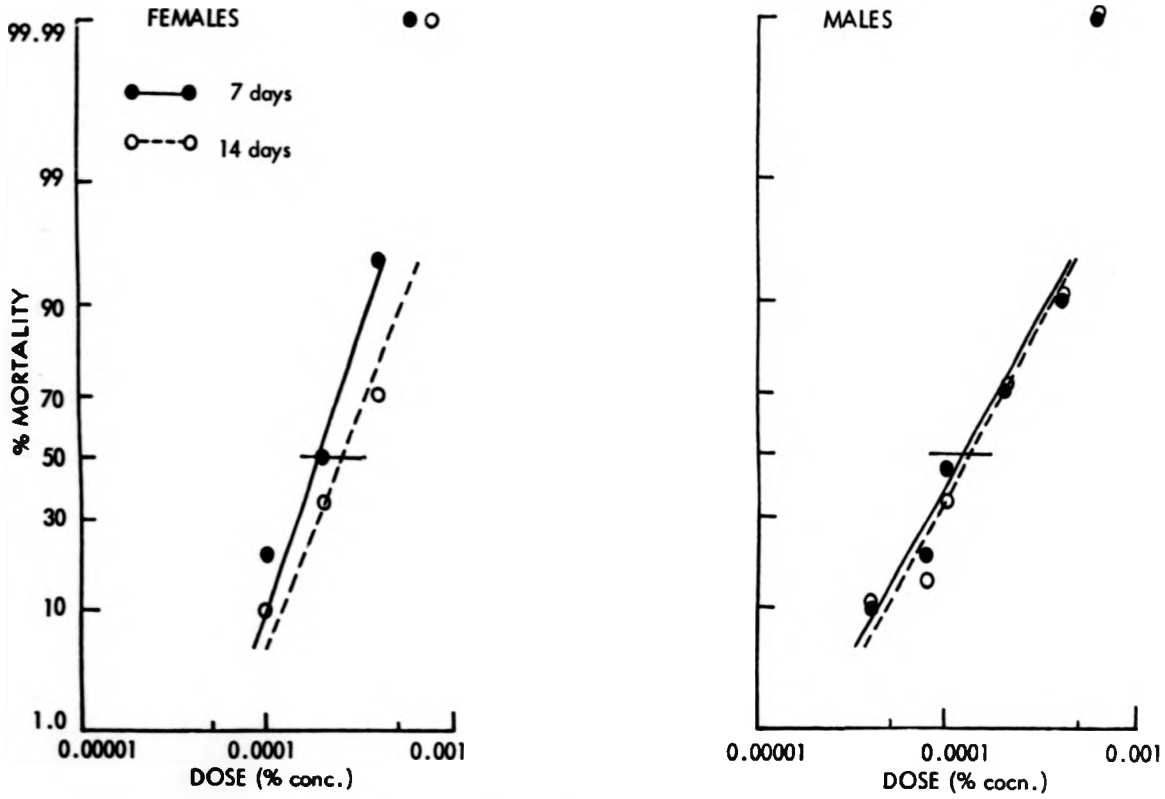


FIG.66

RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH DECAMETHRIN AGAINST UNFED FEMALES AND MALES OF A. VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS AT 7 AND 14 DAYS POST -TREATMENT.

presented in Table 36 and Fig. 66. LC_{50} for females based on 7-day mortality was 0.00019% and for males 0.00013%. While the mortality in males were similar at 7 days and 14 days post-treatment (based on LC_{50} values), the mortality in females at 14 days was slightly higher. The slope of the regression line was steep and 90% kill could be achieved by increasing the LC_{50} by a factor of only 1.9 for females and males, less than the figures of 3.0 and 5.2 for A. hebraeum males and females.

3.6.4.2.4 Comparison of susceptibility of unfed adults of R. appendiculatus, A. hebraeum and A. variegatum to decamethrin

Only decamethrin was tested against all 3 species. The pooling of results from tests done with adults of different ages makes it difficult to assess any change in susceptibility with increasing age. Even so, differences in susceptibility of the 3 species to the compound were similar to those obtained with unfed larvae and unfed nymphs of known ages. On the basis of LC_{50} values for 7 days post-treatment, A. variegatum was the most susceptible species (LC_{50} 0.00019% for females, 0.00013% for males) followed by A. hebraeum (LC_{50} 0.00025% for females, 0.00021% for males). R. appendiculatus with values of 0.0006% for males and females was the most tolerant of the 3 species (Table 37). The increased susceptibility factor of A. variegatum females compared to A. hebraeum females was 1.3, and to R. appendiculatus 3.2. Corresponding figures for males were 1.6 and 4.6. Females and males of R. appendiculatus were 2.4 times and 2.8 times respectively as tolerant as A. hebraeum. The difference in susceptibility of A. variegatum vis-a-vis R. appendiculatus was much more marked with unfed nymphs. Thus, A. variegatum unfed nymphs

TABLE 37 - COMPARISON OF SUSCEPTIBILITY OF UNFED FEMALES AND MALES OF R. APPENDICULATUS, A. HEBRAEUM AND A. VARIEGATUM TO DECAMETHRIN. RESULTS OF TESTS WITH TEA BAG TECHNIQUE*. VALUES OF LC_{50} and LC_{90} (% conc.)

Compound	Days post-treatment	Sex	R. appendiculatus		A. hebraeum		A. variegatum	
			LC_{50} (%)	LC_{90} (%)	LC_{50} (%)	LC_{90} (%)	LC_{50} (%)	LC_{90} (%)
decamethrin	7	F	0.0006	0.001	0.00025	0.0013	0.00019	0.00036
	14	F	0.0006	0.001	0.00022	0.0026	0.00026	0.00053
	7	M	0.0006	0.0008	0.00021	0.00065	0.00013	0.00035
	14	M	0.0006	0.0008	0.00025	0.0011	0.00014	0.00037

* Mortality scored at 7 and 14 days after treatment

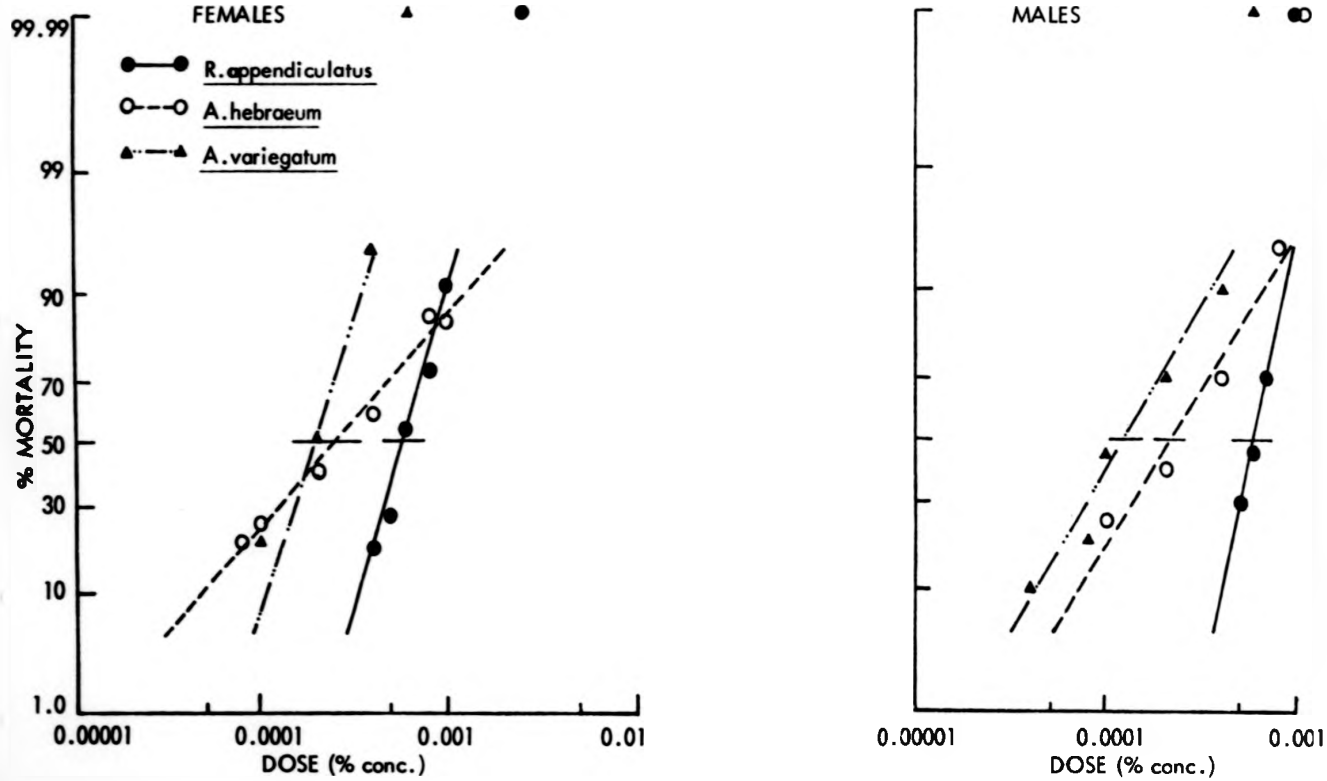


FIG.67

RESULTS OF TESTS BT THE TEA BAG TECHNIQUE WITH DECAMETHRIN AGAINST UNFED FEMALES AND MALES OF R.APPENDICULATUS, A.HEBRAEUM AND A.VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS AT 7 DAYS POST-TREATMENT.

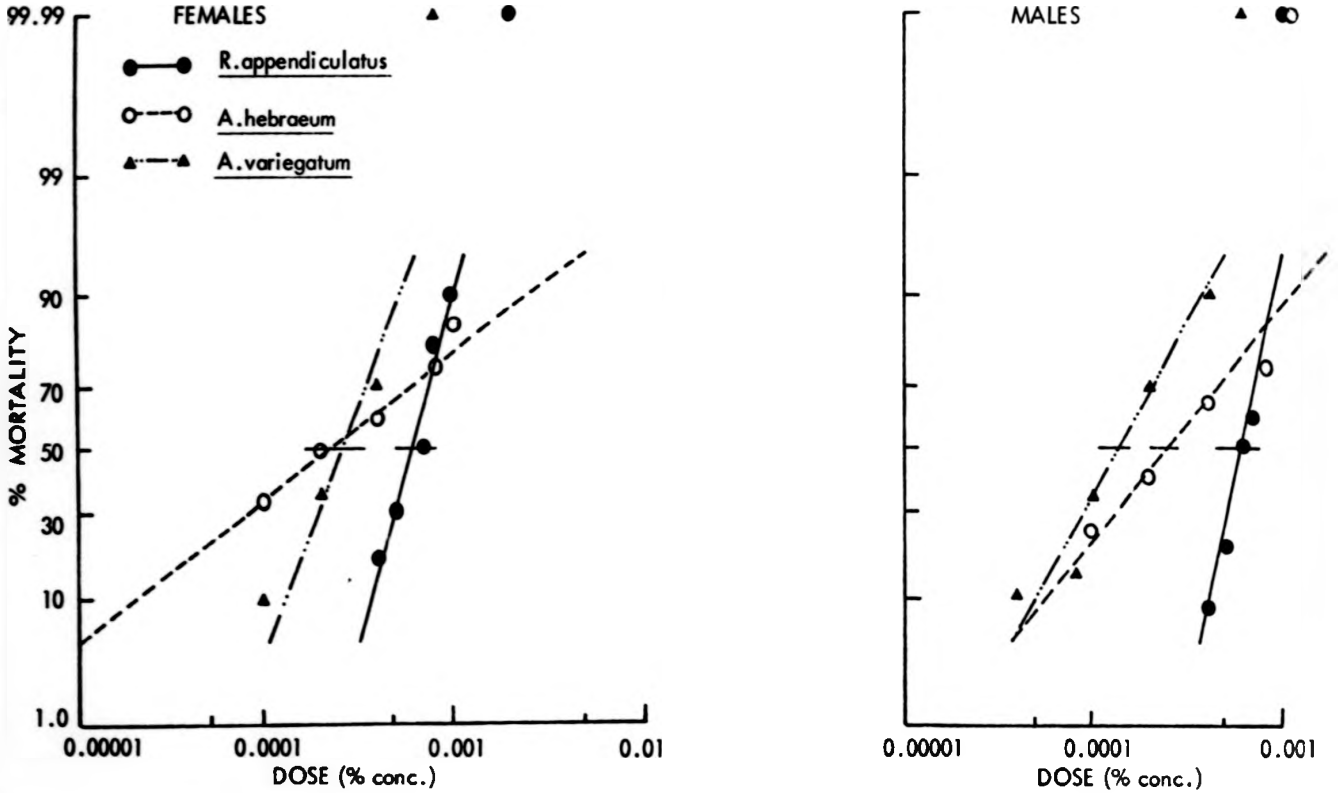


FIG.68
RESULTS OF TESTS BY THE TEA BAG TECHNIQUE WITH DECAMETHRIN AGAINST UNFED FEMALES AND MALES OF R.APPENDICULATUS A.HEBRAEUM AND A.VARIEGATUM. REGRESSION LINES RELATING TO CONCENTRATION AND PERCENTAGE KILLS AT 14 DAYS POST-TREATMENT.

were 6.9 times more susceptible than R. appendiculatus unfed nymphs. While the regression lines for males and females of R. appendiculatus and A. variegatum were steep at 7 days and 14 days post-treatment, the regression lines for A. hebraeum were much shallower, particularly for females (Figs. 67 and 68). This gave an unexpectedly high LC_{90} value for A. hebraeum, which was higher than the value for R. appendiculatus.

3.7 DISCUSSION AND CONCLUSIONS

3.7.1 Developmental periods and feeding behaviour of Rhipicephalus appendiculatus, Dermacentor marginatus, Amblyomma hebraeum and Amblyomma variegatum

In most of the earlier laboratory rearing experiments with ixodid ticks (Nuttall 1913; Lewis 1932), there has been no attempt to rear the ticks under constant conditions of temperature, humidity and photoperiod; in many cases, the conditions are not even mentioned. This has made it extremely difficult to compare the data of different authors for the same species or the biodata for different species. In my experiments the four species were reared under identical conditions. A. hebraeum and A. variegatum had similar developmental periods which were much longer than those for R. appendiculatus and D. marginatus. My data for A. hebraeum are in fairly close agreement with that of Norval (1977) for this species reared at 26 C and complete darkness, except for the minimum oviposition period which was shorter (50 to 53 days) in his experiments. The longest developmental period in both species of Amblyomma occurred during the egg stage (61 - 68 days) and this according to Norval (1977) makes this stage the most susceptible to desiccation.

My data for A. variegatum are close to those obtained by J.B. Walker at 25 - 27 C (quoted by Hoogstraal 1956) except for the shorter duration of the egg stage (minimum 53 days; minimum 61 - 68 days in my experiments) and shorter nymphal and adult premoult periods (minimum 14 days and 19 days respectively; minimum 15 - 20 days and 22 - 28 days in my experiments). Walker does not mention the relative humidity at which the ticks were reared and the shorter periods she obtained could be due to a higher relative humidity, since Norval (1977) has shown that there is a lengthening of the egg stage of A. hebraeum with a drop in the relative humidity as well as temperature. Ilemobade and Mohammed (1978) obtained an even shorter duration of the egg stage, 38 - 45 days, of A. variegatum at 28 C and 80 - 95% relative humidity. The shorter feeding periods of larvae, nymphs and adults which they observed was probably because they were fed on goats and cattle and not on laboratory animals.

Branagan (1973) studied in some detail the developmental periods of R. appendiculatus under laboratory conditions and found that relative humidity did not influence the rate of development. At 25 C and 85 - 87% relative humidity, the minimum duration of the preoviposition period was 6 - 7 days which was longer than the 3 - 6 days obtained by me. Duration of the egg stage was similar in both our experiments as was the duration of the adult premoult but the duration of the nymphal premoult of 8 - 10 days was slightly shorter than the 10 - 14 days in Branagan's experiments. Data for larval, nymphal and adult female feeding periods presented by Tukahirwa (1978) are within the ranges obtained by me; he found that nearly 70% of larvae took 4 days to feed, 70% nymphs took 5 - 6 days and 75% of adults 7 - 9 days.

D. marginatus had the shortest developmental periods of the four

species. Nosek et al. (1967) have given laboratory rearing data for this species at 24 C. While the duration of the preoviposition period, larval feed and nymphal feed were similar to those in my experiments, I obtained a much shorter duration of the egg stage (16 - 17 days; Nosek et al., 29 - 31 days), nymphal premoult (6 - 7 days; Nosek et al., 10 - 15 days) and adult premoult (12 - 13 days; Nosek et al., 20 - 25 days).

The difference in feeding behaviour between R. appendiculatus and the two species of Amblyomma, particularly the excretion of copious amounts of digested blood by nymphs and adults of R. appendiculatus has not been mentioned by previous authors. R. appendiculatus would appear to be a more efficient vector of pathogenic organisms such as rickettsiae which may be excreted with tick faeces, transmission being possible through saliva and faecal contamination, while with the Amblyomma spp. which excrete little or no digested blood during feeding, the faecal route would appear to be less important than bite.

I found a positive linear correlation between weight of engorged females of R. appendiculatus and weight of eggs produced. A similar correlation has been observed in Hyalomma dromedarii Koch by Bassal and Hefnawy (1972), and in A. variegatum and B. microplus by Iwuala and Okpala (1977). Significant correlation between weight of engorged females and number of eggs produced was reported by Drummond, Whetstone and Gladney (1971) for Amblyomma americanum, by Honzakova et al. (1975) for Ixodes ricinus and by Davey et al. (1980) for B. microplus. In my experiments with R. appendiculatus, the conversion efficiency index (wt. of egg mass/wt. of fed female) averaged 52%, a figure remarkably close to the 50 ± 2% which Iwuala and Okpala (1977) obtained for A. variegatum and B. microplus.

3.7.2 Methods of testing acaricides against ticks and scoring of tests

Various methods have been developed for laboratory testing of acaricides against ixodid ticks. The tests have a dual purpose - to compare the acaridical activity of different compounds, and to monitor, measure and compare different populations of ticks for resistance to acaricides. The usual methods employed are, immersion of ticks in acaricidal solutions which also includes the dipping method and the tea bag technique; the principle underlying all three are the same. Two other methods are confinement of ticks in filter paper packets impregnated with acaricides (packet technique) and treatment of ticks with measured doses of acaricides (topical application). A further method, injection of acaricides into ticks has also been reported (Stone and Webber 1960). This is thought to give better effective values, since the compound can reach directly the action sites inside the tick without any loss during transport across the cuticle.

Most of the tests have been on unfed larvae and/or fed females. While larval tests have been extensively used for measurement of resistance, they give no indication of the concentration of acaricides necessary to control infestation on animals. For this, fed females which are the most tolerant stage are tested. The effect on engorged females is not always death. Acaricides may act on the females by inhibiting egg laying, by reduced egg output and by reduced egg viability. The oviposition ratio (%) which is the ratio of number of eggs laid by treated ticks to control ticks has been used by Kitaoka and Yajima (1961) as a measure of the effectiveness of acaricides, while Drummond *et al.* (1971) used the estimated reproduction rate, ER (wt. eggs (g)/wt. female (g) x % hatch x 20000).

The impregnated packet technique of Stone and Haydock (1962) is used for testing unfed larvae for resistance to acaricides. FAO (1971, 1972) recommended that this method be adopted as standard procedure since packets can be prepared in a central laboratory and distributed to different countries and prototype test packets were distributed for assessment (Harris 1977). However, it is extremely difficult to handle, transfer and confine the active larvae and the escaping ticks are a risk to laboratory personnel. Volatility of some acaricides, deterioration on storage of the packets, and standardisation of exposure times and holding times, particularly with synthetic pyrethroids, are further problems. "Self-dosing" of ticks while they walk inside the treated packet may be compared to ticks walking on bodies of treated animals. The packet technique is useful in this context.

