THE EFFECT OF THE PESTICIDE RONNEL ON THE METABOLISM OF THE TICK *Ornithodoros savignyi*

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A THESIS
Submitted to the
AMERICAN UNIVERSITY OF BEIRUT

In partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE IN
TROPICAL HEALTH

September 1968

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Date thesis is presented: September 28, 1968.

EFFECT OF RONNEL ON O. SAVIGNYI

SALIBA

ACKNOWLEDGEMENTS

The writer wishes to thank Dr. Gordon K. Sweatman and Dr. Nasri S. Kawar for their invaluable guidance and assistance during the course of this work.

Thanks are also due to Dr. John F. Schacher, Dr. William K. Lawlor, Dr. Lorne E. Stephen, Dr. George J. Frayha for their encouragement and advice, and Dr. Makram Kaiser for his courtesy in providing the ticks.

The writer wishes to extend his appreciation to Miss Samia Shehadeh, Miss Siranoush Oknayan, Mr. Mtanous Kosa, and Mr. Salim Lutfallah for their appreciated help.

AN ABSTRACT OF THE THESIS OF

Elias Khalil Saliba for Master of Science in Tropical Health

Title: The effect of the pesticide ronnel on the metabolism of the tick Ornithodoros savignyi.

The effect of the pesticide ronnel on the respiration rate of Ornithodoros savignyi was evaluated using a Warburg apparatus. The tick received ronnel in three different methods: (1) fed through a membrane on outdated, citrated whole human blood containing ronnel at the rate of 100 mg/kg, (2) fed on albino rats which have received an oral dose of 100 mg/kg ronnel, and (3) topically treated with 200 ug per tick.

A significant difference in the respiration rate was observed between the means of artificially fed ticks and the controls starting ten days after feeding and continuing for three days. No such difference was observed with ticks fed on treated rats. Ronnel at the rate of 200 ug per tick was found to cause an enhanced oxygen uptake in dermally-treated ticks following a marked latent period which proved to be temperature dependent. Dermally-applied ronnel caused an abnormal decrease in the weight of ticks which was associated with enhanced respiratory rate. Non-treated ticks kept at 40° and 45°C died after 288 and 120 hours respectively.

Gas chromatographic analysis of tick extracts showed that ronnel was more metabolized at 35°C than at 25°C although no appreciable difference between the two temperatures was noted in the rate of absorption of the pesticide through the tick cuticle. Coxal fluid collected from ticks artificially fed on ronnel-treated blood one and 20 hours after feeding showed detectable ronnel as determined by gas chromatography.

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I. INTRODUCTION

The control of ectoparasites with pesticides has been extensively practiced in the last two decades, and until better methods and less toxic and hazardous materials are produced, it seems likely that these chemicals will remain paramount. Organophosphorus compounds constitute a major portion of these pesticides. Furthermore, organic phosphates have replaced chlorinated hydrocarbons where resistance has developed and storage and persistance of the pesticide in fatty tissues and milk have occurred. The effects of pesticides on insects have been investigated by many workers. Such effects on other arthropods, mainly ticks, have by contrast received very little attention, and a comparison between these two classes of arthropods, in this respect, is not possible.

Since no research on the effect of pesticides on the metabolic rate of soft ticks had been previously carried out, the present work was undertaken to study the effect of ronnel on the metabolism of Ornithodoros savignyi Audouin, 1827, an argasid prevalent in the Middle East. Ronnel was chosen for this study since it has been recommended for the control of certain internal and external parasites on warm-blooded animals. Soft ticks feed on

their host for only short periods of time, usually not exceeding two hours, hence, the metabolic processes of these ticks are controlled by ambient conditions on the ground and not by that of the host. As variations in temperature may influence these metabolic processes, different temperatures were used in the present investigation.

II. REVIEW OF LITERATURE

The Tick

Biology

Ornithodoros savignyi known as the "Eyed Tampan" or "Sand Tampan" is locally distributed through the arid parts of North, East, and South Africa, the Middle East, India, and Ceylon. It may be indigenous to the Middle East. Camels are most frequently infested, but all domestic animals may serve as hosts, sometimes even fowls. Human beings are frequently bitten, especially when they sleep in camel yards or sit under trees commonly used by domestic animals for shade. The female *O. savignyi* dig into sandy soil to deposit its eggs. The larvae are non-motile and do not feed. They molt to nymphs in a very short time, not exceeding 24 hours. The number of molts is not yet precisely determined but may vary from four to six (Hoogstraal, 1956). When feeding, nymphs and adults require 15 to 30 minutes to reach repletion with coxal fluid produced both while feeding and after detachment. Most of the coxal fluid is usually produced in the first hour, while some individuals produce coxal fluid for longer periods (Lavoipierre and Riek, 1955). *O. savignyi* shows great ability to limit water loss

at high temperatures, and can exist in deserts where little other life is sustained (Hoogstraal, 1956). Nevill (1964) showed that O. savignyi was stimulated by and attracted to small amounts of carbon dioxide. He was able to collect 4553 ticks in two hours with a small piece of dry ice. He also found that warm objects attracted tampans only after stimulation by carbon dioxide and that moisture increased the attractiveness of the warm objects.

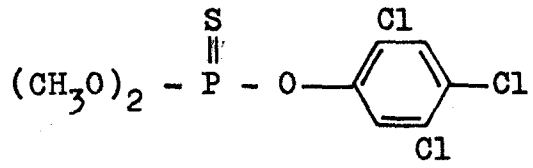
Disease Relations

The bite of O. savignyi may have severely painful sequelae. Camels and cattle may suffer greatly and even be killed by exsanguination, but this tampan has never been found infected with pathogenic organisms in nature (Hoogstraal, 1956). Taylor et al. (1955) found that O. savignyi experimentally infected with Sindbis virus transmitted the organism by bite to infant mice. Hurlbut (1956) found that after parenterally infected, O. savignyi transmitted West Nile virus when biting suckling mice, and isolated the virus from the coxal fluid of these ticks. The tick was also infected from feeding on infected mice but failed to transmit the virus when biting. The potential role of this tick as a disease vector appears therefore to be minimal.

The Pesticide

The organophosphate pesticide ronnel was used in the

current investigation. It is a phosphorothionate used both as an insecticide and acaricide. Ronnel, O, O-dimethyl O- (2, 4, 5-trichlorophenyl) phosphorothionate, having the following structural formula:



is also known as Korlan®; Trolene®; Nankor®; Viozene®; Dow ET-57, and fenchlorfos (Frear, 1965).