Immersion, dipping and the tea bag technique developed by Gladney et al. (1972) all closely parallel field procedures where the ticks on host animals are immersed in or come into contact with acaricidal solutions. A modified tea bag technique was employed by Kigaye and Matthysse (1974) for testing unfed larvae of R. sanguineus. I found the tea bag technique quick, simple and reliable and used it for unfed and fed nymphs as well as for unfed adults. Handling problems are eliminated or reduced to a minimum. Although I did not try this method for unfed larvae it should be possible to remove small batches of eggs into bags, seal them and test the larvae after they have emerged inside the tea bag. This method overcomes the difficulty of handling and transferring actively moving larvae for tests. The tea bag technique as standard testing method for all unfed stages would make it possible to compare the relative susceptibility of larvae, nymphs and adults to acaricides.

In the immersion, dipping and tea bag techniques and in the impregnated packet technique, the dose or concentration of the acaricide is merely the quantity put into the environment of the tick to which the latter is exposed for a standard period. This type of indirect dosing *en masse* is convenient for small arthropods (Busvine 1968b) but does not give the actual amount of acaricide picked up by individual ticks.

Topical application has the advantage that ticks can be treated individually and the dose can be determined with some accuracy and WHO (1975) recommended it as standard procedure for testing susceptibility or resistance of adult ticks to acaricides. For comparing the susceptibility of different species, corrections have to be made for differences in weights and a more reliable criterion will be the LD_{50} expressed as ng of acaricide per mg of tick body weight, rather than per tick.

The scoring of tests with ixodid ticks is beset with many problems which have been referred to in Section 3.4. The criteria for mortality have been discussed by Harris (1977) and Lourens and Tatchell (1979). Drummond *et al.* (1969) placed treated nymphal ticks dorsal side down and scored as dead any that were unable to turn over even after being stimulated by one's breath. While actively moving ticks are no problem to score, motionless ticks may be dead, paralysed or simply inactive. Warming the ticks will often induce inactive ticks to move about or make them more excitable; the latter may often be a sign of intoxication and the ticks will become inactive again when removed from the warm surface. Kitaoka and Yajima (1961) found that some OP compounds had a stimulatory effect on adult *B. microplus* and made them hyperactive. All paralysed and consistently inactive ticks are scored as dead, since they will be unable to perform essential activities such

as host-seeking and feeding and will eventually die. Uspenskij and Levikov (1974) described six stages in acaricidal poisoning in adult ticks, ranging from slight disturbance of motor function through partial or complete paralysis to death.

I found it extremely difficult to score tests with adult ticks. They are usually less active than the immature stages. Furthermore, there was a difference in the activity of adults of different species, the Amblyomma adults being much more sluggish than R. appendiculatus adults. The time taken for the toxic effect to manifest itself in adult ticks may be several days and may vary from species to species, and Privora et al. (1970), WHO (1975) recommended an observation period of 7 days for Ixodes spp. and 10 to 15 days for Dermacentor and Hyalomma, while Uspenskij (1974) did not find any substantial change in mortality from 48 hours to 7 days. I adopted a 7-day post-treatment period for scoring the tests, since there was not a great deal of difference between results obtained at 7 days and 14 days post-treatment.

3.7.3 Relative toxicity of the 3 synthetic pyrethroids to Rhipicephalus appendiculatus, Amblyomma hebraeum and Amblyomma variegatum

The toxicities of the 3 synthetic pyrethroids, permethrin, cypermethrin and decamethrin relative to other acaricides such as DDT and dieldrin, malathion and other OP compounds and carbamates such as carbaryl, have been well documented. However, the relative toxicities may not be of the same order for different groups of arthropods. Barlow and Hadaway (1975) showed decamethrin to be almost 700 times as effective as DDT and about 80 times as effective ad dieldrin against the mosquito Anopheles stephensi, and 1000 times as effective as DDT

and 100 times as effective as dieldrin against the tsetse fly Glossina austeni; against stable flies and larvae of the cattle tick B. microplus however, decamethrin was only 160 times and 262 times respectively as effective as DDT (Barlow and Hadaway 1975; Nolan et al. 1977). The three synthetic pyrethroids have also been shown to be much more toxic than OP compounds such as malathion, endosulfan, fenthion, chlorpyriphos methyl, Abate and coumaphos against various insect species (see Section 3.1.1), although Gladney and Dawkins (1976) found permethrin to be less effective than the OP compounds phoxim and chlorphoxim against unfed nymphs of R. sanguineus. 0.05% or 0.1% permethrin sprayed on to cattle was more effective in controlling adults of A. americanum than the standard treatment with 0.5% toxaphene (Drummond and Gladney 1978).

To my knowledge, there have been no previous studies comparing the effectiveness of the 3 synthetic pyrethroids, the newly developed triazapenta-diene compound, amitraz and the carbamate, carbaryl on R. appendiculatus, A. hebraeum and A. variegatum. Baker et al. (1973) stated that against larvae or R. appendiculatus, 0.0035% amitraz was highly effective in preventing reinfestation, while 0.005% prevented nymphal attachment; 0.00625% to 0.025% amitraz gave excellent control of adult A. hebraeum. In spraying trials against B. microplus on cattle, permethrin and amitraz were more effective than carbaryl and at 0.025% gave 98% to > 99% control respectively, while it needed 0.2% carbaryl to achieve a similar level of control (Nolan 1979). Gladney et al. (1972) tested 1-week old nymphs of R. sanguineus with carbaryl using the tea bag technique and obtained a LC_{50} value of 0.0052%, higher than the value of 0.0046% I obtained with R. appendiculatus, but the LC_{90}/LC_{50} values were the same, 1.7, in both experiments.

I found permethrin, cypermethrin and decamethrin more effective than amitraz and carbaryl against larvae of A. hebraeum and A. variegatum and against nymphs of these 2 species and of R. appendiculatus. Against larvae, the three synthetic pyrethroids were 5 to 68 times as effective as amitraz and 14 to 1618 times as effective as carbaryl; the relative toxicity of the 3 synthetic pyrethroids against nymphs of the 3 species was 4 to 41 times as high as amitraz and 12 to 67 as high as carbaryl. All comparisons are based on LC_{50} values and similar age groups. Although field trials have shown amitraz to be highly effective against ticks, I found this compound one of the more difficult to test in the laboratory. LC_{90} values were generally very high compared to LC_{50} values, giving a shallow regression line, and 100% mortality was obtained only rarely with amitraz.

While decamethrin is the most effective and permethrin the least effective of the 3 compounds, the relative toxicity of the three may vary from one species of arthropd to another. Usually the toxicity of decamethrin compared to permethrin is of a much higher magnitude than that of cypermethrin to permethrin. The relative toxicity of permethrin : cypermethrin : decamethrin for Musca domestica was 1 : 3.5 : 25 and for Glossina austeni, 1 : 4 : 38 (Elliot et al. 1978). Nolan et al. (1977) found that although decamethrin was the most effective, the relative toxicity of the 3 compounds for larvae of a DDT-susceptible strain of B. microplus was only 1 : 2 : 8; against larvae of an OP-resistant strain of this species decamethrin was again 8 times as effective as permethrin. I also found a definite difference in relative toxicity of the 3 compounds and 3 species of ixodid ticks (Table 38). Based on larval results, the toxicity ratios of permethrin : cypermethrin : decamethrin were 1 : 1 : 1.3 for R. appendiculatus, 1 : 5.5 : 8.3 for A. hebraeum and 1 : 5 : 11.5 for

TABLE 38 - TOXICITY RATIOS OF PERMETHRIN, CYPERMETHRIN AND DECAMETHRIN FOR THREE SPECIES OF IXODID TICKS. (TOXICITY OF PERMETHRIN TAKEN AS 1)

Species	Stage	Test method	Compound		
			permethrin	cypermethrin	decamethrin
R. appendiculatus	L	Immersion	1	1	1.3
	N	Tea bag	1	1.1	1.5
	Ad.F	Top. appl.	1	1.4	6.8
	Ad.F	Tea bag	1	2.5	11
A. hebraeum	L	Immersion	1	5.5	8.3
	N	Tea bag	1	1.1	3.6
	Ad.F	Top. appl.	1	1.9	9.2
A. variegatum	L	Immersion	1	5	11.5
	N	Tea bag	1	2.1	4.6

A. variegatum. The ratio for unfed nymphs was 1 : 1.1 : 1.5 for R. appendiculatus, 1 : 1.1 : 3.6 for A. hebraeum and 1 : 2.1 : 4.6 for A. variegatum. The ratio for unfed females of R. appendiculatus by topical application was 1 : 1.4 : 6.8 and 1 : 2.5 : 11 by the tea bag method while the ratio for unfed females of A. hebraeum by topical application was 1 : 1.9 : 9.2. These results confirm that the relative toxicity of the 3 compounds vary not only from species to species, but also from stage to stage of the same species.

A. variegatum was the most susceptible of the 3 species to all 3 synthetic pyrethroids, the different stages being 2.1 to 19.7 times as susceptible as the corresponding stages of R. appendiculatus to the 3 compounds. A. hebraeum which was intermediate in susceptibility was only 1 to 6.5 times as susceptible as R. appendiculatus. Differences in susceptibility to the same acaricides of different species in the same genus has been shown by Drummond et al. (1969) also who found adults of Haemaphysalis papuana kinneari Warburton less susceptible to carbaryl and malathion than adults of H. turturis Nuttall and Warburton and H. spinigera. The higher susceptibility of A. hebraeum relative to R. appendiculatus has been observed by Mathewson and Hughes (1978) also; larvae of A. hebraeum were 1.1 to 4.1 times as susceptible as larvae of R. appendiculatus to chlorpyriphos, carbaryl, chlorfenvinphos, dioxathion and coumaphos.

While my experiments showed that A. variegatum was the most susceptible of the 3 species and R. appendiculatus the most tolerant, and decamethrin the most effective and permethrin the least, the relative toxicities of the compounds were found to vary with the 3 species, the difference in toxicity being least for R. appendiculatus and most for A. variegatum.

3.7.4 Effect of ageing of ticks on susceptibility to acaricides

In the life cycle of ixodid ticks, there is a certain interval, referred to as the prefeeding period, before the freshly emerged ticks are ready to feed. During this period which may vary with species, stage and environmental conditions, the cuticle hardens and the ticks usually remain at the bottom of the vegetation cover. This is followed by excretion of guanin by larvae, nymphs and adults and of residual digested blood by nymphs and adults. Under natural conditions, they would at this stage ascend the stems of grass or other vegetation and quest for host animals. Testing ticks before they have attained this physiological age would be irrelevant to control measures since control by treatment of host animals or by treatment of vegetation by residual acaricides would be ineffectual unless the ticks are actually questing or have passed beyond this stage and boarded a host animal.

The active season during which ticks are questing may last several weeks. During this period they make repeated trips to the vegetation tips. Unless they encounter a host, the heightened metabolic activity which this involves drains their reserves, and there is some evidence that under natural conditions active ticks become exhausted and are short-lived in comparison with individuals maintained in the laboratory under conditions which prevent or restrict their activity (Lees and Milne 1951).

A result of this long active season is that questing ticks and indeed ticks on animals may represent different age groups with varying susceptibilities to acaricides. According to Stone and Haydock (1962) the slight increase in susceptibility of B. microplus larvae from 7 to 28 days, to DDT which they noticed, may be due to a small loss of vigour with increasing age. Shaw (1966) observed in immersion tests with B. decoloratus that mortality with 0.001% dioxathion

had risen from 29% in 3-week old larvae to nearly 50% in 5-week old larvae, suggesting that older larvae were more susceptible than younger larvae. In comparing the susceptibility of Amblyomma americanum of different ages to 16 acaricides, Mount et al. (1970) found 4- to 6-month old nymphs 2.2 times (to dichlorvos) to 35.7 times (to Dursban) as susceptible as 1- to 2- month old nymphs. Rupes et al. (1972a, b) found a positive correlation between age and susceptibility to DDT, Imidan and carbaryl in larvae, nymphs and adults of Ixodes ricinus in Czechoslovakia, older ticks being more susceptible than younger ones, particularly to DDT. In further experiments Rupes et al. (1977) confirmed the higher susceptibility of older larvae and nymphs of this species to fenitrothion; application of 3 kg of fenitrothion per ha at the beginning of the active season in April controlled ticks effectively; by May control could be ensured with 1 kg per ha and in June to August by 0.3 kg per ha. This increased susceptibility of older ticks in nature was paralleled in laboratory experiments also. 6-month old larvae from a laboratory colony were 44 times as susceptible as 1-week old larvae. Nymphs collected in the field and kept in the laboratory showed a nearly 200-fold increase in susceptibility by the end of 6 months. These laboratory experiments would suggest that it is the calender age rather than metabolic activity which is the probable cause for increase in susceptibility of older ticks. Uspenskij (1974) in Russia observed a similar continuous increase in the susceptibility to DDT of natural populations of unfed females of Ixodes persulcatus from the beginning of the active season in May to the end of the active season in June. Harris (1977) noted a progressive increase in mortality with increasing age of B. microplus larvae; 0.04% of the carbamate promacyl killed 43% of 2- to 3- week old larvae, 78% of 4- to 5-week old larvae and 95% of 6- to 7-week old larvae. Mathew-

son and Hughes (1978) tested 1-week, 2-week, 3-week and 4-week old larvae of Hyalomma rufipes, R. appendiculatus, R. evertsi and A. hebraeum to dioxathion, chlorfenvinphos and carbaryl and found that in some cases 1-week old larvae which had not reached the questing stage were the most tolerant. There was a small change in susceptibility over the 4-week period, older larvae being generally more susceptible; the factor of increased susceptibility in A. hebraeum, (2.0 to 2.8) was higher than that in R. appendiculatus (1.4 to 1.5).

In my experiments also, older larvae (5 to 8 weeks) of R. appendiculatus, A. hebraeum and A. variegatum were more susceptible than younger larvae (2 to 4 weeks) to permethrin, cypermethrin and decamethrin, the increased susceptibility factor varying from 1.2 to 3.3 which was slightly higher than the factor of 1.1 observed by Rupes et al. (1977) with I. ricinus of similar age groups. In the more detailed ageing experiments in which I tested larvae every week, 8-week old larvae of A. hebraeum were 4.5 to 8.8 times as susceptible to the 3 pyrethroids as 2-week old larvae, while the increased susceptibility of 8-week old larvae of R. appendiculatus was only 1.5 to 1.6 times that of 2-week old larvae suggesting a specific difference. These experiments also showed that there was a significant and positive correlation between age and susceptibility. The differential susceptibility of the two species with age was seen by Mathewson and Hughes (1978) also, and is probably a reflection of the generally higher tolerance of R. appendiculatus to acaricides. The ticks in my ageing experiments were handled only at the time of testing. It is therefore improbable that the increased susceptibility of the older ticks could have been due to their activity and consequent expenditure of energy resources.

The differential susceptibility of the two species was less marked

with nymphs; older nymphs (8 weeks) of R. appendiculatus and A. hebraeum were 4.4 and 4.6 times, and 4.4 and 4.0 times respectively as susceptible as younger nymphs (2 weeks) to permethrin and cypermethrin. In the detailed experiments where nymphs of R. appendiculatus were tested over a wider range of ages, 12-week old nymphs were 8.3 times as susceptible to permethrin as 1-week old nymphs. Regression analysis showed a positive correlation between age and susceptibility. With decamethrin the increased susceptibility factor of R. appendiculatus (9.6) was higher than that of A. hebraeum (5.6). The reasons for this reversal in the differential susceptibility of larvae and nymphs of the two species is not clear.

From the foregoing account it is clear that the age of ticks is an important factor in assessing their susceptibility to acaricides and that ticks should be tested over a wide range of ages to determine which age group is the most tolerant, for use in standard tests. Field collected ticks may vary widely in their age and there may be substantial variations in results from field collected ticks of unknown ages. Comparative toxicological tests should therefore be done on ticks of the same age. The increased susceptibility of older ticks has practical implications also, since it would require lower concentrations of acaricides to achieve effective control as the active season progresses.

3.7.5 Susceptibility of different stages and of males and females of ixodid ticks to synthetic pyrethroids

To achieve effective control of a multi-host tick species, the concentration of acaricide which will kill 100% of the most tolerant stage has to be determined. Although it is realised that it takes increasingly higher concentrations to kill larvae → nymphs → adults,

there have been few laboratory studies on the relative susceptibility of different stages of the same species to acaricidal compounds.

I compared the relative susceptibility of unfed larvae, nymphs and adults of R. appendiculatus, A. hebraeum and A. variegatum to 3 synthetic pyrethroids. The comparisons are based on LC_{50} values from immersion tests on unfed larvae and from tests by the tea bag technique on unfed nymphs and unfed adults. It is realised that results from two different types of tests such as immersion and tea bag technique are not strictly comparable, and the comparison between nymphs and adults which were tested by the same method would be more reliable than comparison between these stages and larvae. In this comparative study results from ticks of similar age were used.

While decamethrin was the most effective, cypermethrin intermediate in effect and permethrin the least effective against larvae, nymphs and adults of all species, their relative toxicities for larvae, nymphs and adults of each species varied considerably. Larvae of R. appendiculatus were 80 times as susceptible and nymphs 17 times as susceptible as adult females to permethrin (Table 39). This ratio was smaller with decamethrin, larvae and nymphs being only 9 times and 2 times respectively as susceptible as adult females. The toxicity ratio of cypermethrin, 27 : 7 : 1, for larvae, nymphs and adult females was intermediate. Data for comparison of the 3 stages of all 3 species was available only for decamethrin. The variation in toxicity of this compound for the different stages was highest in A. variegatum, larvae being 57 times and nymphs 5 times as susceptible as adult females, while larvae of A. hebraeum were 25 times and nymphs 3 times as susceptible as adult females; larvae of R. appendiculatus were however only 9 times as susceptible and nymphs only twice as susceptible as adult females (Table 39). The relative susceptibility of nymphs compared to

TABLE 39 - TOXICITY RATIOS OF 3 SYNTHETIC PYRETHROIDS TO LARVAE, NYMPHS AND ADULTS OF R. APPENDICULATUS, A. HEBRAEUM AND A. VARIEGATUM

Species	Stage	permethrin	cypermethrin	decamethrin
R. appendiculatus	Larvae	80 | 5	27 | 4	9 | 4
	Nymphs	17	7	2
	Adult F.	1	1	1
A. hebraeum	Larvae	4	20	25 | 8
	Nymphs	1	1	3
	Adult F.	-	-	1
A. variegatum	Larvae	5	12	57 | 11
	Nymphs	1	1	5
	Adult F.	-	-	1

adults was least with decamethrin (2) and R. appendiculatus, intermediate (7) for cypermethrin and highest (17) with permethrin. The relative susceptibility to all 3 compounds of larvae and nymphs of R. appendiculatus were similar, larvae being 4 to 5 times as susceptible as nymphs. Similar values were obtained with permethrin for larvae and nymphs of A. hebraeum and A. variegatum. The values were much higher for cypermethrin and decamethrin and A. hebraeum and A. variegatum, larvae of which were 8 to 20 times as susceptible as nymphs (Table 39). Differences in the relative toxicity of different compounds to unfed larvae, nymphs and adults of the same species was observed by Baker et al. (1978b) also, the toxicity of the OP compound dioxathion for larvae, nymphs and adults of A. hebraeum being much higher than that of another OP compound chlorfenvinphos; the ratio of the LC_{99} for larvae : adults was much higher with dioxathion than with chlorfenvinphos and the difference was of a considerably higher magnitude than that obtained by me with the 3 synthetic pyrethroids.

Males of many ixodid species are found attached and feeding on animals. Since they are capable of transmitting pathogens, their control is important in tick management. Whitnall et al. (1951) found that arsenical treatment of cattle reduced the number of male A. hebraeum, but did not reduce the number of females, which suggests that females were more tolerant than males. However males are not usually included in acaricidal tests probably because the doses used for killing adult females will kill males also. Baker et al. (1977, 1978b) tested males and females of A. hebraeum with toxaphene, dioxathion and chlorfenvinphos, but do not mention any difference in susceptibility. Drummond et al. (1969) found little difference in susceptibility between sexes of Haemaphysalis spinigera to BHC , malathion and carbaryl. However, Uspenskij (1974) using topical application noted that

unfed females of I. persulcatus were more susceptible to DDT than unfed males, when comparisons were made on the basis of LC_{50} per mg of tick body weight. When tested by the tea bag technique, I found males of R. appendiculatus, A. hebraeum and A. variegatum only slightly more susceptible (1.1 to 1.6 times) than females. But the more exact topical application technique where the LD_{50} could be expressed as ng/mg tick body weight, showed that males of R. appendiculatus and A. hebraeum were 1.3 to 3.4 times as susceptible as females to pyrethroids. This suggests that topical application may be more reliable for determining the comparative susceptibility of the two sexes to acaricides.

3.7.6 Effect of acaricides on fed nymphs

One-host ticks such as Boophilus spp. feed and moult on the host animal. In the control of these ticks therefore, the effect of acaricides on unfed, fed and premoult stages of ticks has to be assessed. With multi-host species, unfed ticks as well as ticks in different stages of engorgement up to fully engorged, are found on the host. It is generally accepted that engorged ticks and those in the premoult phase are more tolerant to acaricides than unfed ticks; indeed it takes relatively high concentrations of acaricides to kill engorged female ticks, and the effect of the acaricide on these ticks even then is assessed not only on the basis of kill but also on inhibition of oviposition and failure of eggs to hatch. Acaricidal tests on engorged females is accepted procedure in many laboratories. However, there have been few experiments on the effect of acaricides on fed immature stages, although these are present on host animals and come into contact with acaricidal solutions. Baker and Shaw (1965) tested freshly engorged nymphs of a susceptible strain of R. appendiculatus

for susceptibility to toxaphene and gamma-BHC. The percentage failure of adult emergence was taken as the percentage mortality. On this basis, 100% mortality was obtained with 0.05% toxaphene and with 0.02% gamma-BHC. Baker et al. (1977) tested the susceptibility of freshly engorged larvae and nymphs of A. hebraeum to toxaphene; 99% mortality of unfed larvae was obtained with 0.14% of toxaphene, but it required 3% of this compound to achieve 100% mortality of engorged larvae; 1% toxaphene controlled only just over half the number of treated nymphs.

My experiments also showed that engorged nymphs of R. appendiculatus and A. hebraeum were 315 and 267 times respectively more tolerant to permethrin than unfed nymphs. Results with decamethrin were similar, engorged nymphs of R. appendiculatus, A. hebraeum and A. variegatum being 240 to 641 times as tolerant as unfed nymphs. The effect of decamethrin was manifested not only in failure of adults to emerge, but also in death of the emerged adults, as well as in a significant prolongation of the premoult period in R. appendiculatus, A. hebraeum and A. variegatum. The effect of acaricidal treatment on prolongation of the moult has been noted before but only as a general statement (Wellcome Research Organisation, 1976).

Thus, it is important to include ticks in all stages of engorgement in acaricidal testing if the effect of the compounds on the total biology of ticks is to be assessed, although experiments have been done only with fully engorged ticks. Apart from the conventional criterion of mortality of the fed ticks, additional factors such as failure of ticks in the premoult phase to proceed to moult, death of the emerged ticks and prolongation of the premoult period will have to be included in assessing the acaricidal effect of the compounds.

4. INVESTIGATIONS ON HOST IMMUNITY TO TICK INFESTATION

4.1 INTRODUCTION AND REVIEW OF LITERATURE

Zebu or Brahman cattle (Bos indicus) have survived in Africa and Asia for many years despite infestation by Boophilus and Rhipicephalus ticks and the diseases they transmit. The long host parasite relationship which has evolved in nature has led to the development of a degree of resistance to tick infestation which is not present in exotic European breeds (Bos taurus). This fact has been known for a long time although investigations on the nature of host resistance to ticks or attempts to exploit host resistance for stock improvement have a more recent history. Rajk (1962) observed that individual cattle in a herd without any previous exposure to ticks differed in their resistance to infestation with the cattle tick Boophilus microplus (Canestrini); replete females dropping off Zebu cattle (B. indicus) were significantly lighter than those from European cattle (B. taurus), suggesting a difference between herds. O'Kelly and Spiers (1976) also showed that on their first exposure to ticks from birth, Zebu calves were more resistant to the ticks than British calves. Hewetson (1971; 1972) and Wagland (1975; 1978) on the other hand found that B. indicus cattle before exposure to B. microplus were as susceptible as B. taurus; however, the Brahmans (B. indicus) acquired higher levels of resistance quicker than the Shorthorns (B. taurus), the yield of engorged females decreasing from 25% after the first infestation to 7.5% during the third, while in the Shorthorns, the yield remained unchanged. Wagland (1979) further showed that although there was no obvious loss of feeding B. microplus larvae from Brahman or European cattle with similar levels of resistance, there appeared to be a significant loss of engorged larvae from Brahmans. Strother et al. (1974) exposed purebred Brahman, purebred European

(Hereford) and Brahman x Hereford crossbred steers to Amblyomma americanum (L.) and found that although all breeds acquired high levels of resistance to the ticks following three sequential infestations as evidenced by significant reduction in numbers and weights of replete females, the purebred Herefords were considerably less resistant than both the Brahman x Hereford crossbreeds and the purebred Brahmans. Similar results were reported by Utech et al. (1978) who found that selection for resistance to ticks would be highly effective, resistance following infestation with B. microplus developing more readily in B. indicus cattle and their crosses than in B. taurus animals, showing that resistance in Zebu is dominant. Resistance in B. indicus cattle and in individual B. taurus cattle without previous exposure to ticks is usually referred to as "innate resistance" to differentiate it from "acquired resistance" which develops after exposure to ticks, although the term "acquired immunity" may be more appropriate since this type of resistance has been shown to have an immunological component. Innate or natural resistance is heritable and selection and breeding for resistance are possible not only in Zebu x European crossbreeds but also within European breeds (Wharton 1976), improvement of herds being possible by a combination of breeding for resistance and culling for susceptibility. Strother et al. (1974) suggested that a programme of crossbreeding Brahman with British breeds of cattle may be an effective non-chemical approach to tick management.

Most of the laboratory and field investigations have been on acquired resistance to tick infestation, in which an animal exposed to ticks develops resistance to a subsequent infestation with the same species. Immune response in host animals as a factor in tick management has been reviewed recently by Allen (1979). An earlier review by Nelson et al. (1977) on ectoparasites and hosts includes some work on ticks.

Laboratory investigations on host immunity were first made in the 1930s when Trager (1939a, b) noted that guinea pigs exposed to infestation by larvae of Dermacentor variabilis (Say) became resistant to a subsequent infestation; he further showed that antibody was probably involved in this, since partial immunity could be transferred to naive animals with serum from immune animals. However, most of the work on host resistance to ticks was done in the 1960s and 1970s. Further evidence that host immunity might be antibody mediated was presented by Roberts and Kerr (1976) who induced resistance to B. microplus in cattle by the passive transfer of large volumes of plasma from highly immune cattle; the numbers engorging on such treated animals were significantly less, but numbers engorging on animals treated with plasma from poorly immune or unexposed cattle were not significantly different from those on controls. Brossard (1977) found that Ixodes ricinus L. females feeding on rabbits injected with serum from immune rabbits (on which I. ricinus had fed) weighed significantly less than those from control rabbits, although feeding time was not affected. On the other hand attempts to transfer immunity to I. holocyclus Neumann to naive guinea pigs with immune serum were unsuccessful (Bagnall and Rothwell 1974), although intravenous injection of axillary and prescapular lymph node cells from immune guinea pigs into naive guinea pigs resulted in a significant reduction of feeding performance by I. holocyclus larvae on the immunised animals (Bagnall 1975). Wikel and Allen (1976a) also did not obtain resistance to Dermacentor andersoni Stiles larvae in naive guinea pigs injected with serum from immune donors; but transfer of cervical lymph node cells had a marked effect on fed larval weight, suggesting a cellular as well as a humoral response in host resistance. Wikel and Allen (1976b) also found that immunosuppressant drugs such as methotrexate and cyclophosphamide given to guinea pigs

before infestation with D. andersoni effectively blocked the development of resistance. The role of complement in development of resistance was investigated by Wikel and Allen (1977); cobra venom factor (CoF) which depletes complement, given to immune guinea pigs before and during a second infestation with D. andersoni larvae, increased the number and the weights of larvae although CoF given during primary infestation did not prevent acquisition of resistance to a second infestation. These authors also observed an accumulation of basophils at the site of attachment of ticks to an immune animal; this was absent in complement-depleted animals.

Wikel (1979) went on to demonstrate that C4-deficient guinea pigs became resistant to tick infestation in a manner similar to normal guinea pigs with fully functional complement pathways. These observations and those of Wikel and Allen (1977) on reduction of tick resistance by in vivo reduction of C3 following CoF injection, suggest that an alternative pathway of complement activation is important in the expression of resistance, although its exact role is not known.

Brossard (1976) found that serum γ-globulin concentration was significantly raised in cattle following infestation with B. microplus and indirect immunofluorescence revealed the presence of specific and non-specific antobodies to salivary gland antigen. Although appearance of antibody coincided with development of resistance, a causal relationship was not established. Williams et al. (1977) found that in cattle infested with A. maculatum Koch, significant increases in the total serum protein, serum globulin and plasma fibrinogen and decrease in the total leucocytes occurred, compared to tick-free animals. Willadsen et al. (1978) was able to show specific antibody to B. microplus antigen by indirect haemagglutination in the blood of cattle following tick infestation, but the concentration did not correlate with the degree

of immunity in the animals. Bowessidjaou et al. (1977) showed high titres of antibody to I. ricinus salivary gland antigen in rabbits following a second infestation with the ticks; the titres did not increase thereafter although the animals themselves became progressively more immune.

Production of antibody as a result of tick infestation by other species has been widely reported without any evidence that this was the cause for the development of resistance. Fujisaki (1978) found that rabbits developed precipitating antibody (identified as IgG 7S) as a result of infestation with Haemaphysalis longicornis Neumann, although there was no definite correlation between degree of resistance and antibody. Precipitating and complement fixing antibodies developed in rabbits exposed to Hyalomma anatolicum excavatum Koch and Rhipicephalus sanguineus (Latreille) (Kohler et al. 1967; Weiland and Emokpare 1968).

Delayed hypersensitivity reaction in response to tick bite was studied by Wikel et al. (1978) who found that an extract from partially fed D. andersoni females injected intradermally into resistant guinea pigs produced indurated and necrotic lesions by 48 h; a slight immediate reaction after 30 min. was non-specific. Further evidence of delayed hypersensitivity was obtained by specific lymphocyte blastogenesis to the same antigen in cells from guinea pigs 2 - 4 days after the end of a primary tick infestation. Whether T or B lymphocytes were being stimulated was not shown.

Bagnall (1975) found that lesions developed at the site of attachment of I. holocyclus larvae on resistant guinea pigs. By 48 h after attachment the erythematous lesions were infiltrated with basophils, lymphocytes and neutrophils and by 96 h the basophils and eosinophils were found in equal numbers. The lesions were strikingly different in non-immune hosts and basophils were not seen in any significant numbers

before about 96 h. Intradermal injection of larval extracts produced a local reaction with specific accumulation of eosiniphils and basophils in immune animals. A similar accumulation of basophils and eosinophils was reported by Allen (1973) also in feeding lesions of D. andersoni on guinea pigs and appeared 1 day after infestation in resistant animals.

In these experiments on delayed hypersensitivity reactions, basophil accumulation coincided with peak sensitivity of lymphocytes to salivary gland antigen, suggesting a relationship between the two. The transfer of immunity by lymph node cells from resistant animals (Wikel and Allen 1976a) suggests that T cells may be involved. However it is not certain whether the striking basophil accumulation is responsible for the immunity. Askenase and Worms (1979) reported that immunity and basophil accumulation could both be transferred to naive animals with either serum or peritoneal exudate from resistant animals.

Apart from delayed hypersensitivity, immediate hypersensitivity may be associated with host resistance. Reik (1956, 1962) noted that resistant cattle exposed to B. microplus larvae were intensely irrirated. Injection of histamine beneath attached ticks will make them detach (Kemp 1978) and Kemp and Bourne (1980) provided circumstantial evidence to suggest that earlier detachment of B. microplus larvae from highly resistant cattle was due to histamine release at the attachment site. Tatchell and Bennett (1969) increased the yield of B. microplus from resistant cattle by injecting them with an antihistaminic tranquiliser, promethazine. Intradermal injection of tick eggs or larvae in to cattle, gave immediate oedematous dermal lesions (Reik 1962), but no causal relationship was shown between such reactions and resistance and it appeared doubtful if the immediate hypersensitivity and oedematous lesions were responsible for immunity, particularly in view of the suggestion that oedema consequent to an allergenic reaction and the

resulting increase in blood flow was actually advantageous to the feeding ticks (Tatchell and Moorhouse 1968). Willadsen et al. (1978) have shown that the immediate hypersensitivity in cattle to purified allergens from B. microplus larvae was correlated with levels of immunity in the animals and suggested that it was at least partly responsible for resistance of cattle to this tick species. A major expression of resistance in this tick-host system is rejection of larvae in the first 24 h, when they make repeated attachments on highly resistant cattle. Restriction of grooming actually raises the yield of ticks, suggesting a response which is irritating to the host. Thus immediate hypersensitivity and the resulting grooming activity may well be an expression of resistance and it has been shown that the greater the grooming activity the greater the immunity. It has been established that grooming by resistant cattle can remove up to 50% of B. microplus larvae (Koudstaal et al. 1978). Wikel and Allen (1978) however showed that guinea pigs expressed resistance to D. andersoni even when prevented from grooming. Apart from immediate hypersensitivity, there are other immunological responses in cattle resistant to B. microplus which reduce significantly the weight of engorged females. Involvement of immediate hypersensitivity in host resistance has been suggested also for D. andersoni (Allen 1973). Boese (1974) found that rabbits acquired a persistent immunity to Haemaphysalis leporispalustris (Packard) and high levels of resistance were associated with a non-precipitating, skin-sensitising antibody as shown by high passive cutaneous anaphylaxis titres.

The way in which host immunity is expressed varies with the host and tick species. It may range from simple rejection of the ticks with little or no apparent damage, to death in situ, lower numbers feeding and detaching, prolongation of feeding time, reduction of

engorged weight and in the case of female ticks, in addition, inhibition of egg laying and decreased viability of eggs. An altered sex ratio of adults has also been reported (Musatov 1967), Hyalomma spp. nymphs feeding on resistant rabbits producing more males, while those from control animals tended to produce the same or more females; death in situ was also noted by the author. Death in situ of attached male A. maculatum on rabbits immunised with whole male tick homogenates of A. maculatum was reported by McGowan et al.(1980). Bagnall (1978) also found that larvae of I. holocyclus feeding on immune guinea pigs died in situ between 72 - 96 h.

The first type, i.e., rejection of larvae in the first 24 h has been most frequently reported in cattle and B. microplus (Roberts 1968) but lower weight of engorged females, prolongation of feeding time and reduction of egg output and egg viability have also been reported (Wagland 1978). With the B. microplus-cattle combination death or damage to feeding ticks is minimal, but Loomis (1971) observed that B. microplus feeding on immune rabbits died in situ. Death of feeding ticks in situ, as well as reduction of numbers of engorged females and fall in engorged weight were also reported by Strother et al. (1974) for A. americanum feeding on immune cattle and for I. holocyclus by Doube and Kemp (1975). Repeated infestation of rabbits with I. ricinus resulted in decrease in the percentage of ticks engorging, engorgement weight, percentage of females laying eggs, viability of eggs and in prolongation of feeding time (Bowessidjaou et al. 1977), and the immunity persisted for at least 9 months; although the females engorging on immune rabbits took less blood, this by itself could not account for reduced egg output and a toxic effect on the ticks may have been responsible. Lower percentage of ticks engorging and reduced engorgement weight have been observed for R. appendiculatus Neumann (Branagan 1974),

R. sanguineus (Kohler et al. 1967; Garin and Gabarev 1972), D. variabilis (Trager 1939a), D. andersoni (Allen 1973) and Hy. anatolicum excavatum (Kohler et al.1967). In addition to reduced repletion and prolonged feeding time, prolonged premoult period and increased mortality during premoult were reported by Brown (1977) for nymphs of D. variabilis feeding on resistant guinea pigs. Engorged weight, numbers feeding and feeding rates were reduced when A. testudinis (Conil) were fed on immune amphibian hosts (Schneider et al. 1971). Fujisaki (1978) on the other hand found that when Hae. longicornis were fed on immune rabbits, numbers engorging, feeding rate and egg viability were unaltered although the engorged weight of female ticks was reduced. Sutherst et al. (1979) showed that numbers of Hae. longicornis feeding on resistant cattle were much less than on naive cattle.

Most animals investigated developed immunity to tick bites but Chabaud (1950) reported that domestic rabbits did not develop resistance to Hy. excavatum or Hy. dromedarii Koch. Dogs do not become immune to R. sanguineus (Theis and Budwiser 1974); neither do rabbits and sheep to A. hebraeum Koch larvae and nymphs, although seasonal fluctuations in engorged weights occurred (Norval 1978). Randolph (1979) showed that laboratory mice developed resistance to larvae and nymphs of I. trianguliceps Birula, but field mice (Apodemus sylvaticus), the natural host of the ticks did not. She suggested that this and other similar observations were probably due to the long established association between the tick and its natural host. Bagnall (1978) found that rats did not develop cutaneous immunity to I. holocyclus and suggested that they may be used as the hosts of choice for laboratory rearing. Berdyev and Khudainazarova (1976) reported that lambs exposed to Hy. anatolicum anatolicum under conditions closely resembling those in nature (i.e., long and continuous parasitism by the ticks),

although developing resistance to a primary infestation, became desensitised following repeated feeding by ticks, with similar feeding times and engorgement weights as those from control animals. However Newson (1979) investigating the effect of stocking density of cattle (the natural hosts) on populations of R. appendiculatus found considerable reductions in the tick population when feeding was limited to a single host in the experimental plot. Under natural conditions with many hosts with differing levels of resistance including young animals, population levels would be maintained.

The considerable work done on the histology of tick attachment sites has been reviewed by Balashov (1972) and will not be repeated in this review. More recent investigations on this subject are by Theis and Budwiser (1974), Wikel and Allen (1977), Allen et al. (1977, 1979) and Schleger et al. (1976). Comparative histology of immune and non- immune hosts has not been investigated in most cases.