Ronnel has shown promise as an effective systemic and contact pesticide. When administered orally at about 100 mg/kg body weight to dairy cattle, steers, and heifers, ronnel has been found by many workers, notably McGregor and Bushland (1957), Roth and Eddy (1957), Thurber and Peterson (1960), and Knapp et al. (1960) to be effective against the cattle grubs, Hypoderma lineatum and H. bovis in the backs of these animals. In laboratory tests, McGregor and Bushland (1957) found ronnel toxic to screw-worm, Callitroga hominivorax and stable flies, Stomoxys calcitrans when given orally or subcutaneously to guinea pigs at 100 mg/kg. Drummond (1965) found that the minimal doses (mg/kg) of ronnel required to kill 100% of the screw-worms, secondary screw-worms, Cochliomyia macellaria, and black blow flies, Phormia regina were 100, 50, 100 respectively when administered subcutaneously to guinea pigs. As a feed additive to control dipterous flies, ronnel

was effective against larvae of the house flies, Musca domestica, and Musca autumnalis in cattle dung and chicken and broiler droppings (Sherman and Ross, 1960; Treece, 1962; Ode and Matthysse, 1964; Dorough and Arthur, 1966). Similarly, Sherman et al. (1962) found this method highly effective against maggots of Fannia pusio, Chrysomya megacephala, and Parasarcophaga argyrostoma. When added to rat baits, ronnel killed oriental rat fleas, Xenopsylla cheopis before the rats died in a feeding period of five or more days (Bennington, 1960). Ronnel was highly effective against demodectic mange Demodex folliculorum following systemic treatment of chronically infested dogs (Walton, 1963). Similar results were reported for the control of northern fowl mite, Ornithonyssis sylviarum on chickens (Linkfield and Reid, 1958; Fnapp and Krause, 1960; Foulk and Matthysse, 1963). At the concentration normally fed to animals (100 mg/kg), ronnel was not promising as a systemic pesticide for the control of hard ticks. When fed on goats and sheep receiving 100 mg/kg ronnel, lone star ticks, Amblyomma americanum and stable flies were found to survive (Drummond, 1958). When administered to rabbits at 200 mg/kg, ronnel gave only a 23% kill to the gulf coast tick, Amblyomma maculatum while producing a 100% mortality to stable flies and bedbugs, Cimex lectularis (Fnapp and Eden,1960). Harvey and Brethour (1961) found that when ronnel was fed to steers at 30 mg/kg, it

failed to control the ear tick, Otobius megnini. Ronnel appears therefore to be less effective systemically against ticks than some insects.

Ronnel proved to be effective when administered as a contact toxicant. Drummond et al. (1960) found that a spray of 0.75% ronnel gave equal protection as a standard 0.5% toxaphene against the lone star tick. When tested as single dips, ronnel at 0.5% was only partially effective against Boophilus annulatus and B. annulatus microplus, but when the cattle were dipped a second time, the treatment was complete and no nymphs molted (Drummond et al., 1964). Highly effective treatment for Dermacentor nitens, the tropical horse tick, was obtained with 5% ronnel smear (Drummond and Graham, 1964). Excellent control of larval and nymphal ear tick, Otobius megnini was obtained with ronnel either as 0.75% spray or 5% dust and smear (Drummond et al., 1967). Ronnel was the first organophosphorus compound effective as a cord impregnant for house fly control (Kilpatrick and Schoof, 1959a; Weinburgh et al., 1961). As a spray, it was also very effective and rapidly acting against female house flies (Eddy, 1961). Excellent control of horn flies, Haematobia irritans has been reported from the use of back rubbers impregnated with ronnel (Dobson and Peterson, 1963; Hoffman and Roberts, 1963). Similar results were obtained when ronnel was used either as a dust (Johnson and Langford , 1960) or as a spray (Dorsey

et al., 1962).

As to its mammalian toxicity, ronnel is considered one of the safest organophosphorus toxicants. The acute oral LD_{50} to female rat is 2630 mg/kg, and the dermal LD_{50} to rabbits is 1000 mg/kg (Vanderkar, 1968). When fed at the rate of 100 mg/kg, ronnel was generally safe to goats, sheep, cattle, and horses (Radeleff and Woodard, 1957; Drummond, 1958; Jackson et al., 1960; Radeleff et al., 1963).

Linkfield and Reid (1958) and also Fnapp and Krause (1960) found neither toxic symptoms on birds treated with ronnel for the control of ectoparasites nor unfavorable taste in their eggs. Furthermore, the eggs hatched normally. Knowles and Arthur (1966) found no detectable residues in the milk of dairy cows where the back rubber application of ronnel was practiced. Kawar et al. (1968) reported that ronnel disappeared from milk four days after spraying of cows with 0.75% ronnel. Thus, ronnel appears to be a safe pesticide for the control of ectoparasites on warm-blooded animals.

It is generally accepted that cholinesterase is the enzyme most significantly involved in organic phosphate poisonings (Metcalf, 1955; West and Hardy, 1961; O'Brien, 1967), although other esterases have been implicated in the poisoning process (van Asperen, 1958; Winteringham and Lewis, 1959). Many organophosphorus compounds are poor inhibitors

of cholinesterase *in vitro*, yet are potent inhibitors *in vivo* (O'Brien, 1960). One group of such compounds is phosphorothioate (- thionate) containing the thionosulfur group (P = S) exemplified by ronnel. These compounds are themselves inactive and have to be converted in biological systems to their corresponding oxygen analogs or oxons (P = O) which are active cholinesterase inhibitors (O'Brien, 1960; West and Hardy, 1961). Metcalf and March (1949) found that paraoxon was more toxic than parathion to adult female house flies and worker bees, *Apis mellifera*, and produced toxic effects much more rapidly. They also observed that compounds with phosphoryl group were more toxic than those with thiophosphoryl group. Diggle and Gage (1951) noted that parathion underwent a change to an active inhibitor *in vivo* and demonstrated that it was converted by the liver to an unstable potent anticholinesterase agent. O'Brien (1957) found that in *in vitro* studies, malaoxon was a more potent inhibitor of various cholinesterases than malathion. He also reported that malaoxon was a product of malathion metabolism in the cockroach and the mouse. March *et al.* (1955) found a secondary pathway in the case of thiono-isomer of systox involving the oxidation of the thiono-sulfur to produce the phosphate, its sulfoxide and sulfone. Plapp and Casida (1958) obtained evidence for the partial conversion *in vivo* of all the phosphorothioates they studied (parathion, methylparathion, diazinon, ronnel, chlorthion,

and dicapthon) to their corresponding phosphates. Hopkins (1962) was able to identify the oxygen analog of ronnel in the extracts of ronnel-treated Madeira cockroach, Leucophaea maderae, though such identification was not demonstrated in the earlier studies of Plapp and Casida (1958) and Hopkins (1961). Lovell (1964) found that if the dimethoate studied for in vitro fly head cholinesterase inhibition contained 0.187% of its oxygen analog, then the inhibitory effectiveness would increase ten fold. The reason for the high anticholinesterase activity of the oxygen analogs of thiophosphates appears to be in the electron factors in these compounds, that is, the P = S may be less electrophilic than P = O and consequently inadequately positive to undergo a rapid reaction with cholinesterase (O'Brien, 1960).