Various attempts have been made to exploit the development of resistance in animals by immunising them artificially by injection of tick-derived antigens. Trager (1939a) and Gregson (1941) obtained partial immunity in guinea pigs by the injection of D. variabilis and D. andersoni extracts respectively. Schneider et al. (1971) found that injection of homogenates of unfed nymphs of A. testudinis into tortoises prevented feeding by the ticks. Salivary gland extracts have been commonly used as antigens for inducing resistance in animals. Injection of salivary gland extract from Hy. anatolicum excavatum into a rabbit prevented ticks from feeding (Kohler et al. 1967) and Garin and Gabarev (1972) successfully immunised rabbits and guinea pigs against R. sanguineus by injection of salivary glands. Injection of calves with salivary glands from partially engorged females of B. microplus gave a lower yield of engorged ticks than did controls

(Brossard 1976). Bagnall (1975) in a comprehensive series of experiments consistently produced immunity in guinea pigs by the injection of I. holocyclus larval extracts with or without adjuvants although the immunity was less than naturally acquired immunity. Partial immunity was also obtained by Musatov (1967) against Rhipicephalus bursa Canestrini and Fanzago, in rabbits by the injection of salivary gland extracts, although here again it was less than naturally acquired immunity. Perhaps the most exciting and promising of the artificial immunisation experiments are those of Allen and Humphreys (1979). D. andersoni females fed on guinea pigs immunised with extracts of midgut and reproductive organs from partially fed female D. andersoni, produced significantly fewer eggs than those from controls and no larvae hatched from the eggs that were laid. Extracts from all internal organs produced more dramatic results and females feeding on guinea pigs injected with such extracts failed to engorge and produced no eggs. When adult D. andersoni were fed on calves immunised with extracts of midgut and reproductive organs, there was no significant difference in the number of ticks recovered from immunised and control calves, although females from the immunised animals had a lower engorged weight and produced fewer eggs and larvae. One of the interesting aspects of these experiments was that extracts from unfed ticks were ineffective.

More recently, McGowan et al. (1980) fed nymphs and adults of A. maculatum on rabbits immunised with whole tick homogenates of unfed male A. maculatum. The homogenate showed a range of 22 to 24 proteins and protein subunits with molecular weights from 6,000 to 340,000 daltons. When nymphs were fed on immunised rabbits and naive rabbits, there was no significant difference in mean engorged weights or % moulting. However adult females feeding on immunised animals

weighed significantly less and had lower egg mass weights than those feeding on controls. The authors suggest that the difference in feeding performance of nymphs and adults on immunised animals was probably due to the fact that the immunogen was made from adult ticks. When adults, which as nymphs had fed on immune rabbits, were fed on non-immune rabbits, their engorged weights were significantly lower than those of adults which had fed as nymphs on non-immune animals; the mean egg mass weights were also lower. The depression in mean engorged weights was even more marked when the adult feed was on immunised rabbits. On immunised hosts, females which as nymphs had fed on immunised rabbits, took significantly smaller blood meals than those which as nymphs had fed on naive rabbits; however there was no difference in the egg mass weight of females from the two groups. Adults developing from nymphs which had fed on immune rabbits, did not take significantly larger blood meals from non-immune rabbits than adults feeding for the first time on immune animals; the egg mass weights of females feeding on the immune rabbit were however significantly lower.

Two aspects of host immunity to ticks which have received little attention are the effect of infections for e.g., by blood pathogens, in the host animals on the development of resistance to ticks, and the occurrence of heterospecific or cross immunity in host animals when they are infested sequentially with two different species of ticks. Both are important since animals in nature in many tropical countries are constantly exposed to various pathogens and ticks, both of which provoke an immune response, and animals are often parasitised by more than one species of tick. The immunosuppressive effect of Babesia bovis infection on feeding of B. microplus on cattle was shown by Callow and Stewart (1978); they found that when cattle were infected

with Babesia bovis at about the time of first exposure to B. microplus, more ticks matured on the infected than on uninfected cattle. They suggested that this may have an important epidemiological aspect, since by reducing host resistance to ticks, Babesia bovis may be improving its own chances of survival.

Heterospecific infestation and immunity has been less studied. Musatov (1967) fed ticks of various species (Hy. plumbeum plumbeum = Hy. marginatum Koch), Hy. asiaticum asiaticum, R. bursa and R. sanguineus) in succession on rabbits and found that the ticks fed in the same way as control ticks suggesting the lack of heterospecific or cross immunity, but no details are given.

The two primary objectives of my investigations were to study the homospecific and heterospecific infestation of rabbits and guinea pigs with 4 different species of ixodid ticks and immunity in the animals, and to study the effect of blood pathogens on immunity in rabbits to tick infestation. Cattle in Africa are exposed to more than one species of tick and all three African species used in my studies are common cattle ectoparasites in Africa; A. hebraeum and A. variegatum have long mouth parts while R. appendiculatus has short mouth parts. A fourth species D. marginatus with short mouth parts although European in distribution was included in the studies to see if depth of penetration by the mouth parts had any effect on development of resistance. The pathogen I chose for study was Trypanosoma congolense, a common cattle parasite in Africa. Although Callow and Stewart (1978) used a tick-borne parasite, Babesia bovis in their immunosuppressive studies, it was of interest to see if a non tick-borne pathogen which was nevertheless a cattle parasite will have any effect on acquired immunity to ticks. Both these studies, although using laboratory animals, have some relevance to natural situations involving cattle, cattle parasites and cattle ticks.

Experiments were also done on artificial immunisation of rabbits and guinea pigs using tick-derived antigens including cells from a continuous line from R. appendiculatus.

The terms "immunity" and "resistance" have been used interchangeably by various authors to refer to the same phenomenon and in the review I have used both terms. Since the responses in the animals following tick infestation have an immunological basis, the term "immunity" is probably more meaningful and in the rest of the thesis I have used this term exclusively. When primary and secondary infestation of an animal are with the same species, I have used the term homospecific infestation; when primary and secondary infestation have been with different species, the term heterospecific infestation has been used; similarly the term homospecific immunity is used when primary infestation with one species produces immunity to a secondary infestation with the same species; heterospecific immunity or cross immunity (cross resistance of other authors) denotes immunity developed as a result of primary infestation with one species, against secondary infestation with a second species.

4.2 MATERIALS AND METHODS

4.2.1 Ticks

Four species of ticks, R. appendiculatus, A. hebraeum, A. variegatum and D. marginatus were used in the investigations on host immunity. Details on maintenance and feeding of all four are given in Section 3.2.1.

4.2.2 Experimental animals

Outbred female albino guinea pigs (Dunkin Hartley strain) and female New Zealand white rabbits obtained from commercial suppliers

were used for homospecific as well as heterospecific infestations. Weight of the guinea pigs ranged from 600 to 800 g and weight of the rabbits from 2 to 3 kg. To avoid any possible bias of weight of animals on feeding performance of ticks, every attempt was made to ensure that naive animals used as controls were approximately the same weight as experimental animals experiencing a secondary infestation. Details of preparation of animals for tick feeding are given in Section 3.2.1.2.

4.2.3 Homospecific infestation

With each species a total of nine combinations are possible when one of the three stages is used for the primary infestation and the same or a different stage is used for the secondary infestation. For example, when primary infestation is with larvae, the secondary infestation could be with larvae, nymphs or adults. In the majority of experiments on homospecific infestation, primary and secondary infestations were with nymphs; in a few, primary infestation was with larvae or adults. The interval between primary and secondary infestation ranged from 49 to 97 days for R. appendiculatus, 62 to 85 days for A. hebraeum and 63 to 90 days for A. variegatum; in a single experiment with D. marginatus the interval between primary and secondary infestation was 56 days. Since primary and secondary infestations were usually on the same site, i.e., the shaved dorsum of the animals, a minimum interval of 5 to 6 weeks was necessary to make sure that tick bite lesions had healed completely before the animals were exposed for a second time. The number of nymphs used for each infestation was usually 200 of R. appendiculatus and D. marginatus and 150 of A. hebraeum and A. variegatum. Numbers of larvae and adults used for each infestation was not strictly controlled.

4.2.4 Heterospecific infestation

In heterospecific infestations, 27 combinations of primary/ secondary infestations are possible, using larvae, nymphs or adults of one species for the primary, and larvae, nymphs or adults of another species for the secondary infestation. In most of the experiments primary and secondary infestations were with nymphs, the numbers used being similar to that in the homospecific infestation experiments. It was not possible to investigate all the different combinations during the time available; nevertheless experiments with R. appendiculatus and A. hebraeum primary infestation, and secondary infestation with the remaining three species were done. With O. marginatus primary infestations, secondary infestations with R. appendiculatus and A. variegatum only were investigated. Heterospecific infestation following a first infestation with A. variegatum was studied only in a single experiment when A. hebraeum was used for the secondary infestation. The interval between primary and secondary infestations varied from 40 to 82 days.

In homospecific and heterospecific infestations, each experimental animal was infested only twice. The effect of more than two sequential infestations was not investigated.

4.2.5 Experimental infection of animals with Trypanosoma congolense

4.2.5.1 Source and method of inoculation

Only rabbits were used in experiments with T. congolense strain "S 104 Fly". The strain was originally isolated in 1966 from the blood of a Grant's gazelle at Grumeti in North Tanzania and had been passed several times in rats and mice and also through tsetse flies before being frozen in liquid N_2. Cryobanked stabilates (mouse blood) of the

strain were inoculated intraperitoneally (IP) into two adult mice (T O strain). Six to seven days later when tail blood of the mice was strongly positive, blood was taken by cardiac puncture and 0.2 to 0.5 ml inoculated subcutaneously (SC) into the shaved right scapular area of rabbits. The estimated number of parasites in the inoculum ranged from 2.4×10^6 to 1.1×10^8.

4.2.5.2 Examination for parasitaemia

Tail blood from mice, and venous blood from the ear of rabbits obtained by pricking with a sterile disposable lancet, was examined as fresh wet smear preparations, under a magnification of x 400. Five, 10 or 20 fields were examined and the number of parasites per ml estimated according to the method of Herbert and Lumsden (1976). When no organisms were seen in 20 fields the preparation was scored as negative, although in reality it may have been positive, but the parasites could not be seen by the method employed. Ear blood from rabbits was examined every other day from the date of patent parasitaemia till the termination of the experiments.

4.2.6 Immunisation of animals with tick-derived antigens

Rabbits and guinea pigs were inoculated with extracts of cells from a continuous cell line from R. appendiculatus, or with homogenates of R. appendiculatus unfed larvae. Nymphs or adults of R. appendiculatus were fed on these animals and their feeding performance assessed to determine if immunity had developed as a result of inoculation with antigens.

4.2.6.1 Immunisation of rabbits and guinea pigs with cell extracts

The cells used in these experiments were from a continuous cell

line (RA-243) established from developing adults of R. appendiculatus (Varma et al. 1975). Cells were grown in 8 oz. glass bottles and when a confluent monolayer had formed (usually seven days after seeding), the medium was decanted and the cell sheet washed twice in 10 ml of phosphate buffered saline (PBS). The cells were then scraped into 5 ml of PBS, centrifuged (2500 rpm for 10 min.) and the packed cells from each bottle resuspended in 1 ml of sterile distilled water and stored in plastic screw cap ampoules at -70 C. Protein concentrations of the cell extracts or of the larval homogenates were not estimated.

For inoculation of animals, the ampoules of stored cells were taken out of -70 C, thawed and freeze-thawed 2x, using a mixture of dry ice (solid CO_2) and methanol for freezing. For each inoculation, the contents of one or two ampoules were mixed with an equal volume of Freund's Complete Adjuvant (FCA, Difco Batch 626970 or Gibco-Biocult Batch C983014) and inoculated SC or IP. Control animals received only FCA or were not inoculated at all. An additional one or two inoculations were given at weekly intervals and 8 to 12 days after the last inoculation the animals were exposed to ticks.

In one experiment two ampoules of stored cells were taken out of -70 C and after two cycles of freeze-thawing, the contents were pooled and concentrated to 0.5 ml by placing in a dialysis bag and covering with polyethylene glycol (mol. wt., 6000 from Sigma Chemical Company). 0.25 ml of the concentrate was mixed with an equal amount of FCA and inoculated into the ventral footpad of a guinea pig. Nine days later the remaining 0.25 ml (which had been stored at -70 C) was mixed with FCA and inoculated into the footpad of the same guinea pig. A second guinea pig received first and second inoculations of FCA only. Twenty-two days after the second inoculation, R. appendiculatus nymphs were released on both animals.

4.2.6.2 Immunisation of guinea pigs with whole tick homogenates

Approximately 3500 unfed larvae of R. appendiculatus were killed by chilling and homogenised using a mortar and pestle. Two ml of PBS was added, mixed well and the homogenate left undisturbed for about 30 min. for the particulate matter to settle down. The supernate was inoculated SC into a guinea pig. One week later larval homogenate (2 ml) from ca 3800 unfed larvae was lightly centrifuged (800 rpm for 5 min.) and the supernate inoculated SC into the same guinea pig. Nine days after the second inoculation, R. appendiculatus nymphs were released on the inoculated guinea pig and on an uninoculated guinea pig.

4.2.7 Serological tests

Rabbits were bled by cardiac puncture eight to 85 days after a primary infestation with ticks. The sera were separated by centrifugation (3000 rpm for 10 min.) and stored frozen at -20 C until tested.

The sera after heat inactivation (56 C for 30 min.) were tested for antibody to tick salivary gland antigen by the complement fixation test (CF) and by the indirect immunofluorescence test (IIFT) by Mr. Alex Chanas. Normal rabbit serum was included as a control in all the tests.

4.2.7.1 Complement fixation test

Salivary gland antigen used in the test was a crude lysate from homogenised salivary glands of R. appendiculatus and A. hebraeum. Forty pairs of salivary glands from 40 partially fed females of R. appendiculatus (five days after attachement on a rabbit) and 20 pairs of glands from 20 partially fed A. hebraeum (seven days after attachment on a rabbit) were dissected out and homogenised in 2 ml each of cold barbitone buffered saline (pH 7.2) in a glass tissue grinder. The

homogenates were dispensed in 0.5 ml amounts in screw cap plastic ampoules and stored at -70 C. Just before use, the antigens were thawed, freeze-thawed a further two times and sonicated.

The tests were performed according to the method of Bradstreet and Taylor (1962). Antigen was used at serial two-fold dilutions starting at 1/10 upto 1/1280 and test sera and normal rabbit serum at serial two-fold dilutions starting from 1/2 to 1/1024.

4.2.7.2 Immunofluorescence test

Salivary gland from a partially fed female of R. appendiculatus was dissected out, placed in a drop of embedding medium for frozen tissue specimens (Tissue - Tek O.C.T. compound from Ames Company, USA) on a cork base and fixed by quick immersion in liquid N_2. Frozen sections 6 - 8 μ thick were cut using a cryostat, positioned on glass slides and stored at -20 C till used. 1/50 dilution of the experimental sera and normal rabbit serum were layered on to the salivary gland sections and examined by the indirect immunofluorescence test using fluorescein isothiocyanate conjugated anti-rabbit sheep immunoglobulin (Burroughs Wellcome) at dilutions of 1/30 to 1/40. The sections were examined by epifluorescence using a Leitz fluorescent microscope.

4.2.8 Parameters used in assessing tick feeding performance

Most of the experimental infestations, primary and secondary, were with nymphs and three parameters were used in assessing feeding performance of ticks on animals. These were: recovery rate of fed nymphs when the number released was known, average feeding time (in days) and mean engorged weight (in mg). Average feeding times were calculated from the number of fed ticks dropping off every day. For calculating the mean engorged weight, all fed ticks or at least 75% of fed ticks

were weighed, using an Oertling R 52 analytical balance. The smaller nymphs of R. appendiculatus and D. marginatus were weighed in batches of 5 to provide more reliable figures and the larger nymphs of A. hebraeum and A. variegatum were weighed individually. Accordingly, weights recorded in the tests for R. appendiculatus and D. marginatus nymphs are for groups of five and for A. hebraeum and A. variegatum for individual nymphs. All weights are in mg. Fed adult females were weighed individually. Two additional parameters used in assessing feeding performance of adults of R. appendiculatus were mean weight of egg mass in mg and % conversion efficiency index (CE1, see Section 3.6.1.5) which is a measure of the ability of females to convert body weight to egg weight.

4.2.9 Statistical analysis of data

The Chi^2 test was used for comparing recovery rates of fed ticks from experimental and control infestations. The probability (P) value for Chi^2 was then read off from Fisher and Yates' statistical tables. The recovery rates were considered not significantly different when P was > 0.05.

The Student's t-test was employed for comparing the mean engorged weights of ticks and of egg masses, the average feeding times and the % conversion efficiency index. The value of P for the appropriate degrees of freedom was obtained from Fisher and Yates' tables. The results were considered not significantly different when P was > 0.05 and significantly different when P was 0.05 or less.

4.3 RESULTS

4.3.1 Recovery rate of fed ticks

Where the number of ticks released on animals was known (200 nymphs of R. appendiculatus and D. marginatus, 150 nymphs of A. hebraeum and A. variegatum and 25 females of R. appendiculatus), the recovery rate of fed ticks in the different experiments ranged from 70% to 99%. In 12 out of 16 homospecific and 9 out of 11 heterospecific infestation experiments, there was no significant difference ($P > 0.05$) in recovery rates between primary and secondary infestations. In the rest of this thesis therefore, no further mention of this particular parameter will be made.

4.3.2 Site of primary infestation and development of immunity to secondary infestation

Since in almost all the experiments, ticks in the primary and secondary infestations were fed on the backs of rabbits or guinea pigs, it was important to determine whether the immunity which developed was local and confined to the site of the primary infestation or whether it was general.

Data from two separate experiments clearly showed that immunity was not confined to the site of primary infestation. In the first experiment nymphs of R. appendiculatus were released on the back of a rabbit. The mean engorged weight was 59.3 ± 0.8 mg (range 56 to 63). 90 days later R. appendiculatus nymphs were released on the back as well as on the ears of the same rabbit and on the back and ears of a naive rabbit. The mean engorged weights of nymphs feeding on the ears (18.44 ± 0.97 mg, range 14 to 23) and on the back (22.44 ± 0.35 mg, range 17 to 31) of the immune rabbit were significantly lower ($P < 0.001$) than of those feeding on the ears (61.5 ± 1.1 mg, range 48 to 70) and

on the back (62.48 ± 1.3 mg, range 50 to 74) of the control rabbit; the mean weight of nymphs from the immune rabbit was only about $^1/_3$ that of the nymphs from the control rabbit.

In the second experiment, nymphs of R. appendiculatus were released on the back of two rabbits 68 days after adults of R. appendiculatus had fed on the ears. Nymphs released on the back of a naive rabbit served as controls. Ticks feeding on the immune rabbits took significantly smaller blood meals ($P < 0.001$) than those on the control animal. The mean engorged weight of nymphs from the immune rabbits (25.03 ± 1.22 mg and 24.33 ± 0.9 mg, range 17 to 40) was less than ½ that of nymphs from the control rabbit (54.55 ± 1.03 mg, range 46 to 64).

4.3.3 Age of ticks and feeding performance

In the homospecific infestation experiments using the same stage of ticks, feeding performance during the secondary infestation could be compared either with that during primary infestation of the same animal, or with feeding performance on a naive animal used as a control. If ticks from the same batch are used for both infestations, by the time of the secondary infestation, they will be much older, since the interval between the two feeds is often considerable. This introduces an age factor and it was important to find out if significant differences in feeding performance were due to acquired immunity of the animals or due to the older age of the ticks.

To determine if age of ticks did in fact affect feeding performance, nymphs of R. appendiculatus and A. variegatum were assessed when ticks of the same age (i.e., from the same batch) were fed simultaneously on rabbits, when ticks from the same batch but of different calendar ages were fed on rabbits and when ticks of the same calendar age but from different batches were fed on rabbits. For these base line data feeding performance has been assessed on mean engorged weight of the nymphs.

4.3.3.1 Engorged weights of R. appendiculatus nymphs from the same batch fed simultaneously on two rabbits

In each of five experiments two naive rabbits were exposed to nymphs from the same batch. In one experiment the age of the nymphs was unknown. The age of the nymphs in the remaining four varied from 29 to 128 days. Age of the nymphs, mean engorged weight ± SE, and values of t and P are given in Table 40. There was no significant difference ($P > 0.1$ to > 0.8) in the engorged weight of nymphs from the same batch fed simultaneously on two rabbits, the five different sets of weight being 51.34 ± 0.72 and 52.82 ± 0.77 mg; 51.02 ± 0.75 and 50.06 ± 0.88 mg; 50.14 ± 0.61 and 50.30 ± 0.62 mg; 47.28 ± 1.03 and 48.4 ± 1.25 mg and 50.40 ± 0.66 and 51.99 ± 0.88 mg. The range in weights was 36 to 65.2 mg in the five experiments.

4.3.3.2 Engorged weights of R. appendiculatus and A. variegatum nymphs from the same batch fed at different times on rabbits

The age of the R. appendiculatus nymphs from three different batches were 19 and 127 days, 49 and 92 days, and 69 and 97 days and of the A. variegatum nymphs 60 and 89 days. The results are presented in Table 41. With the three batches of R. appendiculatus, there was a significant difference (P 0.001 or less) in the engorged weight of younger and older nymphs, older nymphs weighing 8.7 to 9.4% less than younger nymphs. The reverse was the case with A. variegatum nymphs, where older nymphs (89 days) weighed 11.7% more than younger nymphs (60 days). A similar highly significant difference ($P < 0.001$) was also obtained between mean engorged weight (61.94 ± 1.13 mg) of 47-day old nymphs and 16-day old nymphs (47.13 ± 0.87 mg) of A. hebraeum.

4.3.3.3 Engorged weights of A. variegatum nymphs of the same calendar age but from different batches fed on rabbits

Unfed nymphs from different batches may not always be of the same

TABLE 40 - MEAN ENGORGED WEIGHTS ± SE OF R. APPENDICULATUS NYMPHS FROM THE SAME BATCH FED SIMULTANEOUSLY ON TWO RABBITS

Serial No.	Age of nymphs (days)	Rabbit number	Mean engorged weight ± SE (mg)	t	P
1	29	R 52 R 53	51.34 ± 0.72 52.82 ± 0.77	1.40	>0.1
2	42	R 56 R 57	51.02 ± 0.75 50.06 ± 0.88	0.83	0.4
3	93	R 36 R 37	50.14 ± 0.61 50.30 ± 0.62	0.17	>0.8
4	128	R 32 R 33	47.28 ± 1.03 48.40 ± 1.25	0.69	0.5
5	Unknown	R 60 R 61	50.40 ± 0.66 51.59 ± 0.88	1.08	>0.2

TABLE 41 - MEAN ENGORGED WEIGHTS ± SE OF R. APPENDICULATUS AND A. VARIEGATUM NYMPHS FROM THE SAME BATCH FED AT DIFFERENT TIMES ON RABBITS

Serial No./Sp.	Age of nymphs (days)	Rabbit number	Mean engorged weight ± SE (mg)	t	P
1/ R.a.	19	R 10	53.00 ± 1.07	3.49	0.001
	127	R 33	48.40 ± 1.25		
2/ R.a.	49	R 47	55.29 ± 0.92	4.62	<0.001
	92	R 37	50.30 ± 0.62		
3/ R.a.	69	R 54	51.53 ± 0.88	4.01	<0.001
	97	R 68	46.68 ± 0.82		
4/ A.v.	60	R 62	47.29 ± 1.03	4.11	<0.001
	89	R 63	53.54 ± 1.12		

TABLE 42 - MEAN ENGORGED WEIGHTS ± SE OF A. VARIEGATUM NYMPHS OF SAME CALENDAR AGE BUT FROM DIFFERENT BATCHES FED ON RABBITS

Age of nymphs (days)	Rabbit number	Mean engorged weight ± SE (mg)	t	P
86	R 63	53.54 ± 1.12	3.58	<0.001
83	R 78	48.18 ± 1.0		

size and this may have a bearing on engorged weight. When nymphs of similar age (86 and 89 days) were fed on two rabbits, the mean engorged weights, 48.18 ± 1.0 mg and 53.54 ± 1.12 mg were significantly ($P < 0.001$) different (Table 42).

In view of these results it was considered that a more valid comparison of feeding performance in the homospecific infestation experiments would be of ticks from the same batch and of the same age released simultaneously on a previously exposed animal (secondary infestation) and on a naive animal without previous exposure to ticks (primary infestation).

4.3.4 Homospecific infestation of animals

4.3.4.1 Homospecific infestation with R. appendiculatus

The feeding performance of R. appendiculatus during the secondary infestation of rabbits and guinea pigs previously exposed to a primary infestation was significantly reduced. This was equally true when primary infestation was with larvae and secondary infestation with nymphs, when primary and secondary infestations were with nymphs, when primary infestation was with adults and secondary infestation with nymphs and when both infestations were with adults.

4.3.4.1.1 Primary infestation with larvae and secondary infestation with nymphs (Table 43)

In each of 2 experiments two rabbits were exposed to 2,000 to 3,000 larvae. Seventy and 97 days later R. appendiculatus nymphs were released on both rabbits. At the same time two naive rabbits (R 37 and R 75) were also exposed to a primary infestation with nymphs. The mean engorged weights and average feeding times of nymphs during the secondary infestation were compared to mean engorged weights and average feeding times of nymphs on the control rabbits (Table 43). The reduction in

TABLE 43 - RESULTS OF HOMOSPECIFIC INFESTATION OF RABBITS WITH LARVAE (PRIMARY INFESTATION) AND NYMPHS (SECONDARY INFESTATION) OF R. APPENDICULATUS. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Rabbit-number	Infes-tation	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
25	R 35	2^{o}	25.02 ± 0.49	30.69	<0.001	5.97 ± 0.05	14.58	<0.001
	R 37	1^{o}	50.30 ± 0.62			5.05 ± 0.02		
42	R 73	2^{o}	28.39 ± 0.94	22.26	<0.001	6.64 ± 0.08	14.79	<0.001
	R 75	1^{o}	56.57 ± 0.84			5.21 ± 0.04		

weights and increase in feeding times during the secondary infestation were significantly different ($P<0.001$) in both cases. Mean engorged weight of nymphs during secondary infestation (25.02 ± 0.49 mg and 28.39 ± 0.34 mg, range 17.0 to 51.4) was about half that of nymphs during a primary infestation on the control rabbits (50.30 ± 0.62 mg and 56.57 ± 0.84 mg, range 40.9 to 65).

In a single experiment, a guinea pig was exposed to ca 4,000 larvae. Fifty-five days later nymphs were released on the same guinea pig as well as on a naive guinea pig. As in the case of rabbits, mean engorged weight during the secondary infestation (28.65 ± 0.99 mg, range 13 to 41) was significantly less ($P<0.001$) than that during the primary infestation on a control guinea pig (49.31 ± 0.7 mg, range 38 to 64). The increase in average feeding time in days (7.23 ± 0.09) during the secondary infestation was also significantly longer ($P<0.001$) than during the primary infestation (6.83 ± 0.06).

4.3.4.1.2 Primary and secondary infestations with nymphs of *R. appendiculatus* (Tables 44 and 45)

In each of four experiments a rabbit was exposed to infestation with *R. appendiculatus* nymphs. Forty to 83 days later *R. appendiculatus* nymphs were released on the same rabbits; nymphs released on four naive rabbits (R 43, R 47, R54 and R 69) at the same time served as controls. The mean engorged weights during the secondary infestation of the rabbits ranged from 22.67 ± 0.72 to 31.43 ± 1.11 mg. The mean engorged weights of nymphs during primary infestation of the same rabbits (46.68 ± 0.82 to 51.34 ± 0.72 mg) and during the primary infestation of the naive rabbits (47.88 ± 0.80 to 55.29 ± 0.92 mg) were significantly higher ($P<0.001$). Reduced feeding performance of the nymphs during the secondary infestation was also manifested in increase in the average feeding times. These ranged from 5.14 ± 0.04 to 6.54 ± 0.07 days during

TABLE 44 - RESULTS OF HOMOSPECIFIC INFESTATION OF RABBITS WITH NYMPHS (PRIMARY AND SECONDARY INFESTATIONS) OF R. APPENDICULATUS. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Rabbit number	Infestation	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
27	R 42	1°	48.88 ± 0.64	13.10	<0.001	5.68 ± 0.06	5.41	<0.001
		2°	31.43 ± 1.11	12.12	<0.001	6.37 ± 0.11	8.31	<0.001
	R 43	1°	49.71 ± 0.88			5.48 ± 0.04		
31	R 33	1°	48.40 ± 1.25	11.59	<0.001	5.14 ± 0.04	11.02	<0.001
		2°	28.93 ± 1.03	18.45	<0.001	6.88 ± 0.15	9.87	<0.001
	R 47	1°	55.29 ± 0.92			6.54 ± 0.07		
34	R 52	1°	51.34 ± 0.72	18.65	<0.001	5.31 ± 0.06	11.36	<0.001
		2°	31.04 ± 0.82	16.97	<0.001	6.61 ± 0.09	12.23	<0.001
	R 54	1°	51.53 ± 0.88			5.21 ± 0.06		
38	R 68	1°	46.68 ± 0.82	21.41	<0.001	7.49 ± 0.07	7.15	<0.001
		2°	22.67 ± 0.72	22.77	<0.001	6.52 ± 0.12	9.89	<0.001
	R 69	1°	47.88 ± 0.80			5.14 ± 0.07		

TABLE 45 - RESULTS OF HOMOSPECIFIC INFESTATION OF GUINEA PIGS WITH NYMPHS (PRIMARY AND SECONDARY INFESTATIONS) OF R. APPENDICULATUS. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Guinea pig	Infestation	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
9	GP	2^0	21.29 ± 0.71	18.27	<0.001	8.79 ± 0.14	13.92	<0.001
		1^0	43.6 ± 1.02			6.73 ± 0.06		
21	GP	2^0	26.2 ± 1.48	8.69	<0.001	7.27 ± 0.13	4.57	<0.001
		1^0	42.68 ± 1.20			6.5 ± 0.1		

primary infestations to 6.37 ± 0.11 to 6.88 ± 0.15 during the secondary infestation. The differences were highly significant ($P < 0.001$) between primary and secondary infestation of the same rabbit, (in the case of R 68 the average feeding time was longer during the primary infestation) and between secondary infestation of the experimental rabbits and primary infestation of the naive rabbits.

In two separate experiments two guinea pigs were exposed to R. appendiculatus nymphs 35 and 62 days after the primary infestation. Feeding performance during the primary infestation was not noted and two naive guinea pigs exposed to nymphs at the same time as the secondary infestation served as primary infestation controls. In both experiments the reduction in mean engorged weights (21.29 ± 0.71 and 26.2 ± 1.48 mg) and the increase in average feeding times (7.27 ± 0.13 and 8.79 ± 0.14 days) during the secondary infestation were significantly different ($P < 0.001$) from those during a primary infestation (Table 45).

4.3.4.1.3 Primary infestation with adults and secondary infestation with nymphs of R. appendiculatus

Sixty days after a primary infestation with 30 females and 40 males on the ears of a rabbit, nymphs were released on the back. A naive rabbit was not included as control; however, the mean engorged weight of nymphs during the secondary infestation (24.82 ± 1.61 mg) was well within the range usually obtained during a secondary infestation (see previous Sections).

In a second experiment nymphs were released on the back of a rabbit 68 days after a primary infestation of the ears with 25 females and 40 males. Nymphs were released on the back of a naive control rabbit. The reduction in mean engorged weight (24.33 ± 0.99 mg) and increase in average feeding time (7.8 ± 1.81 days) during the secondary

infestation were significantly different ($P < 0.001$) from the mean engorged weight (54.55 ± 1.03 mg) and average feeding time (5.28 ± 0.04 days) during the primary infestation.

4.3.4.1.4 Primary and secondary infestations with adults of R. appendiculatus (Table 46)

In the first of 2 experiments, 25 females and 30 males were released on the ears of a rabbit (R 39). Sixty-four days later 25 females and 30 males were released on the ears of the same rabbit. The mean engorged weight of females during the secondary infestation (110.52 ± 17.92 mg) was significantly ($P < 0.001$) less than that during the primary infestation (289.2 ± 31.15 mg). Curiously enough the average feeding time during the primary infestation (9.45 ± 0.49 days) was significantly ($P < 0.001$) longer than during the secondary infestation (7.2 ± 0.21 days).

In the second experiment 25 females and 30 males were released on the ears of a rabbit (R 6) 68 days after primary infestation with 25 females and 30 males. The same number of adults were released on the ears of a control rabbit (R 13). Mean engorged weight of females during the secondary infestation (99.63 ± 14.91 mg) was significantly lower ($P < 0.001$) than the weights during primary infestations of the same rabbit (299.48 ± 22.73 mg) and of the control rabbit (330.21 ± 22.20 mg). There was no significant difference ($P > 0.2$) in the average feeding times. The mean weight of egg mass laid by females after the secondary infestation (41.54 ± 7.67 mg) was significantly lower than that laid by females after a primary infestation (116.79 ± 15.14 mg and 160.35 ± 15.78 mg). A significant reduction was also found in the ability of females to convert body weight to egg mass after feeding on an immune rabbit. Percent conversion efficiency

TABLE 46 - RESULTS OF HOMOSPECIFIC INFESTATION OF RABBITS WITH ADULTS (PRIMARY AND SECONDARY INFESTATIONS) OF *R. APPENDICULATUS*. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES OF FEMALES, MEAN EGG MASS WEIGHTS AND MEAN CONVERSION EFFICIENCY INDEX

Serial number	Rabbit number	Infestation	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P	Mean weight of egg mass ± SE (mg)	t	P	Mean conversion efficiency index (%)	t	P
1	R 39	1°	289.2 ± 31.15	5.21	<0.001	9.45 ± 0.49	4.47	<0.001	-			-		
		2°	110.52 ± 17.92			7.20 ± 0.21			-			-		
2	R 6	1°	299.48 ± 22.73	7.14	<0.001	8.28 ± 0.35	1.24	>0.2	116.79 ± 15.14	3.92	<0.001	42.03 ± 2.53	3.34	<0.01
		2°	99.63 ± 14.91			8.86 ± 0.29			41.54 ± 7.67			32.65 ± 1.82		
	R 13	1°	330.21 ± 22.20	3.83	<0.001	9.55 ± 0.55	1.13	>0.2	160.35 ± 15.76	6.77	<0.001	45.63 ± 2.63	4.06	<0.001

index (CEI) was lower with females of a secondary infestation (32.65 ± 1.82 %) than with females of a primary infestation (42.09 ± 2.53 % and 45.63 ± 2.63%).

4.3.4.2 Homospecific infestation with D. marginatus

In a single experiment, nymphs of D. marginatus were released on a guinea pig 56 days after primary infestation with 10 females and 15 males. At the same time, nymphs of D. marginatus were released on a control guinea pig. The mean engorged weight of nymphs during the secondary infestation (56.24 ± 1.99 mg, range 35 to 75) was significantly lower ($P < 0.001$) than the mean engorged weight during the primary infestation (68.71 ± 1.79 mg, range 50 to 53). The average feeding time during the secondary infestation (5.34 ± 0.05 days) although slightly longer than that during the primary infestation (5.31 ± 0.03 days), was not significantly different from it.

4.3.4.3 Homospecific infestation with A. hebraeum (Table 47)

Eighty-five days after primary infestation with nymphs a rabbit (R 58) was exposed to a secondary infestation with nymphs. Nymphs were also released at the same time on a naive rabbit (R 64). The mean engorged weight of nymphs during the secondary infestation (37.96 ± 0.88 mg, range 16.5 to 61.7) was significantly lower ($P < 0.001$) than those during primary infestations (61.94 ± 1.13 mg and 47.12 ± 0.87 mg, range 30.8 to 78.0). The difference in weights during the two primary infestations are most probably due to the different batches of ticks used. The average feeding time during the secondary infestation (7.49 ± 1.26 days) was significantly longer ($P < 0.001$) than during the primary infestations (6.78 ± 0.07 days and 6.54 ± 0.56 days).

A poorer feeding performance was also observed when nymphs were released on a guinea pig 62 days after a primary infestation with nymphs. The mean engorged weight during the secondary infestation (30.11 ± 2.24 mg) was significantly lower ($P < 0.001$) than that of nymphs feeding on a naive control guinea pig (55.48 ± 2.04 mg). The average feeding time in this experiment was however significantly longer during the primary infestation (8.55 ± 0.11 days) than during the secondary infestation (7.75 ± 0.09 days).

4.3.4.4. Homospecific infestation with A. variegatum (Table 48)

Nymphs were released on a naive rabbit (R 79) at the same time as on a rabbit which had been exposed 63 days previously to a primary infestation with A. variegatum nymphs (R 78). Mean engorged weight of nymphs from the secondary infestation (35.59 ± 1.13 mg, range 9.8 to 81.0) was significantly lower ($P < 0.001$) than the weight obtained with nymphs during primary infestation of the same rabbit (48.18 ± 1.0 mg, range 21.7 to 79.0) and the naive rabbit (48.80 ± 1.36 mg, range 10.6 to 77.4). The average feeding time during the secondary infestation (9.45 ± 0.19 days) was also significantly longer than during the primary infestations (7.63 ± 0.08 and 7.29 ± 0.11 days).

In a second experiment nymphs were released on a guinea pig 90 days following a larval infestation. A second guinea pig was used as a control host for nymphs. Primary infestation of the guinea pig produced nymphs with a mean engorged weight of 57.11 ± 2.11 mg which was significantly higher than that of nymphs from the secondary infestation (28.65 ± 0.99 mg). There was no significant difference in the average feeding times in this experiment.

Thus in experiments with all four species, primary infestation with one species conferred immunity to secondary infestation with the

TABLE 47 - RESULTS OF HOMOSPECIFIC INFESTATION OF RABBITS WITH NYMPHS (PRIMARY AND SECONDARY INFESTATIONS) OF A. HEBRAEUM. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Rabbit number	Infes-tation	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
48	R 58	1°	61.94 ± 1.13	17.00	<0.001	6.78 ± 0.07	5.83	<0.001
		2°	37.96 ± 0.88	7.38	<0.001	7.49 ± 1.26	8.30	<0.001
	R 64	1°	47.12 ± 0.87			6.54 ± 0.56		

TABLE 48 - RESULTS OF HOMOSPECIFIC INFESTATION OF RABBITS WITH NYMPHS (PRIMARY AND SECONDARY INFESTATIONS) OF A. VARIEGATUM. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Rabbit number	Infes-tation	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
50	R 78	1°	48.18 ± 1.0	8.34	<0.001	7.63 ± 0.08	9.24	<0.001
		2°	35.59 ± 1.13	7.68		9.45 ± 0.19	9.38	
	R 79	1°	48.80 ± 1.36			7.29 ± 0.11		

same species (homospecific immunity). This immunity was not total because ticks were still able to feed on the animals although the feeding performance was impaired. Rabbits as well as guinea pigs developed immunity and there did not appear to be any difference in the immunogenic potential of larvae, nymphs or adults, although more experiments are needed to confirm this. Fed nymphs from primary and secondary infestations moulted normally and the lower blood meals did not appear to have any effect on duration of the premoult period or sex ratio of the adults.

4.3.5 Heterospecific infestation of animals

4.3.5.1 Primary infestation with R. appendiculatus, secondary infestation with D. marginatus, A. hebraeum and A. variegatum (Table 49)

In six experiments rabbits or guinea pigs were exposed to a secondary infestation with nymphs of D. marginatus, A. hebraeum and A. variegatum, 43 to 82 days following primary infestation with R. appendiculatus nymphs. Nymphs were also released on naive animals at the same time to provide control primary infestation data. In all cases except one primary infestation with one species did not produce cross immunity to secondary infestation with another species. The single exception was Rabbit R 69 in Expt. 51 which developed a heterospecific immunity to D. marginatus following primary infestation with R. appendiculatus nymphs. The mean engorged weight of D. marginatus nymphs from this rabbit was 58.09 ± 1.39 mg which was significantly lower ($P < 0.001$) than that of D. marginatus nymphs from a primary infestation (68.2 ± 1.69 mg). The average feeding time however was significantly longer ($P < 0.001$) on the immune rabbit (5.85 ± 0.06 days) than on the naive rabbit (5.34 ± 0.05 days). In a similar experiment

(Expt. 18) using guinea pigs the mean engorged weights (67.04 ± 1.58 and 69.71 ± 1.79 mg) of D. marginatus nymphs feeding on immune and naive animals were not significantly different ($P > 0.4$); neither was there a significant difference in the average feeding times.