Oxygen Uptake and Pesticides

Nothing has been written on the effects of a pesticide on the respiration of ticks. For insects, however, Lord (1950) found that most of the insecticides tested on Tribolium castaneum at 25°C caused an increase in oxygen uptake at some stage before death. Chlordane and toxaphene were outstanding for the long period elapsing before any increase in oxygen uptake occurred, with peak times at 1000 and 2400 minutes respectively. The peaks for BHC, DDT, DNC, HETP, and pyrethrins appeared in less than 100 minutes,

and the time-lag for parathion was 140 minutes. He also noted that an increase in the concentration of applied parathion, up to a certain level, shortened the latent period. This did not appear to hold for DNC, γ-BHC, and the pyrethrins. At their lowest toxic concentrations, these insecticides killed T. castaneum without causing any considerable change in oxygen uptake.

Harvey and Brown (1951) studied the effects of 26 insecticidal compounds upon the rate of oxygen consumption of the German cockroach, Blattela germanica, at 27°C. The chlorinated hydrocarbons were characterized by a rapid rise in oxygen consumption. In the case of DDT and methoxychlor, the increase occurred immediately after injection, and reached a peak within 30 minutes. With lindane the respiratory rate continued to rise for a longer period to reach a peak in 60 minutes when it was five times the normal (0.8 cu mm O_2/min./roach). The three compounds caused paralysis of the insect 60 minutes after the peak of respiration, halfway on its return to the normal level. With toxaphene a three-fold increase was preceded by a latent period of 60 minutes with paralysis commencing one hour after the peak of respiration was complete. The "Chlordane compounds" (chlordane, heptachlor, aldrin, and dieldrin) were characterized by a long latent period followed by an abrupt rise in respiration. With heptachlor the latent period overaged 150 minutes, and the peak of

respiration was about five times the normal level. The latent period was greatly prolonged in the case of ∝ - chlordane and B-chlordane and caused a six-fold increase after latent periods of at least 400 minutes and 250 minutes respectively. Both aldrin and dieldrin caused a five-fold increase in respiration, with latent periods of 200-300 minutes for aldrin and 100 minutes for dieldrin. The organic phosphates tested in the experiment also induced a respiratory stimulation of about three times the normal. There was a latent period of 60 minutes in the case of parathion while with TEPP the stimulation appeared immediately. Similar results were obtained by spray application of these insecticides. Harvey and Brown (1951) also reported that the two insecticides of plant origin ryania and rotenone, had a depressing effect after causing a slight initial rise in respiration. Summarizing the effects of rotenone on respiration, O'Brien (1966) stated that:

> "Rotenone appears to inhibit specifically the coupled oxidation of $NADH_2$ and reduction of cytochrome-b. A sequence is that rotenone inhibits that part of oxygen uptake which goes via $NADH_2$ (and probably $NADPH_2$), for example, that part caused by glutamate or pyruvate oxidation".

Winteringham and Harrison (1956) found that the rate of oxygen uptake of house flies treated with DEP reached double the normal level after two hours and fell steadily to about the original level after five hours. Ezzat and Highland (1957) as reported by Keister and Buck (1964) found that malathion caused a slight

depression in the respiration rate of adult Pseudococcus maritimus while a slight increase was obtained with systox. Similarly, malathion caused no elevation in respiratory rate of the German cockroach (O'Brien, 1956).

Studying the effects of DDT on oxidative metabolism in susceptible and DDT-resistant Aedes aegypti, Micks and Murthy (1961) found that susceptible larvae exhibited a higher respiratory rate than the DDT-resistant ones. They also noted that whereas various concentrations of DDT increased the rate of oxygen consumption of resistant larvae, low concentrations of the insecticide increased oxygen uptake of the susceptible strain and high concentrations decreased it. When the temperature was increased from 28°C to 37.1°C, the oxygen consumption of the resistant larvae was higher. Bennett and Dowden (1966) found the mean oxygen consumption of the swamp crawfish, Procambarus clarki, treated with chlordane very high at 1 ppm, very low at 2 ppm, increased at 4 and 8 ppm, and decreased from 8 to 16 ppm.

Relating anticholinesterase action of organophosphorus compounds and respiration of house flies, Winteringham and Lewis (1959) stated that:

> "The acute and lethal effects of anticholinesterase action in mammals may be largely ascribed to the failure of the neuromusculature of respiration and anoxia. This suggests some additional lesion in the insect, since there was no respiratory failure of the paralyzed insect in terms of cholinesterase inhibition".

O'Brien (1960) commenting on elevated respiratory rates in

insects after exposure to insecticides mentioned that such effects are perhaps a consequence of hyperactivity of the poisoned insects. Keister and Buck (1964) believe that the symptomatology of DDT poisoning, involving incoordination, high frequency "jitters", prostration and spasm, seems ample explanation for the quickly apparent and often greatly enhanced concurrent rate of oxygen uptake. The effect of pesticides on oxygen uptake was best summarized by Keister and Buck (1964), when stated that:

> "Many of the bewildering array of contemporary insecticides affect respiration rate. It is difficult to systemize these effects because of the number of chemical groups involved, the greatly different rates of penetration into different insects, and the problem of achieving comparable concentrations at the only place where it is meaningful - the site of action".

III. MATERIALS AND METHODS

The Parasite

The ticks used in the present investigation were adult female O. savignyi obtained from Dr. Makram Kaiser, Medical Zoology Department, Naval Medical Research Unit No. 3, Cairo, United Arab Republic. All ticks were collected from Dahshur, El-Badrshein, Giza Governorate in August 1967 where spraying with pesticides has not been practiced. In the laboratory, the ticks were kept at 25°C and 80% relative humidity (R.H.). Partially engorged ticks were used in all topical application experiments. For feeding experiments ticks were labelled either with small number attached to their bodies or with a non-toxic stain. For the purpose of the experiment, the tick was considered "paralyzed" when it could not move when stimulated with carbon dioxide and heat, and "dead" when the difference in its manometric reading for 24 hours was less than 10 mm (0.009 ul O_2/mg/hr) since heat-killed ticks demonstrated a similar difference when run at 25°C and above.