During secondary infestation of rabbits and guinea pigs with A. hebraeum nymphs (Expts. 35 and 9) the ticks took either similar, 55.24 ± 1.93 mg, (not significantly different) or a larger, 66.90 ± 1.16 mg (significantly different) blood meal than those during a primary infestation (55.48 ± 2.04 mg and 61.94 ± 1.13 mg), again demonstrating the lack of cross immunity between R. appendiculatus and A. hebraeum. The average feeding times (6.70 ± 0.08 days and 6.78 ± 0.07 days) were not significantly different in the rabbit experiment (Expt. 35), while in the guinea pig experiment (Expt. 9) the average feeding time of A. hebraeum nymphs on previously exposed rabbit (7.45 ± 0.09 days) was in fact significantly lower than that of A. hebraeum nymphs during the primary infestation (8.55 ± 0.1 days).

Lack of cross immunity between R. appendiculatus and A. variegatum also was demonstrated in two experiments with rabbits, in which the mean engorged weight of A. variegatum nymphs from primary infestations (52.28 ± 1.28 mg and 47.29 ± 1.03 mg) did not differ significantly from those from a secondary infestation (53.87 ± 1.11 mg and 49.49 ± 0.97 mg) on previously exposed rabbits. The average feeding times of nymphs during the secondary infestation (7.91 ± 0.06 days and 6.68 ± 0.06 days) were significantly different, shorter in one experiment and longer in the other, from those during the primary infestation (7.36 ± 0.09 days and 7.10 ± 0.06 days).

4.3.5.2 Primary infestation with D. marginatus, secondary infestation with R. appendiculatus and A. variegatum (Table 50)

In one of two experiments, a guinea pig was exposed to a primary

TABLE 49 - RESULTS OF HETEROSPECIFIC INFESTATION OF ANIMALS WITH THREE SPECIES FOLLOWING PRIMARY INFESTATION WITH R. APPENDICULATUS NYMPHS. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Animal	Infestation/ Tick Sp.	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
51	R 69	2°/D.m.	58.09 ± 1.39	3.68	<0.001	5.85 ± 0.06	6.32	<0.001
	R 82	1°/D.m.	66.2 ± 1.69			5.34 ± 0.05		
18	GP	2°/D.m.	67.04 ± 1.58	0.69	>0.4	5.37 ± 0.04	1.05	>0.2
	GP	1°/D.m.	68.71 ± 1.79			5.31 ± 0.03		
35	R 57	2°/A.h.	66.90 ± 1.16	2.99	<0.01	6.70 ± 0.08	0.68	0.5
	R 58	1°/A.h.	61.94 ± 1.13			6.78 ± 0.07		
9	GP	2°/A.h.	55.24 ± 1.93	0.08	>0.9	7.45 ± 0.09	7.24	<0.001
	GP	1°/A.h.	55.48 ± 2.04			8.55 ± 0.10		
30	R 31	2°/A.v.	53.87 ± 1.11	0.94	>0.3	7.91 ± 0.06	5.12	<0.001
	R 46	1°/A.v.	52.28 ± 1.28			7.36 ± 0.09		
39	R 61	2°/A.v.	49.49 ± 0.97	1.55	>0.1	6.68 ± 0.06	5.99	<0.001
	R 62	1°/A.v.	47.29 ± 1.03			7.10 ± 0.06		

infestation with larvae of D. marginatus. Forty days later R. appendiculatus nymphs were released on the guinea pig as well as on a control. In the second experiment nymphs of A. variegatum were released on a rabbit (R 51) 69 days following primary infestation with D. marginatus; at the same time a control rabbit (R 63) was also exposed to A. variegatum nymphs. Judging from the mean engorged weights, the primary infestation with D. marginatus gave partial protection against secondary infestation with R. appendiculatus and A. variegatum. The mean engorged weight of R. appendiculatus nymphs on the previously exposed guinea pig was significantly lower (38.71 ± 1.16 mg) than that from the naive guinea pig (42.68 ± 1.19 mg). A similar significant difference was found with A. variegatum nymphs on rabbits also, nymphs from a secondary infestation (49.09 ± 1.02 mg) weighing significantly less than those from a primary infestation (53.54 ± 1.12 mg). There was no significant difference in the average feeding times on the two guinea pigs (6.46 ± 0.07 days and 6.50 ± 0.1 days); the average feeding time of A. variegatum nymphs was significantly longer on the naive rabbit (7.34 ± 0.07 days) than on the previously exposed rabbit (7.08 ± 0.08 days).

There was some evidence of reciprocal cross immunity between R. appendiculatus and D. marginatus. Primary infestation of a rabbit with R. appendiculatus gave some immunity against secondary infestation with D. marginatus; in a second experiment with a guinea pig however there was no evidence of cross immunity. Primary infestation of a guinea pig with D. marginatus protected it to some degree against a subsequent feed by R. appendiculatus. But judging by the reduction in mean engorged weights, the protection seen in heterospecific infestations with the two species was of a much lower order than that obtained with homospecific infestations. Thus with R. appendiculatus

TABLE 50 - RESULTS OF HETEROSPECIFIC INFESTATION OF ANIMALS WITH R. APPENDICULATUS AND A. VARIEGATUM NYMPHS FOLLOWING PRIMARY INFESTATION WITH D. MARGINATUS. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Animal	Infestation/ Tick Sp.	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
21	GP	2♀/R.a.	38.71 ± 1.16	2.25	<0.05	6.46 ± 0.07	0.31	>0.7
	GP	1♀/R.a.	42.68 ± 1.19			6.50 ± 0.1		
43	R 51	2♀/A.v.	49.09 ± 1.02	2.95	<0.01	7.08 ± 0.08	2.38	<0.02
	R 63	1♀/A.v.	53.54 ± 1.12			7.34 ± 0.07		

nymphal engorged weights during a homospecific secondary infestation were reduced by 36% to over 50% while with a primary D. marginatus infestation, the reduction in engorged weight of R. appendiculatus during the secondary infestation was only about 10%. Similarly, the reduction in engorged weight of D. marginatus nymphs during a secondary homospecific infestation was about 20% and the reduction following a primary infestation with R. appendiculatus nymphs only about 12%. Although primary infestation with D. marginatus gave some protection against secondary infestation with A. variegatum (reduction in mean engorged weight 8%), it was much less than when the primary and secondary infestations were with A. variegatum (reduction in mean engorged weight 38%). More experiments are needed to confirm these observations.

4.3.5.3 Primary infestation with A. hebraeum, secondary infestation with R. appendiculatus, D. marginatus and A. variegatum (Table 51)

A guinea pig and two rabbits (R 50 and R 65) were exposed to infestation with A. hebraeum nymphs. Fifty to 58 days later R. appendiculatus nymphs were released on the guinea pig as well as on a naive control guinea pig. D. marginatus and A. variegatum nymphs were released on the two rabbits and on two naive rabbits (R 51 and R 66) for control primary infestation data. Primary infestation with A. hebraeum nymphs did not protect against secondary infestation with D. marginatus or A. variegatum nymphs. The mean engorged weight of D. marginatus nymphs during the primary infestation (62.91 ± 1.4 mg) was not significantly different from that of nymphs during the secondary infestation (61.76 ± 1.16 mg). A. variegatum nymphs feeding on the previously exposed (to A. hebraeum) rabbit took nearly the same quantity of blood (mean weight 48.15 ± 0.99 mg) as those feeding on a naive rabbit (mean weight 49.63 ± 1.05 mg). Previous infestation of the guinea pig with A. hebraeum nymphs however appeared to provide some

TABLE 51 - RESULTS OF HETEROSPECIFIC INFESTATION OF ANIMALS WITH R. APPENDICULATUS, D. MARGINATUS AND A. VARIEGATUM NYMPHS FOLLOWING PRIMARY INFESTATION WITH A. HEBRAEUM. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Animal	Infestation/ Tick Sp.	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
9	GP	2♀/R.a.	38.69 ± 1.18	3.04	<0.01	6.91 ± 0.08	1.79	>0.05
	GP	1♀/R.a.	43.60 ± 1.02			6.73 ± 0.06		
33	R 50	2♀/D.m.	61.76 ± 1.16	0.63	>0.5	5.43 ± 0.39	4.37	<0.001
	R 51	1♀/D.m.	62.91 ± 1.40			5.70 ± 0.21		
37	R 65	2♀/A.v.	48.15 ± 0.99	1.02	>0.3	7.13 ± 0.09	3.53	<0.001
	R 66	1♀/A.v.	49.63 ± 1.05			7.53 ± 0.06		

protection against secondary infestation with R. appendiculatus nymphs, the lower mean weight (38.69 ± 1.18 mg) during secondary infestation being significantly different from the mean weight during the primary infestation (43.6 ± 1.02 mg). The reduction in weight (11%) was however much less than the 45.2% in a homospecific R. appendiculatus infestation. There was no consistent pattern in the average feeding times. On the rabbits average feeding times during the secondary infestation (5.43 ± 0.39 days and 7.13 ± 0.09 days) were significantly shorter than during the primary infestations (5.70 ± 0.21 days and 7.53 ± 0.06 days); the reverse was the case with the guinea pigs.

4.3.5.4 Primary infestation with A. variegatum, secondary infestation with A. hebraeum (Table 52)

In a single experiment a rabbit (R 46) was exposed to A. hebraeum nymphs 63 days after a primary infestation with A. variegatum nymphs. The primary infestation appeared to provide a measure of protection, the reduction in mean engorged weight of nymphs being 14% compared to 35% in a homospecific A. hebraeum infestation. The mean engorged weight of A. hebraeum nymphs during the secondary infestation (58.18 ± 1.06 mg) was significantly less than that of nymphs from a primary infestation (67.5 ± 1.04 mg). The average feeding time also was significantly longer during the secondary infestation (7.08 ± 0.92 days) compared to 6.65 ± 0.65 during the primary infestation.

It was interesting to note that the cross immunity was one-way, A. variegatum protecting against secondary infestation with A. hebraeum while A. hebraeum did not protect against secondary infestation with A. variegatum (see Section 4.3.5.3).

4.3.6 Results of serological tests

In the CF tests, normal rabbit serum reacted with 1/10 dilution of

TABLE 52 - RESULTS OF HETEROSPECIFIC INFESTATION OF A RABBIT WITH A. HEBRAEUM NYMPHS FOLLOWING PRIMARY INFESTATION WITH A. VARIEGATUM. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Rabbit number	Infestation/ Tick Sp.	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
32	R 46	2♀/A.h.	58.18 ± 1.06	6.25	<0.001	7.06 ± 0.92	3.44	<0.001
	R 48	1♀/A.h.	67.50 ± 1.04			6.65 ± 0.65		

TABLE 53 - RESULTS OF COMPLEMENT FIXATION TESTS WITH RABBIT SERA

Rabbit number	Infesting tick Sp.	Days post-infestation	CF titre	
			R. appendiculatus*	A. hebraeum*
R 51	D. marginatus	48	1/64	1/128
R 58	A. hebraeum	31	1/64	1/32
	A. hebraeum	85	1/64	1/128
R 60	R. appendiculatus	84	1/64	1/128
R 62	A. variegatum	17	1/64	1/64
R 65	A. hebraeum	31	1/32	1/64
R 68	R. appendiculatus	8	1/32	1/64
R 71	R. appendiculatus	49	1/16	1/64
Normal rabbit serum	-	-	1/32	1/64

* Salivary gland antigen

FIG. 69A
T.S. SALIVARY GLAND OF R. APPENDICULATUS
+ NORMAL RABBIT SERUM

FIG. 69B
T.S. SALIVARY GLAND OF R. APPENDICULATUS
+ R. APPENDICULATUS RABBIT SERUM (R 60)

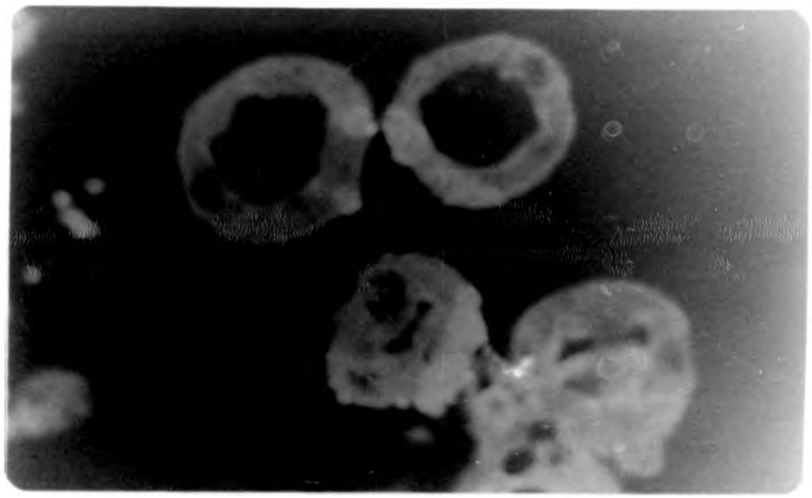


FIG. 69A
T.S. SALIVARY GLAND OF R. APPENDICULATUS
+ NORMAL RABBIT SERUM

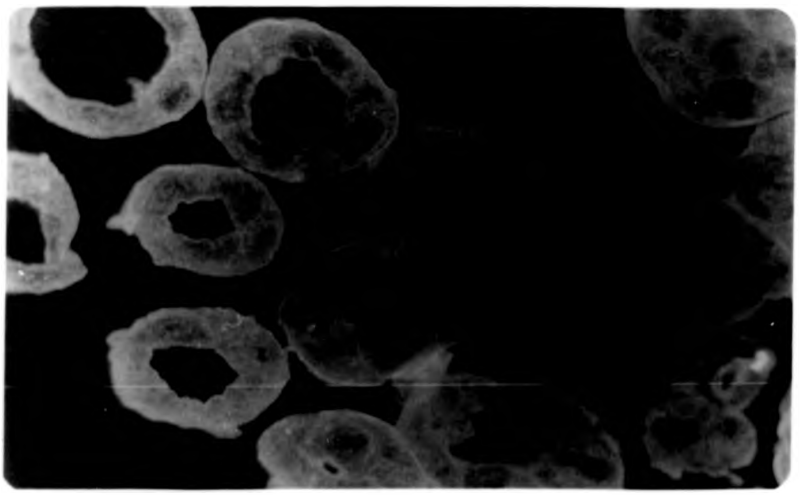


FIG. 69B
T.S. SALIVARY GLAND OF R. APPENDICULATUS
+ R. APPENDICULATUS RABBIT SERUM (R 60)

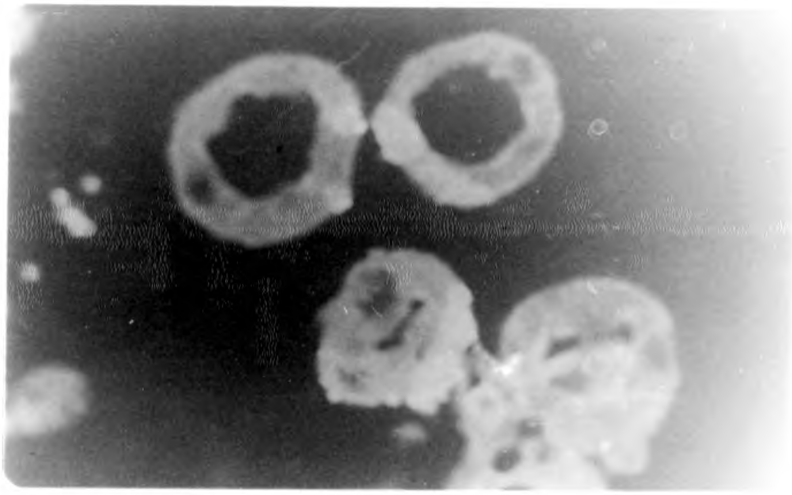


FIG. 69A
T.S. SALIVARY GLAND OF R. APPENDICULATUS
+ NORMAL RABBIT SERUM

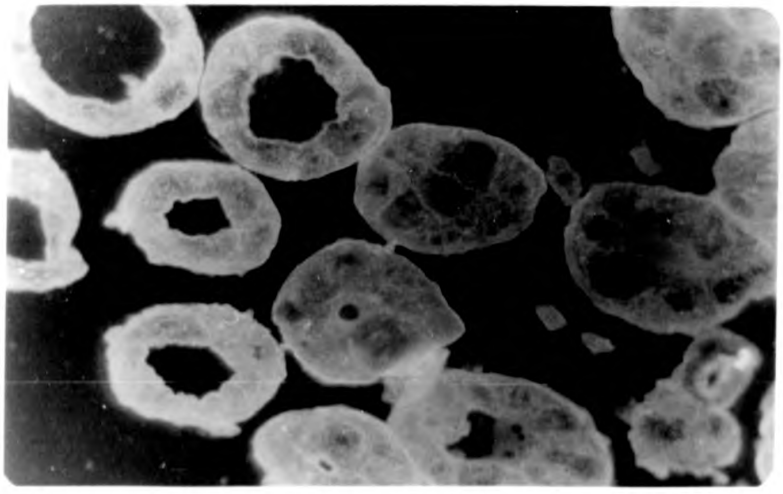


FIG. 69B
T.S. SALIVARY GLAND OF R. APPENDICULATUS
+ R. APPENDICULATUS RABBIT SERUM (R 60)

FIG. 69C
T.S. SALIVARY GLAND OF R. APPENDICULATUS
+ A. HEBRAEUM RABBIT SERUM (R 58)

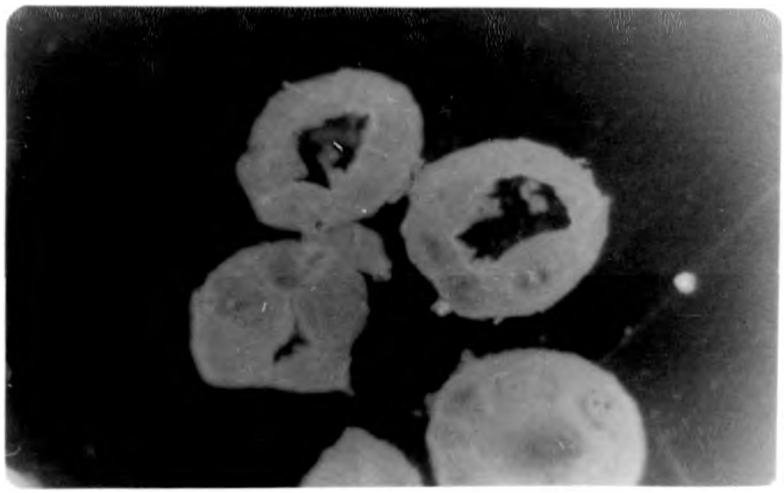


FIG. 69C
T.S. SALIVARY GLAND OF R. APPENDICULATUS
+ A. HEBRAEUM RABBIT SERUM (R 58)

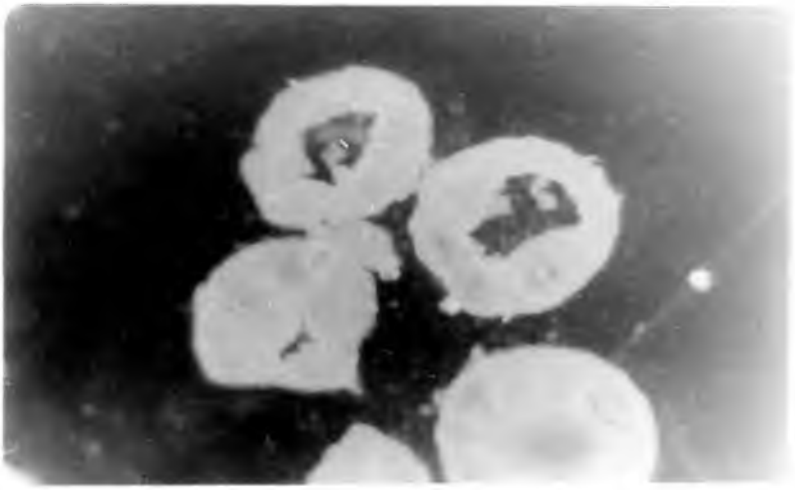


FIG. 69C
T.S. SALIVARY GLAND OF R. APPENDICULATUS
+ A. HEBRAEUM RABBIT SERUM (R 58)

salivary gland antigens of both R. appendiculatus (1/32) and A. hebraeum (1/64). None of the sera from the experimental rabbits had a titre above 1/64 to R. appendiculatus antigen and above 1/128 to A. hebraeum antigen and the two-fold increase in titre is not considered significant (Table 53). The only rabbit to show a significant (i.e., four-fold) rise in antibody titre was R 58 infested with A. hebraeum, in which the titre rose from 1/32 at 31 days to 1/128 at 85 days, suggesting that CF antibody developed later than 31 days. This is supported by the lack of significant levels of antibody in R 65, 31 days after infestation with A. hebraeum. Rabbit 58 developed immunity to a secondary homospecific infestation with A. hebraeum at 85 days but whether the rise in CF titre could be correlated with the development of immunity is not known.

When salivary gland sections were examined by indirect immunofluorescence, a bright fluorescence was obtained with sera from rabbits which had been exposed to R. appendiculatus as well as to A. hebraeum, suggesting a lack of specificity; however no fluorescence was obtained with normal rabbit serum (Figs. 69A, B, C).

4.3.7 Effect of Trypanosoma congolense infection in rabbits on homospecific immunity to R. appendiculatus

4.3.7.1 Primary infestation with larvae and secondary infestation with nymphs (Table 54)

In each of two experiments two rabbits were exposed to 2,000 to 4,000 larvae each. Sixty and 82 days later two of the four rabbits were inoculated with T. congolense infected mouse blood. The ear blood of the rabbits showed parasites eight to nine days after inoculation. Seventy-two to 90 days after the primary infestation and 10 to 15 days after the inoculation of parasites, R. appendiculatus nymphs

were released on both rabbits. At the same time nymphs were also released on the two uninfected rabbits with primary infestation. Naive rabbits exposed to R. appendiculatus nymphs at the same time provided control primary infestation data. The number of parasites in the blood of the previously exposed rabbits during the secondary infestation ranged from 4×10^6/ml to 2.4×10^8/ml.

In both experiments the mean engorged weights of nymphs from the secondary infestation on the infected rabbits (48.05 ± 0.51, range 40.3 to 55.7 mg and 39.33 ± 0.79, range 31.2 to 45.6 mg) were lower than of nymphs from the primary infestation (50.30 ± 0.62, range 40.9 ± 56.3 mg and 56.57 ± 0.84, range 44.1 to 65.0 mg); however they were significantly higher than those of nymphs from a secondary infestation of uninfected rabbits (25.02 ± 0.49, range 17.0 to 33.1 mg and 28.39 ± 0.94, range 20.1 to 51.6 mg). This clearly showed that infection with T. congolense altered the immunity of the rabbits to ticks, allowing the nymphs to take a significantly larger quantity of blood. The increase in the mean engorged weights of ticks feeding on the infected rabbit was not paralleled by a decrease in the average feeding time; in one experiment it was actually longer and in the second experiment not significantly shorter.

4.3.7.2 Primary and secondary infestations with nymphs (Table 55)

In the first of two experiments two rabbits were exposed to a primary infestation with 200 nymphs each. There was no significant difference in the mean engorged weights of nymphs on the two rabbits (51.34 ± 0.71 and 52.82 ± 0.77 mg). Fifty-three days later one of the rabbits was inoculated with T. congolense infected mouse blood and a further 10 days later R. appendiculatus nymphs were released on both rabbits. A third naive rabbit served as control. During the second-

TABLE 54 - FEEDING PERFORMANCE OF R. APPENDICULATUS NYMPHS ON RABBITS PREVIOUSLY EXPOSED TO R. APPENDICULATUS LARVAE AND INFECTED WITH TRYPANOSOMA CONGOLENSE. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Rabbit number	Infection/ Infestation	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
25	R 34	T.c./2^{o}	48.05 ± 0.51	32.0	<0.001	6.35 ± 0.09	3.9	<0.001
	R 35	2^{o}	25.02 ± 0.49	30.69	<0.001	5.97 ± 0.05	14.6	<0.001
	R 37	1^{o}	50.30 ± 0.62			5.05 ± 0.03		
42	R 72	T.c./2^{o}	39.33 ± 0.79	8.86	<0.001	6.44 ± 0.12	1.40	>0.05
	R 73	2^{o}	28.39 ± 0.94	22.26	<0.001	6.64 ± 0.08	14.79	<0.001
	R 75	1^{o}	56.57 ± 0.84			5.21 ± 0.04		

ary infestation the parasitaemia level in the infected rabbit was lower than in the previous experiment and the number of parasites ranged from undetectable levels to 2.8×10^7/ml. Although there was no significant difference in the engorged weight of nymphs from the two experimental rabbits during the primary infestation, during the secondary infestation nymphs feeding on the T. congolense infected rabbit took a significantly higher blood meal (mean engorged weight 36.16 ± 1.05, range 22.0 to 46.2 mg) than those feeding on the uninfected rabbit (31.04 ± 0.82, range 21.5 to 39.9 mg). These figures were still lower than the mean weights of nymphs (51.53 ± 0.99 mg) during a primary infestation (Table 55, Rabbit 54).

A slightly different protocol was followed in the second experiment, although the objective was the same. One of two rabbits was inoculated with T. congolense infected mouse blood. Three days later R. appendiculatus nymphs were released on both rabbits (primary infestation). The number of parasites in the blood during the primary infestation ranged from undetectable levels to 7.9×10^6/ml. The infection did not appear to have any effect on feeding performance during the primary infestation, the mean engorged weights of the nymphs from the two rabbits being 48.89 ± 0.84 and 46.68 ± 0.82 mg. Forty-nine days after the primary infestation both rabbits were exposed to R. appendiculatus nymphs for a second time. Nymphs were also released on a third naive rabbit (control primary infestation). Parasite numbers in the infected rabbit during the secondary infestation ranged from undetectable levels to 2.8×10^7/ml. As in the first experiment T. congolense infection significantly increased the amount of blood taken by nymphs during the secondary infestation, mean engorged weight of nymphs from the infected rabbit being 28.32 ± 0.70 mg (range 19.7 ± 35.2) and from the uninfected rabbit 22.87 ± 0.72 mg (range 16.7 to

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A slightly different protocol was followed in the second experiment, although the objective was the same. One of two rabbits was inoculated with T. congolense infected mouse blood. Three days later R. appendiculatus nymphs were released on both rabbits (primary infestation). The number of parasites in the blood during the primary infestation ranged from undetectable levels to 7.9×10^6/ml. The infection did not appear to have any effect on feeding performance during the primary infestation, the mean engorged weights of the nymphs from the two rabbits being 48.89 ± 0.84 and 46.68 ± 0.82 mg. Forty-nine days after the primary infestation both rabbits were exposed to R. appendiculatus nymphs for a second time. Nymphs were also released on a third naive rabbit (control primary infestation). Parasite numbers in the infected rabbit during the secondary infestation ranged from undetectable levels to 2.8×10^7/ml. As in the first experiment T. congolense infection significantly increased the amount of blood taken by nymphs during the secondary infestation, mean engorged weight of nymphs from the infected rabbit being 26.32 ± 0.70 mg (range 19.7 ± 35.2) and from the uninfected rabbit 22.67 ± 0.72 mg (range 16.7 to

28.8); both were lower than the mean weight of nymphs feeding on the naive rabbit (See Table 55, Rabbit 69).

Eighty-one days after the primary infestation and 32 days after the secondary infestation, Rabbit 67 (infected) and Rabbit 68 (uninfected) were exposed to nymphs for the third time. The number of parasites in the blood of the infected rabbit during the time the ticks were feeding ranged from 1.2×10^6/ml to 5.9×10^6/ml. During this tertiary infestation also nymphs took a significantly larger ($P < 0.001$) blood meal on the infected rabbit, (mean engorged weight 32.82 ± 0.65, range 25.9 to 41.2 mg) than on the uninfected rabbit (27.75 ± 0.70, range 22.9 to 36.8 mg); the average feeding time on the infected rabbit (6.93 ± 0.11 days) was significantly longer ($P < 0.001$) than on the uninfected rabbit (6.3 ± 0.06 days).

Thus, in all four experiments, feeding performance of nymphs of R. appendiculatus based on mean engorged weight, improved during a secondary infestation when the host rabbits were infected with T. congolense after the primary infestation; parasitaemia did not seem to affect the course of the primary infestation. Mean engorged weights were still lower during the secondary infestation than during the primary, but the higher weights on the infected rabbits compared to those on uninfected rabbits would suggest that parasitaemia in some way blocked the expression of immunity of the animal to a secondary infestation; this was also seen in tertiary infestations of infected and uninfected rabbits.

The difference in mean engorged weights of nymphs feeding on infected and uninfected rabbits during the secondary infestation was greater when the primary infestation was with larvae than when it was with nymphs; in the former case nymphs from the infected rabbits were on average 1.4 to 1.9 times as heavy as those from the uninfected

TABLE 55 - FEEDING PERFORMANCE OF *R. APPENDICULATUS* NYMPHS ON RABBITS PREVIOUSLY EXPOSED TO *R. APPENDICULATUS* NYMPHS AND INFECTED WITH *TRYPANOSOMA CONGOLENSE*. MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES

Expt. number	Rabbit number	Infection/ Infestation	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
34	R 53	*T.c.*/2^{o}	36.16 ± 1.05			5.74 ± 0.08		
	R 52	2^{o}	31.04 ± 0.82	3.81	<0.001	6.61 ± 0.1	7.01	<0.001
	R 54	1^{o}	51.53 ± 0.88	16.97	<0.001	5.22 ± 0.06	12.23	<0.001
38	R 67	*T.c.*/2^{o}	26.32 ± 0.70			7.76 ± 0.17		
	R 68	2^{o}	22.67 ± 0.72	3.63	<0.001	6.52 ± 0.12	5.85	<0.001
	R 69	1^{o}	47.88 ± 0.8	22.77	<0.001	5.14 ± 0.07	9.5	<0.001

rabbits; in the latter case they were only 1.2 times as heavy.

4.3.8 Effect of host immunisation with tick-derived antigens

4.3.8.1 Feeding performance of R. appendiculatus on animals immunised with cell extracts from a cell line from R. appendiculatus (Table 56)

One hundred R. appendiculatus nymphs were released on each of two guinea pigs, one inoculated SC three times with cell extract + FCA and the other with FCA only. Nymphs were also released on a third uninoculated guinea pig (Expt. 5). Recovery rate of fed nymphs from all three animals was nearly 100% and there was no significant difference in the average feeding times. The engorged nymphs were not weighed, but 10 of the emerging females from each batch were weighed individually. Females from the guinea pig receiving the cell extract weighed less (mean weight 4.42 ± 0.24 mg) than those from the guinea pig receiving only FCA (5.57 ± 0.25 mg) and from the uninoculated control (5.30 ± 0.24 mg). The differences were highly significant ($P < 0.001$).

The protective effect of cell extract immunisation was seen in a further two experiments. In the first (Expt. 6) 200 nymphs of R. appendiculatus were released on each of two rabbits, one receiving two inoculations of cell extract + FCA and the other FCA only. There was no difference in the recovery rate of fed nymphs from the rabbits. Reduced feeding performance of the nymphs on the rabbit inoculated with cell extract was shown by a significant reduction in mean engorged weight of nymphs (52.81 ± 1.45, range 43.0 to 59.0 mg) and by a significant prolongation of average feeding time (6.90 ± 0.07 days). The corresponding figures for nymphs from the rabbit inoculated with FCA only were 64.77 ± 1.1 (range 57.0 to 72.0 mg) and 6.31 ± 0.08 days.

In the second experiment (Expt. 17), a rabbit received two IP inoculations of cell extract with FCA. An uninoculated rabbit served as control. Twenty-five females and 30 males of R. appendiculatus

were released on the ears of each rabbit. Twenty-three and 25 fed females were recovered from the two rabbits. The mean engorged weight of females from the experimental rabbit was 318.72 ± 25.87 mg (range 195 to 570); this was significantly lower than the mean engorged weight of females from the control rabbit (441.95 ± 23.15, range 282 to 648 mg). There was no significant difference in the average feeding times. The mean egg mass weight laid by females from the experimental rabbit (180.61 ± 9.64 mg) was significantly lower ($P < 0.001$) than that laid by females from the control rabbit (243.47 ± 15.13 mg). This is probably a direct result of the lower blood meal. There was no signigicant difference in the % CEI of females from the two rabbits, which were 49.98 ± 1.72% and 52.91 ± 1.42%.

In the last experiment in the series (Expt. 28), there was no significant difference between the mean engorged weight of nymphs feeding on a guinea pig inoculated with cell extract concentrate + FCA (39.87 ± 0.7 mg) and on a guinea pig receiving FCA only (41.74 ± 0.82 mg).

4.3.8.2 Feeding performance of R. appendiculatus on animals immunised with larval homogenates (Table 56)

The immunising potential of larval homogenates was clearly shown in a single experiment (Expt. 26) in which a guinea pig received two SC inoculations of larval homogenate without FCA. Two hundred nymphs of R. appendiculatus were released on this and on a control guinea pig. There was no difference in the recovery rate of fed nymphs, but on the inoculated guinea pig, nymphs took a significantly lower blood meal (mean engorged weight 34.29 ± 0.87, range 14.0 to 42.5 mg) than on the control guinea pig (mean engorged weight 39.50 ± 0.57, range 33.0 to 46.0 mg). The average feeding time on the inoculated guinea pig (6.96 ± 0.08 days) was also significantly longer than that on the

TABLE 56 - MEAN ENGORGED WEIGHTS AND AVERAGE FEEDING TIMES OF R. APPENDICULATUS NYMPHS ON ANIMALS INOCULATED WITH TICK-DERIVED ANTIGENS

Expt. number	Animal number	Antigen	Inoculation protocol	Route	Stage fed	Mean weight ± SE (mg)	t	P	Average feeding time ± SE (days)	t	P
5	GP 1	FCA[1]	$1^0,2^0,3^0$[2]	SC	Ny	5.57 ± 0.25[3]	9.69	<0.001	7.51 ± 0.12	0.48	>0.6
	GP 2	Cells + FCA	$1^0,2^0,3^0$	SC	Ny	4.42 ± 0.24			7.59 ± 0.10		
	GP 3	-	-	-	Ny	5.30 ± 0.24	7.78	<0.001	7.38 ± 0.07	1.88	>0.05
6	R 1	Cells + FCA	$1^0,2^0$	SC	Ny	52.61 ± 1.45	6.71	<0.001	6.90 ± 0.07	6.2	<0.001
	R 2	FCA	$1^0,2^0$	SC	Ny	64.77 ± 1.1			6.31 ± 0.06		
17	R 23	Cells	$1^0,2^0$	IP	F	318.72 ± 25.87	3.53	0.001	9.04 ± 0.3	0.14	>0.8
	R 29	-	-	-	F	441.95 ± 23.15			9.10 ± 0.29		
28	GP 1	Cell conc.[4] + FCA	$1^0,2^0$	Foot pad	Ny	39.87 ± 0.7	1.7	>0.05	6.53 ± 0.05	2.27	<0.05
	GP 2	FCA	$1^0,2^0$	Foot pad	Ny	41.74 ± 0.82			6.76 ± 0.05		
26	GP 1	Larval homo-genate	$1^0,2^0$	SC	Ny	34.29 ± 0.87	5.05	<0.001	6.96 ± 0.06	9.6	<0.001
	GP 2	-	-	-	Ny	39.50 ± 0.57			6.14 ± 0.06		

1/ Freund's Complete Adjuvant
2/ First, second and third inoculations
3/ Weight of emerging females
4/ Cell concentrate

control animal (6.14 ± 0.06 days).

In summary, rabbits and guinea pigs could be immunised against infestation with R. appendiculatus nymphs and adults by injection of cell extract antigen from R. appendiculatus and with R. appendiculatus larval homogenates. The immunisation did not prevent infestation and the reduction in mean engorged weight was not so dramatic as that obtained when the ticks were fed on animals naturally immunised by a primary infestation. Whether immunisation with antigens from one species will confer some protection against a second species remains to be investigated.

Although the addition of complete adjuvant is usually considered essential for the development of immunity, I found that immunity could be induced by inoculation of larval homogenates without FCA.

4.4 DISCUSSION AND CONCLUSIONS

4.4.1 Age of ticks and feeding performance

One of the factors to be considered in the design of sequential infestation experiments is the age of ticks released on the host animals. The possible effects of age on feeding performance and the need to keep the age of ticks relatively constant during a given series of infestations have been noted by Norval (1978) and Boese (1974). I obtained significant differences in the engorged weights of *R. appendiculatus*, *A. hebraeum* and *A. variegatum* nymphs from the same batch but of different ages, but no significant difference between nymphs from the same batch and of the same age. There was also a significant difference in the engorged weights of nymphs of similar ages but from different batches. These differences in weights although significant, were of a much lower order than those obtained during a homospecific secondary infestation, and it should not be difficult to distinguish between differences in weight due to differing age of ticks and due to feeds on immune animals during a secondary infestation. Where the interval between two infestations is only one or two weeks, ticks from the same batch could be used for both primary and subsequent infestations of the same animal. But where the interval between two sequential infestations is considerable as was the case in my experiments, a more valid assessment of feeding performance will be comparison of engorged weights of ticks from the same batch and age released simultaneously on the previously exposed animal (secondary infestation) and on a naive animal without previous exposure to ticks (primary infestation). Both Wikel and Allen (1977) and Randolph (1979) included control animals along the test animals to provide data on primary infestation. Animals used simultaneously in my infestation experiments were nulliparous and

nearly of the same age and weight to reduce any effect of age and physiological condition of host animals on feeding performance of the ticks.

4.4.2 The expression of host immunity to tick infestation

Animals immune to tick infestation show at least two types of response. The first, probably induced by irritation of tick attachment, is actual physical removal of the ticks before they have attached or before the attached ticks have completed feeding, and the second, reduction in feeding performance. Reduction in feeding performance is usually shown by reduced recovery rate of fed ticks, death of feeding ticks *in situ*, prolonged feeding time and lower engorged weight. In the case of engorged immature stages, further effects such as prolonged premoult period and death of ticks in the premoult phase have also been reported (Brown 1977). An altered sex ratio of adults emerging from nymphs feeding on immune animals is mentioned by Musatov (1967) but this has not been confirmed by any of the other workers. Adult females feeding on immune animals show in addition to prolonged feeding time and lower engorged weight, reduction in numbers laying eggs, lower egg mass weight and lower viability of eggs. Effects on males such as impairment of mating capabilities have not been reported. But McGowan *et al.* (1980) observed that male *A. maculatum* feeding on artificially immunised rabbits died prematurely, and suggested that this may have been responsible for failure of a number of females to become replete and lay eggs.