Feeding

Artificial Feeding

Feeding of ticks was carried out in an apparatus modified from that described by Tarshis (1958). The

apparatus consisted of a thermostatically controlled water bath maintained at 35°C, a chamber to hold blood, and a feeding chamber to which a membrane was attached to contain the ticks (Figure 1). The blood chambers consisted of 30 ml sterile glass beakers which were filled with 20 ml blood at the time of feeding. The feeding chambers consisted of glasstubes 7 cm in height and 4 cm in diameter opened from both sides. This was wide enough to accomodate about eight ticks. The membrane, made into two layers to prevent leakage of blood, was secured to one side with a rubber band. "Fourex", a commercial prophylactic skin prepared from caeca of lambs purchased from Julius Schmid, Inc., N.Y., was used.

Reference grade ronnel (Dow Chemicals Co.) was added at the rate of 100 mg/kg, to outdated, citrated whole human blood contained in close sterile tubes and obtained from the American University Hospital, American University of Beirut. The blood was mechanically shaken for 15 minutes at 35°C to insure complete solubility of the pesticide and then transferred to the blood chambers in the water bath. Eight weighed ticks were placed in the feeding chambers and were allowed to engorge, after which they were removed and left for one hour at room temperature to allow for the excretion of coxal fluid which was collected with small pieces of filter paper and dipped in 10 ml normal hexane and stored at -20°C for later extraction. The ticks

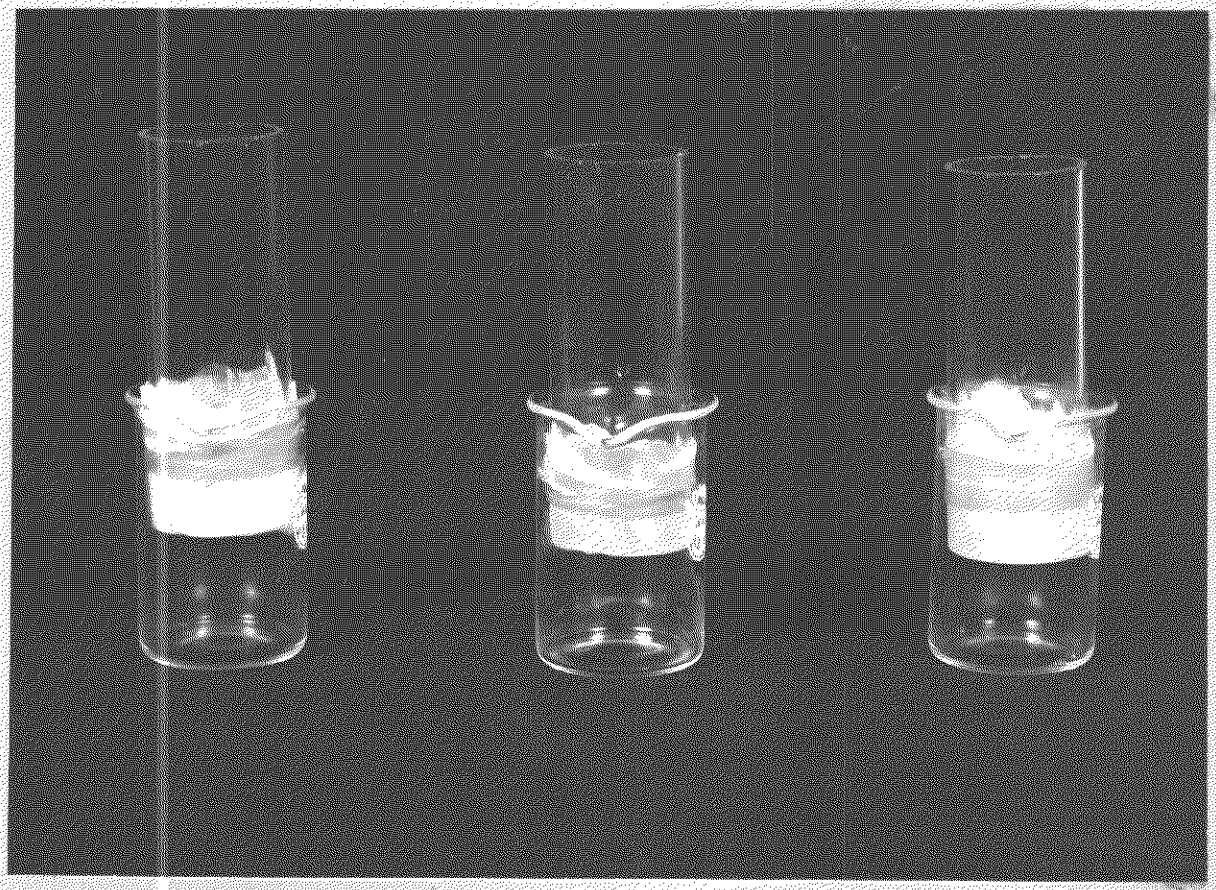


Figure 1. Apparatus for tick feeding.

were re-weighed and placed in silk bags to prevent their mobility in the Warburg flasks. One hour was needed for equilibration of pressure before readings were recorded. Eight ticks used as controls were similarly fed on clean blood. This experiment as well as all others was performed in duplicate.

Rat Feeding

Ronnel at the rate of 100 mg/kg body weight dissolved in 3 ml corn oil (Mazola) was given to a 200 g albino male rat through a feeding tube (size 5 french, length 38 cm). Ten hours later the rat was anaesthetized with sodium barbiturate (Nembutal) using 29 mg/kg. This time-lapse between administration of ronnel and tick feeding was necessary to allow for maximum concentration of the pesticide in the blood of rat (Plapp and Casida, 1958). Eight weighed ticks were placed on the shaved abdomen of the rat until engorged, re-weighed after an hour and placed in the Warburg flasks as described earlier. One ml of rat blood was removed from the heart with a one ml heparinized syringe immediately after removing the ticks and stored at -20°C for analysis. Controls were similarly fed on a rat receiving corn oil alone.

Topical Application

A ronnel solution of 200 ug in 2 ul acetone was applied by microcapillary tubes (Desaga Co.), to the

mid-dorsal part of each tick. The ticks were then placed in manometric flasks for oxygen determination. Controls received 2 ul of acetone each. Application of the pesticide was carried out at room temperature (22 ± 1°C).

Oxygen Uptake Determination

Oxygen uptake was measured in a Warburg apparatus (American Instruments Co.),using 15 ml manometric flasks. In all experiments ticks were individually placed in uniform silk bags and placed in the flasks. A 0.4 ml solution of 20% potassium hydroxide was added to the central well of the flask to keep carbon dioxide pressure zero and provide 80% R.H. A filter paper was dipped in the alkali to increase its surface area and insure better carbon dioxide absorption. For each experiment two manometers were used as thermobarometers.

Pesticide Absorption Through Cuticle

To determine the degree of ronnel absorption through tick cuticle with time at 25°C and 35°C and 80% R.H., a group of ticks, four for every time interval, and each receiving ronnel solution of 200 ug in 2 ul acetone, were placed in silk bags and kept at the required temperature and humidity in a desiccator containing a saturated solution of $CaHPO_4$ and placed in regulated incubators. At either 0, 24, 48, 96, or 120 hours, each tick and its bag were slowly

dipped in 10 ml acetone for 10 seconds to wash the insecticide. The ticks and their washings were refrigerated at -20°C for extraction and analysis.

Pesticide Extraction and Determination

Extraction

The ticks were extracted according to a modified procedure of Hopkins (1962). They were individually ground with dry ice in a porcelain mortar for about a minute, then 5 g of anhydrous sodium sulfate and 5 ml acetone were added and grinding continued till a fine powder was obtained. The contents were warmed to room temperature and filtered into 25 ml graduated evaporation flasks. The filtrate with three equal washings of the mortar and the filter paper were evaporated, except for few drops, with a gentle flow of nitrogen gas passing over a glass tube with anhydrous calcium sulfate. The sides of the flask were then washed with n-hexane (chromatographic grade) and re-evaporated until one ml of the solution was obtained. The extracts were refrigerated at -20°C for the determination of the pesticide with gas liquid chromatography. Similarly, coxal fluid-containing haxane and acetone washings of ticks were evaporated with the final n-hexane solutions stored at -20°C for analysis. Rat blood and that for percent recovery determination were extracted with three equal volumes of n-hexane and stored at -20°C for later gas

chromatographic determination. Clean ticks and those treated for estimation of percent recovery were extracted in a manner similar to the treated ones.

Determination

All determinations were carried out in a gas liquid chromatograph. The analytical instrument was a Varian Aerograph Hi-Fi model 600 C gas chromatograph equipped with an electron capture detector containing a 250 mc tritium ionization source. The instrument was operated with a 90 volt potential across the detector. The recorder used was a 1-mv Leeds and Northrup model H with a disc integrator unit. The analytical column was a coiled Pyrex glass 3 mm od and 150 cm long and packed with 5% Dow 11 Silicone on 60/80 mesh hexamethyldisilazane (HMDS)-treated Chromosorb W. Both compounds were obtained from Varian Aerograph, the supplier of the gas chromatograph. Oven temperature was maintained at 205°C and that of the injector at 250°C. Nitrogen gas flow rate was kept at 40 ml per minute. Samples were injected with a 10 ul syringe (Hamilton Co.) and the areas under the curve, measured by the disc integrator, were compared with a standard curve established from standard insecticide solutions.

Treatment of Data

Calculations of oxygen uptake consumed per mg body weight during a given time, one minute, were made according to the method described by Umbriet et al. (1964) as presented

in the following formulas:

$$Ko_2 = \frac{V_g \frac{273}{T} + V_f a}{P_o}$$

$$x = Ko_2 h \text{ (ul)}$$

Where:

Ko_2 = flask constant (mm).

h = the observed change in the manometer reading in mm.

x = ul gas (0^oC, 760 mm Hg pressure).

V_g = volume of gas phase in flask (mm^3).

V_f = volume of tick, silk bag, filter paper, and KOH. Volumes of ticks were considered equivalent to their weights (mm^3).

T = temperature of bath in absolute degrees (273 + temperature in ^oC).

a = solubility in reaction liquid of oxygen gas.

P_o = standard pressure which is 10,000 mm Brodie's fluid.

All the above calculations were carried out on a Friden[2] automatic calculator. Calculations of means, standard deviations and the t-value were facilitated by use of an IBM 1620[R] Computer.

IV. RESULTS AND DISCUSSION

Feeding

Artificial Feeding

Ticks were found to feed equally well on treated and non-treated blood. However, there were individual differences among ticks as to the amount of blood ingested, but with no apparent effect upon the respiration rates among the treated ones. The means of the oxygen consumption rate for both treated and control ticks are presented in Figure 2. When ticks were kept at 25°C a significant difference in oxygen consumption of the treated ones as compared to the controls appeared nine days after engorgement and continued until the 13th day (Table 1). This increase is most probably due to the activation of ronnel to a more potent anticholinesterase resulting in hyperactivity of the tick. When the temperature was raised to 35°C both groups exhibited a very sharp increase in respiration rate but with no significant difference between the two groups indicating that probably most of the ronnel was already metabolized to a non-toxic form.