It is difficult to assess the relative importance of these various parameters of the expression of immunity since it may vary in the different tick-host combinations. The most effective expression of immunity, whether innate, naturally acquired or artificially induced, is prevention of establishment of infestation. Since immunity can not

be expressed unless the animal is challenged by antigen (tick saliva), it is doubtful if tick bite and transmission of tick-borne pathogens can be prevented. Removal or rejection of ticks during the first 24 h or so due to self grooming is an expression of immediate hypersensitivity. It is usually seen in Boophilus larvae - cattle combinations and is probably one of the major and most effective expressions of immunity. But during the short time that the ticks are on immune cattle, they make repeated attachments (Kemp et al. 1976) during which transmission of pathogens might already have taken place. A considerable proportion of B. microplus larvae (up to 50%) may be removed by grooming activity with minimal damage to the ticks. Removal of other tick species such as I. holocyclus from immune cattle by self grooming has also been reported (Doube and Kemp 1975). In most of the experiments involving immunity to tick infestation, including mine, it has not been possible to assess the importance of this particular parameter, because the animals are restrained from grooming, but it is quite possible that removal of ticks by grooming of immune hosts occurs in other tick-host combinations also.

Death of attached ticks in situ has been reported by several authors for different tick species (see Section 4.1) but was never observed in any of the tick species used in my experiments.

Reduction in recovery rates i.e., in number of ticks feeding and detaching has been observed by several authors and according to Hewetson (1972) this is the most important and the easiest parameter for assessing immunity of cattle to B. microplus. But Fujisaki (1978) found no difference in the number of Hae. longicornis females feeding on immune rabbits and naive rabbits. A modification of this parameter, reduction in proportion of ticks maturing on the host, based on length of the attached ticks, was used by Sutherst et al. (1979) for Hae.

longicornis feeding on immune cattle. In my experiments, with a few exceptions, there was no significant reduction in the numbers engorging during a homospecific or heterospecific secondary infestation and at least in the experimental tick-host combinations I used, this parameter would appear to be unimportant in assessing immunity.

Prolongation of feeding time of the infesting ticks is one of the most common expressions of host immunity and has been observed by most workers except Brossard (1977) and Fujisaki (1978). In homospecific infestation experiments, with few exceptions (4 out of 17), I found that the average feeding time of ticks feeding on immune animals was significantly longer than that of ticks feeding on naive animals. However this was not the case in heterospecific infestation experiments in which reduction in engorged weight of ticks on immune animals was not always accompanied by a significantly prolonged feeding time.

The most consistent and reliable parameter in the expression of immunity would appear to be reduced engorged weight and it has been invariably reported by all workers irrespective of whether the infesting stage was larva, nymph or adult. I also found this the most consistent and reliable parameter.

In the case of females feeding on immune animals, reduced egg mass weight and even reduced egg viability may be a direct result of reduced engorged weight. But according to Bowessidjaou et al. (1977), lower blood meal could not by itself account for reduced egg viability which in the case of I. ricinus females feeding on a repeatedly exposed rabbit was reduced to 3.1%. I found that adults of R. appendiculatus feeding on immune rabbits had in addition to lower engorged weights and lower egg mass weights, a significantly lower conversion efficiency index, suggesting that the ability of the females to convert blood meal into egg mass was impaired.

When immunity results in removal or rejection of a considerable proportion of the ticks, in significant reduction of attached and feeding ticks (in some cases to zero) or in death of the attached ticks, it may be regarded as "total". But in most cases, immunity may only be "partial" since many of the ticks attach, feed and develop normally, although the feeding and reproductive performances may be reduced. The effects of partial immunity of host animals on tick populations may not be dramatic but cumulative, resulting in a gradual decline in the tick population provided no non-immune hosts are introduced into the population from time to time. Whether rejection or removal of ticks would have occurred in my experiments is not known since the animals were prevented from self grooming.

4.4.3 Host immunity in homospecific and heterospecific infestations

There is general acceptance that animals exposed to ticks develop immunity to a homospecific secondary and subsequent infestations; failure to develop homospecific immunity has been ascribed to a long established association between the tick and the natural vertebrate host; for e.g., dogs do not become immune to the dog tick R. sanguineus (Theis and Budwiser 1974) but guinea pigs and rabbits do (Chabaud 1950). Goodrich et al. (1978) have shown that guinea pigs rapidly develop immunity to I. holocyclus whereas long nosed bandicoots (Perameles nasuta), a natural host of the tick, could be infested several times before any appreciable immunity develops. A similar difference between laboratory mice and field mice, the natural host of I. trianguliceps has been noted by Randolph (1979). However cattle, the natural hosts of the cattle ticks, Boophilus spp. can and do develop an immunity to the ticks.

There are some aspects of host immunity which need appraisal;

for e.g., do different species of ticks and different stages of the same species produce different levels of immunity; do immunity levels increase with repeated infestations; for how long after a primary infestation does immunity last and finally do host animals develop heterospecific immunity to different species of ticks.

It is difficult to evaluate the levels of immunity conferred by different stages unless the total amount of antigen injected and the immunogenicity of the antigen are known. In my experiments infestation with one stage conferred immunity to infestation with another stage. Thus nymphs of R. appendiculatus feeding on animals previously exposed to R. appendiculatus larvae, nymphs or adults had their engorged weight reduced by 36% to 55%; the reduction in weight of adult females feeding on animals previously exposed to adults was 62% to 70%. In homospecific infestation with adults of B. microplus, Hewetson (1971) found that engorged weights were reduced by 23% to 35%; the reduction obtained in the experiments of Wagland (1975) was less, 11% to 21%. Homospecific infestation with I. ricinus females resulted in reduction of engorged weights by 28% to 68% (Bowessidjaou et al. 1977). Wikel and Allen (1976a; 1977) however obtained a much greater reduction, 6 - 7 fold, in weights of D. andersoni larvae feeding on a previously exposed guinea pig, when the interval between infestations was 7 days.

Norval (1978) reported that ticks with long deeply penetrating mouth parts (Amblyomma and Hyalomma) are less susceptible to host immunity mechanisms than ticks with superficially attaching short mouth parts (Rhipicephalus and Boophilus). He indicated that this was probably one of the reasons why A. hebraeum larvae and nymphs were able to feed normally on sheep and rabbits previously exposed to the ticks. However in my experiments contrary to Norval's findings, animals did develop homospecific immunity to A. hebraeum nymphs. A. hebraeum nymphs

and A. variegatum nymphs with long mouth parts of similar length (0.46 ± 0.003 mm and 0.41 ± 0.003 mm respectively) had engorged weights during a secondary homospecific infestation reduced by 35% and 38% respectively. This was not much less than the reduction of 45.2% obtained with R. appendiculatus nymphs with very much shorter mouth parts (0.18 ± 0.002 mm). However D. marginatus nymphs with short mouth parts (0.2 ± 0.001 mm) similar in length to that of R. appendiculatus, had mean engorged weights during a secondary infestation reduced by only about 20%. Thus from my experiments it would appear doubtful if length of penetration of mouth parts is one of the factors responsible for different levels of immunity.

In all my experiments, animals were infested only twice and the effect of repeated infestations was not investigated. A single infestation was able to confer immunity to a second infestation. This was also noted by Wikel and Allen (1977) and Fujisaki (1978). Several authors have reported that immunity is enhanced by repeated infestations (for e.g., Boese 1974; Wagland 1975; Brossard 1976; Bowessidjaou et al. 1977) but Berdyev and Khudainazarova (1976) observed that long and continuous infestation actually desensitised animals. Randolph (1979) found that repeated infestations of field mice and sometimes of laboratory mice with I. trianguliceps actually resulted in an improved feeding performance.

Boese (1974) noted that host immunity was of short duration, becoming maximal between 10 to 16 days after exposure to ticks and then declining unless the animals were reinfested. He also observed that immunity appeared to depend less on number of ticks per infestation than on frequency of infestation. Bowessidjaou et al. (1977) found that with repeated infestations, immunity lasted for up to nine months. In my experiments, a single infestation conferred immunity for at least

three months. Immunity may appear soon after the ticks have started feeding (Brossard 1976) but in the case of immature stages with short feeding times it may have no effect on the feeding ticks. With females which feed for much longer periods, immunity produced as a result of the bites, may have an effect on females engorging towards the end of the infestation (Boese 1974).

To my knowledge heterospecific infestation and immunity has been investigated only by Musatov (1967) in spite of its importance in natural tick infestations of animals. He demonstrated the lack of heterospecific immunity in rabbits exposed in succession to two species of Hyalomma and two species of Rhipicephalus. I was not able to investigate cross immunity with different combinations of the four tick species. There was no cross immunity between R. appendiculatus and A. hebraeum and between R. appendiculatus and A. variegatum or between A. hebraeum and D. marginatus and A. hebraeum and A. variegatum. However, there was evidence of reciprocal cross immunity between D. marginatus and R. appendiculatus, although judging by reduction in engorged weights, heterospecific immunity between the two species was of a much lower order than homospecific immunity. Similarly, primary D. marginatus infestation gave some protection against secondary infestation with A. variegatum, although here again it was of a much lower order than homospecific immunity to A. variegatum. While R. appendiculatus primary infestation did not protect against a secondary infestation with A. hebraeum, a primary with A. hebraeum gave marginal protection against a subsequent infestation with R. appendiculatus. Cross immunity between A. hebraeum and A. variegatum was one-way, A. variegatum giving some protection against secondary infestation with A. hebraeum, but not vice versa.

In summary, rabbits and guinea pigs developed homospecific immunity to a secondary infestation following primary infestation with the same

species. In heterospecific infestations, there was either no cross immunity between the two species used for primary and secondary infestation, or if there was, it was of a much lower order than homospecific immunity. It will be interesting to see if cross immunity exists between species in the same genus and between different genera.

4.4.4 Serological tests for antibody in tick-infested animals

Ever since Trager (1939 a) showed that partial immunity to D. variabilis could be transferred to naive guinea pigs with serum from immune animals, various authors have attempted to demonstrate circulating antibodies to tick antigens in the blood of animals previously exposed to a tick infestation or injected with tick antigens. Several standard serological tests have been used for this purpose, but rarely have circulating antibodies been demonstrated in all infested animals tested (Nelson et al. 1977).

The most commonly used test for detecting circulating antibody is the gel immunodiffusion technique. Precipitating antibodies were found by this technique in a number of amphibia exposed to A. testudinis (Schneider et al. 1971) and Kohler et al. (1967) used the test for demonstrating antibody to salivary gland and gut extracts of Hy. anatolicum excavatum and R. sanguineus in the sera of rabbits exposed to the ticks. The antibodies were specific to each species. Precipitating antibody to salivary gland extracts has also been shown by immunoelectrophoresis in the blood of cows following infestation with B. microplus (Brossard 1976). Fujisaki (1978) and Allen and Humphreys (1979) obtained precipitation bands with sera of rabbits exposed to Hae.longicornis infestation and with that of cattle inoculated with whole tick antigen from D. andersoni, using whole tick extract from the respective species as antigens. Boese (1974) on the other hand could not demonstrate preci-

pitating antibodies to whole tick extracts of Hae. leporispalustris in rabbits following an infestation. However, skin-sensitising antibodies could be shown by homologous passive Cutaneous anaphylaxis (PCA) test when areas of normal rabbit's skin were injected intradermally with test serum and subsequently challenged with antigen. Bagnall (1978) found that skin-sensitising antibodies (as detected by PCA) as well as precipitating antibodies developed in guinea pigs following infestation with I. holocyclus.

The complement fixation test was used by Trager (1939 b) to demonstrate antibody to whole larval extract of D. variabilis in the blood of guinea pigs exposed to the ticks and Weiland and Emokpare (1968) obtained complement fixing antibodies against salivary gland extracts of Hy. anatolicum excavatum to a titre of up to 1/320 in rabbits infested with this species. However low titres of CF antibodies were also obtained against R. sanguineus salivary gland extracts in naive rabbits before infestation. The observation by Musatov (1976) that CF tests were clearly positive in the majority of cases, even in those done on control animals, throws some doubt on the reliability of the test. My experiments showed that a positive reaction could be obtained even with normal rabbit serum and that any interpretation on the development of CF antibody should be based on at least a four-fold increase in titre in sequential samples of serum, or compared to normal controls. In my studies CF tests did not prove to be specific and in any case gave generally inconclusive results.

Bowessidjaou et al. (1977) used the indirect immunofluorescence test with salivary gland sections of I. ricinus and serum of rabbits infested with this species and showed that antibody could be detected as early as seven days after infestation and eventually reached a titre as high as 1/387. The direct fluorescent antibody technique was used

by Kohler et al. (1967) to demonstrate antibody in the sera of rabbits infested with Hy. anatolicum excavatum and R. sanguineus using sections of tick gut and salivary gland as antigen; titres up to 1/80 were obtained.

The fluorescent antibody test is accepted to be highly specific and the cross reactivity I obtained with sera from rabbits exposed to R. appendiculatus and A. hebraeum with R. appendiculatus salivary gland sections is surprising. However, unlike the CF test in which normal rabbit serum reacted with antigens, the immunofluorescence test was able to distinguish between naive rabbits and exposed rabbits.

An indirect haemagglutination test was able to detect specific antibody in cattle exposed to B. microplus (Willadsen et al. 1978); antibody was absent in unexposed animals but its concentration did not correlate with degree of immunity in the exposed animals. McGowan et al. (1980) also used passive haemagglutination for measuring antibody in rabbits immunised with whole tick extracts from A. maculatum; the injected rabbits developed HA antibody within seven days.

Erythrocyte sedimentation rate was mentioned by Musatov (1976) for measuring antibody in animals exposed to ticks, but details are not given.

Other sophisticated techniques such as lymphocyte transformation test have been rarely used to study host immunity to ticks and these and cutaneous hypersensitivity tests may give a better picture of the immune status of animals than demonstration of circulating antibody by more conventional serological tests. The type of antigen also may be critical and the presence of antigen in gut sections would suggest that whole tick extracts may be a better source of antigen for serological tests as well as for experimental immunisation of animals.

4.4.5 The effect of parasitic infections on immunity to tick infestation

Antibody and complement are among the various immunological responses implicated in immunity to ticks. Animals under natural conditions are exposed to various pathogens which also provoke an immune response and it is surprising that the interaction between infection by pathogens and infestation by ticks has not been extensively investigated. Wikel and Allen (1976 b) found that immunosuppressive drugs such as methotrexate and cyclophosphamide effectively blocked the development of resistance to ticks in guinea pigs. Callow and Stewart (1978) demonstrated the immunosuppressive effect of Babesia bovis infection on feeding of B. microplus on cattle and suggested that this was probably a device on the part of the parasite to improve its chances of survival.

The improved feeding performance of R. appendiculatus which I observed during a secondary infestation of rabbits when they were infected with T. congolense long after a primary infestation, was more like the effect of complement depletion due to Cobra Venom Factor (CoF) observed by Wikel and Allen (1977) rather than an immunosuppressive phenomenon. In this context, it is interesting to note that reduction of complement occurs in cattle chronically infected with T. congolense (Nielsen et al. 1978).

Trypanosoma congolense inoculated into animals before a primary infestation did not prevent the development of immunity to a secondary infestation, but parasitaemia before and during secondary infestation resulted in the ticks taking a significantly larger blood meal on the infected rabbits, than on uninfected rabbits. I also found that T. congolense infection was able to block the expression of immunity in rabbits to a greater extent when the primary infestation was with larvae than when it was with nymphs.

Whether the effect of blood pathogens is immunosuppressive or depletion of complement, their role in the expression of immunity to tick infestation seems to be certain. My preliminary experiments were limited in number and confined to laboratory animals. More laboratory experiments as well as field trials in cattle would be required to assess the interrelationship between parasite infection and tick infestation.

There was some evidence from my experiments (not reported in this thesis) that tick infestation actually increased the number of parasites in the peripheral circulation of rabbits. This and other aspects of tick-parasite-vertebrate host interactions are the subject of an ongoing programme of research here.

4.4.6 Artificial immunisation of animals against tick infestation

Several attempts have been made to immunise animals against tick infestation, most of them with laboratory animals such as rabbits and guinea pigs, which are not the natural hosts of the ticks. Antigens used for immunisation have been extracts of salivary glands, homogenates of unfed larvae, unfed nymphs or unfed adults and homogenates of internal organs of partially fed females. Salivary gland extracts have been most commonly used as antigens to immunise animals against an infestation with the same species of tick (Kohler *et al.* 1967; Musatov 1967; Garin and Gabarev 1972; Brossard 1976). Larval and nymphal homogenates gave some protection against a homospecific infestation (Trager 1939 a; Gregson 1941; Schneider 1971; Bagnall 1975). The experiments of Allen and Humphreys (1979) on immunisation of guinea pigs and calves with extracts from midgut and reproductive organs and from all internal organs of partially fed *D. andersoni* has been referred to in Section 4.1. Extracts from tissues of unfed ticks were ineffective, although McGowan *et al.* (1980) found that extracts of unfed males of *A. maculatum*

inoculated into rabbits reduced the feeding performance of adult female ticks but not of nymphal ticks, presumably because the antigen was derived from adults; however there was a delayed effect on nymphs, adults emerging from nymphs fed on immune animals taking less blood than those emerging from nymphs fed on non-immune animals.

Immunity developing as a result of artificial immunisation has always been noted to be of a lower order than naturally acquired immunity following tick infestation. This was seen in my experiments also where the reduction in engorged weight of R. appendiculatus feeding on artificially immunised animals was 13% to 28% compared to 36% to over 50% obtained during a homospecific secondary infestation. I was able to induce immunity in animals by the injection of cell extracts from a continuous cell line of R. appendiculatus. This is the first time that this has been accomplished and cell extracts have the advantage in that they are "clean" and cells can be grown in bulk under controlled conditions in the laboratory.

Two additional considerations in artificial immunisation are the use of adjuvants, and cross immunity. Freund's complete adjuvant is commonly used in laboratory procedures but may not be acceptable in practice because of severe reactions. In one of my experiments larval homogenates without adjuvant gave some protection. We are currently experimenting with another adjuvant, muramyl dipeptide (MDP) in a liposome carrier. Preliminary results are promising.

It is doubtful if artificial immunisation with extracts from one species will produce heterospecific immunity. If it does not, the value of artificial immunisation with "single" extracts will be limited in areas where animals are exposed to more than one species of ticks. Under such circumstances a "cocktail" vaccine made of extracts from the different species might be more effective. Finally, we do not know

whether the effects of parasitic infections on artificially induced immunity are the same as those on naturally acquired immunity. Our laboratory is currently investigating these problems also.

5. GENERAL CONCLUSIONS

Three species of economically important African ixodid ticks, Rhipicephalus appendiculatus, Amblyomma hebraeum and Amblyomma variegatum were highly susceptible to three synthetic pyrethoids, permethrin, cypermethrin and decamethrin. Decamethrin was the most effective followed by cypermethrin and permethrin. A. variegatum was the most susceptible species and R. appendiculatus the most tolerant with A. hebraeum intermediate. Two other acaricides, carbaryl and amitraz were much less effective than the pyrethroids. With the increasing problem of resistance to other acaricides, the new synthetic pyrethroids have a considerable potential in tick control.

Rabbits and guinea pigs developed homospecific immunity to infestation with four species of ticks, R. appendiculatus, A. hebraeum, A. variegatum and Dermacentor marginatus. Heterospecific or cross immunity was seen with some species, but was always of a much lower order than homospecific immunity.

Some protection against R. appendiculatus infestation was obtained by immunising animals with cell extracts and whole larval homogenates, but this was less than the immunity acquired after tick infestation.

Trypanosoma congolense injected into rabbits blocked the expression of immunity; the effect is probably due to depletion of complement by the parasites and appears to be similar to that obtained by injecting Cobra Venom Factor into immune animals.

In tick management on domestic animals by artificial immunisation with tick-derived antigens, the effect of infection by pathogens and infestation by other tick parasites should be important considerations, since there may be no cross immunity or at best only low levels of heterospecific immunity.

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