Rat Feeding

Results of the rat feeding experiment presented in

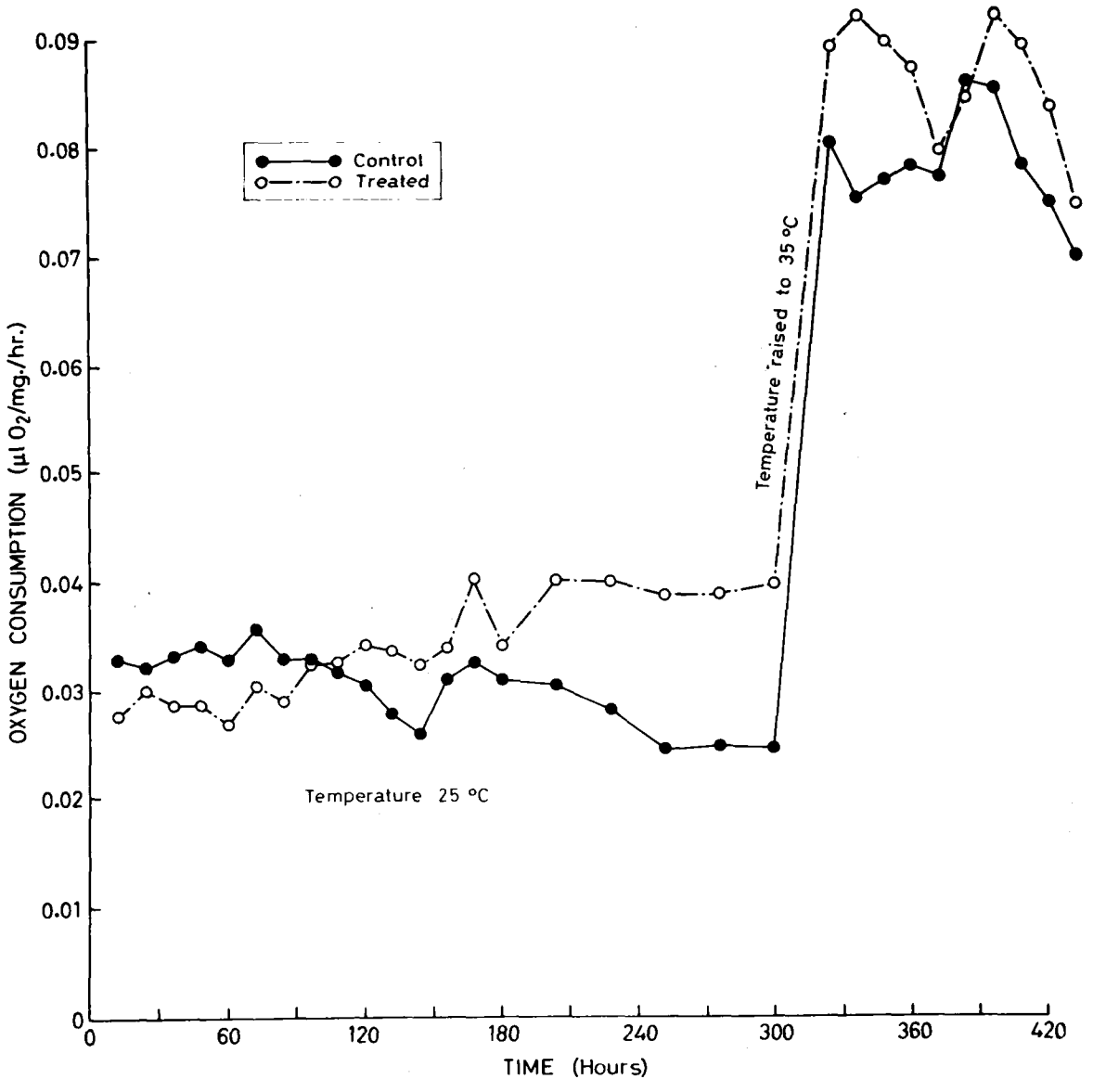


Figure 2. Mean of oxygen consumption rate of *O. savignyi* artificially fed blood containing ronnel at the rate of 100 mg/kg.

Table 1. Mean, standard deviation, t-value, and probability of being from the same universe of the respiration rate of ticks artificially fed on ronnel-containing blood at the rate of 100 mg/kg at 25°C.

Temperature °C	Hours after application	Mean (ul/mg/hr) Treated	Mean (ul/mg/hr) Control	S.D.	t-value	Probability
25	0 - 12	.028	.033	.010	1.003	0.300
	12 - 24	.030	.032	.017	0.235	0.800
	24 - 36	.029	.034	.012	0.735	0.500
	36 - 48	.028	.034	.012	0.922	0.400
	48 - 60	.027	.033	.010	1.090	0.300
	60 - 72	.031	.036	.011	0.795	0.400
	72 - 84	.029	.033	.009	0.810	0.400
	84 - 96	.032	.033	.009	0.071	0.900
	96 -108	.033	.031	.008	0.235	0.800
	108 -120	.034	.031	.008	0.821	0.400
	120 -132	.034	.028	.008	1.377	0.200
	132 -144	.033	.026	.008	1.437	0.200
	144 -156	.034	.031	.008	0.776	0.500
	156 -168	.041	.033	.009	1.616	0.100
	168 -180	.034	.031	.008	0.791	0.400
	180 -192	.040	.031	.006	2.944	0.010
	204 -228	.040	.028	.006	3.692	0.010
	228 -252	.039	.025	.006	4.054	0.001
	252 -276	.039	.025	.006	4.011	0.001
	276 -300	.040	.025	.006	4.327	0.001

Table 1 (Continued).

Temperature °C	Hours after application	Mean (ul/mg/hr) Treated	Control	S.D.	t-value	Probability
35	300-324	.089	.081	.025	0.624	0.500
	324-336	.093	.076	.020	1.494	0.200
	336-346	.090	.077	.023	1.030	0.300
	348-360	.088	.079	.024	0.715	0.500
	360-372	.080	.077	.023	0.214	0.800
	372-384	.085	.087	.021	0.103	0.900
	384-396	.093	.086	.022	0.542	0.600
	396-408	.090	.079	.021	0.959	0.300
	408-420	.084	.075	.022	0.733	0.500
	420-444	.075	.071	.023	0.356	0.700

Figure 3 and Table 2 show that when the ticks were fed on rats receiving 100 mg/kg ronnel no significant differences in oxygen consumption were exhibited. At the time of feeding, the concentration of ronnel extracted from the rat blood was 3 ppm as determined by gas chromatography. Therefore, it seems apparent that the amount of the pesticide found in the blood of rat at the time of engorgement was not high enough to cause any effect or injury to the tick.

Topical Application of the Pesticide

The results obtained with dermal applications of the pesticide at a concentration producing 100% mortality (200 ug/tick) exhibited a definite increase in oxygen uptake at the different temperatures investigated except with 15°C where the consumption rate was very low. At this temperature about 60% of the treated ticks (five out of eight) showed no rise in oxygen consumption for 20 days. The other 40% showed higher respiration rates after a latent period of 200 hours. When all the ticks were later placed at 35°C, the 60% group exhibited a respiration rate three to five times that of the controls or even the 40% group and with no appreciable latent period as shown in Figures 4 and 5. All treated ticks died at the end of the experiment.

Results of the experiments carried out at 25°, 35°, and 45°C respectively are summarized in Table 3. The table shows that both the latent period and the time lapse for

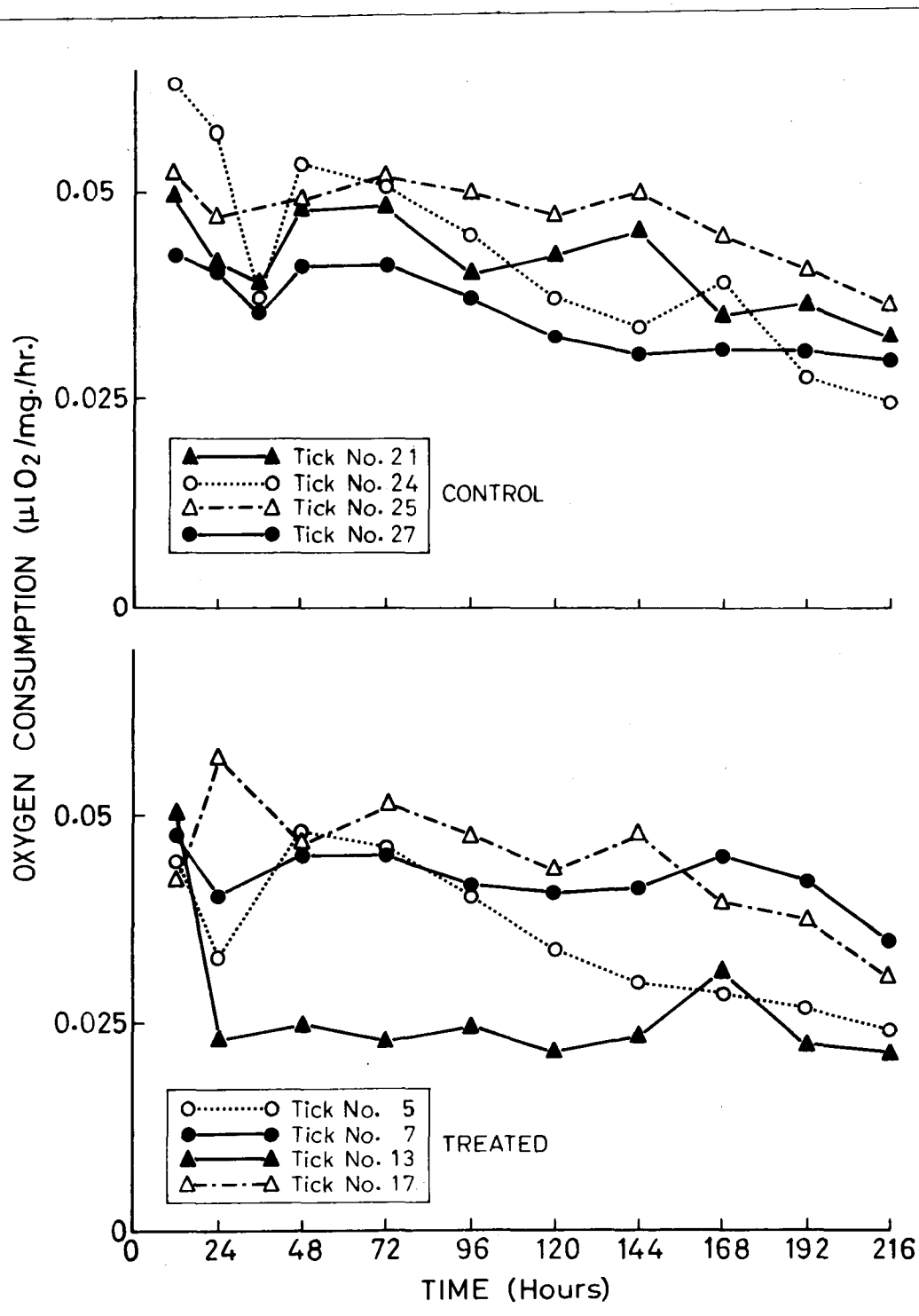


Figure 3. Rate of oxygen consumption of *O. savignyi* fed on rats receiving 100 mg/kg ronnel at 25°C.

Table 2. Mean, standard deviation, t-value, and probability of being from the same universe of the respiration rate of ticks fed on rats receiving 100 mg/kg ronnel at 25°C.

Hours after application	Mean (ul/mg/hr) Treated	Control	S.D.	t-value	Probability
0 - 12	.043	.042	.009	0.101	0.9
12 - 24	.037	.044	.011	1.313	0.2
24 - 48	.038	.037	.010	0.189	0.8
48 - 72	.041	.040	.013	0.116	0.9
72 - 96	.044	.038	.010	1.102	0.3
96 - 120	.039	.039	.013	0.306	0.8
120 - 144	.035	.034	.014	0.249	0.8
144 - 168	.035	.032	.012	0.440	0.7
168 - 192	.033	.031	.011	0.365	0.7
192 - 216	.030	.027	.007	0.659	0.5

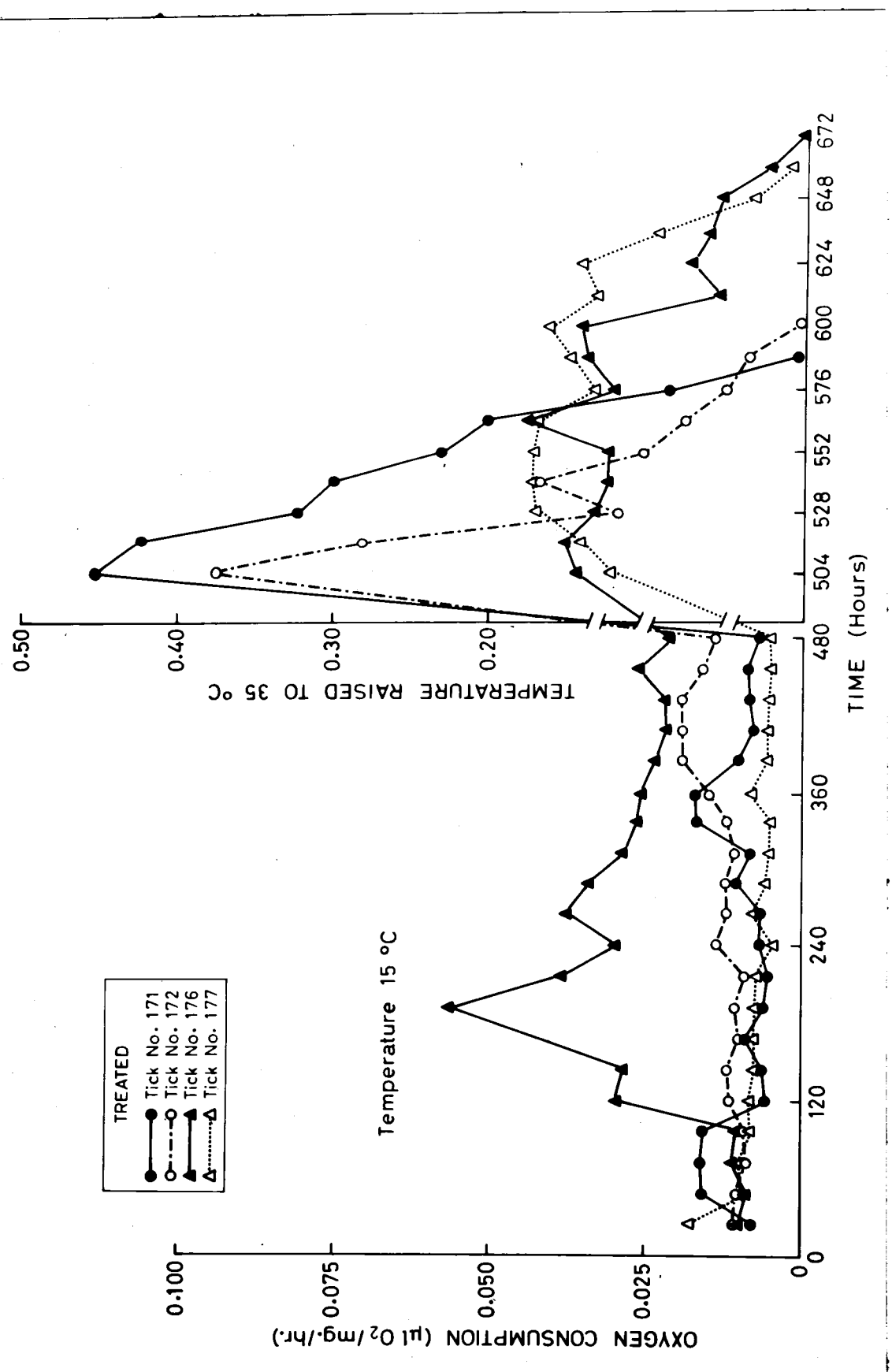


Figure 4. Rate of oxygen consumption of O. savignyi dermally treated with 200 ug ronnel at 15°C.

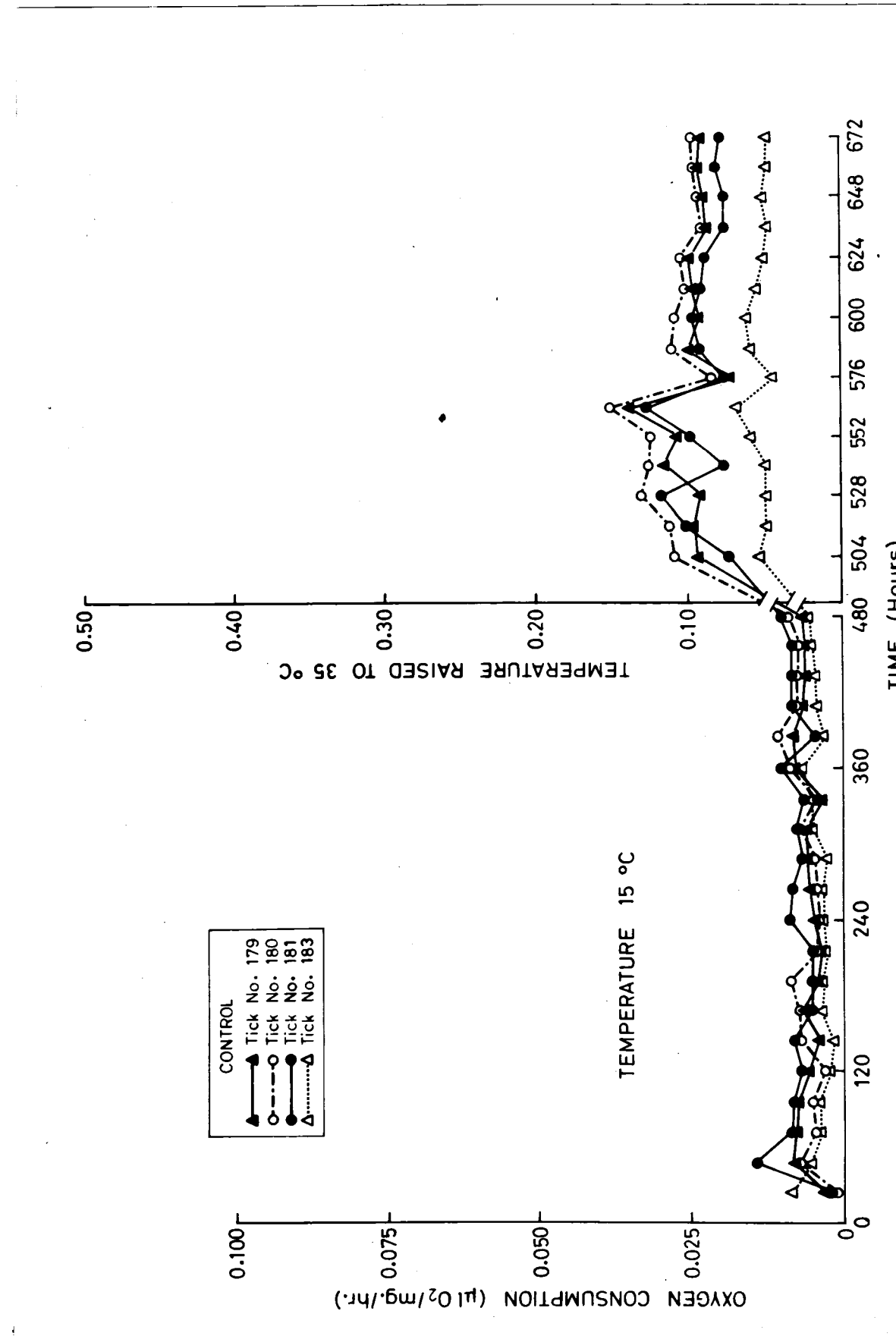


Figure 5. Rate of oxygen consumption of non-treated *O. savigmyi* at 15°C.

Table 3. Effect of temperature on O. savignyi dermally-treated with 200 ug ronnel.

Temperature °C	Mean O_2 uptake (ul/mg/hr)		Increase in O_2 uptake compared to normal	Average latent period (hrs)	Days from treatment to death
	Treated	Control			
25	.2160	.0422	5.0x	96	18 - 24
35	.5446	.1132	5.0x	60	10 - 15
45	.1981	.1321	1.5x	30	2 - 5

the death of ticks were directly related to increase in temperature, the higher the temperature, the shorter the latent period and the sooner death occurred.

Figures 6, 7, and 8, and also Table 3 indicate that the metabolic rates of both treated and control ticks were temperature dependent, the higher the temperature the higher the metabolic rate except at 45°C where the rate of the treated ticks at the peak hours decreased appreciably. Reference to Figures 8 and 9 shows that the temperatures 40°C and 45°C caused the death of non-treated ticks within 288 and 120 hours respectively. Moreover, at both temperatures, these ticks showed enhanced oxygen consumption rate before dying. Ronnel affected the respiration rate of O. savignyi in a manner similar to that observed for many pesticides except that it had an appreciable longer latent period (Lord, 1950; Harvey and Brown, 1951; Bennett and Dowden, 1966). It can be concluded that within the temperature range that permitted normal life for this poikilothermic animal, the increase in the respiration rate of treated ticks as the temperature increased and before the symptoms of poisoning appeared seems ample explanation for the decrease in the latent period, that is, the higher the temperature, the higher the metabolic rate and the faster the change of the pesticide to its potent inhibitor of cholinesterase. The elevated respiration rate of treated ticks, even at the paralysis state, as well as the enhanced rate of normal

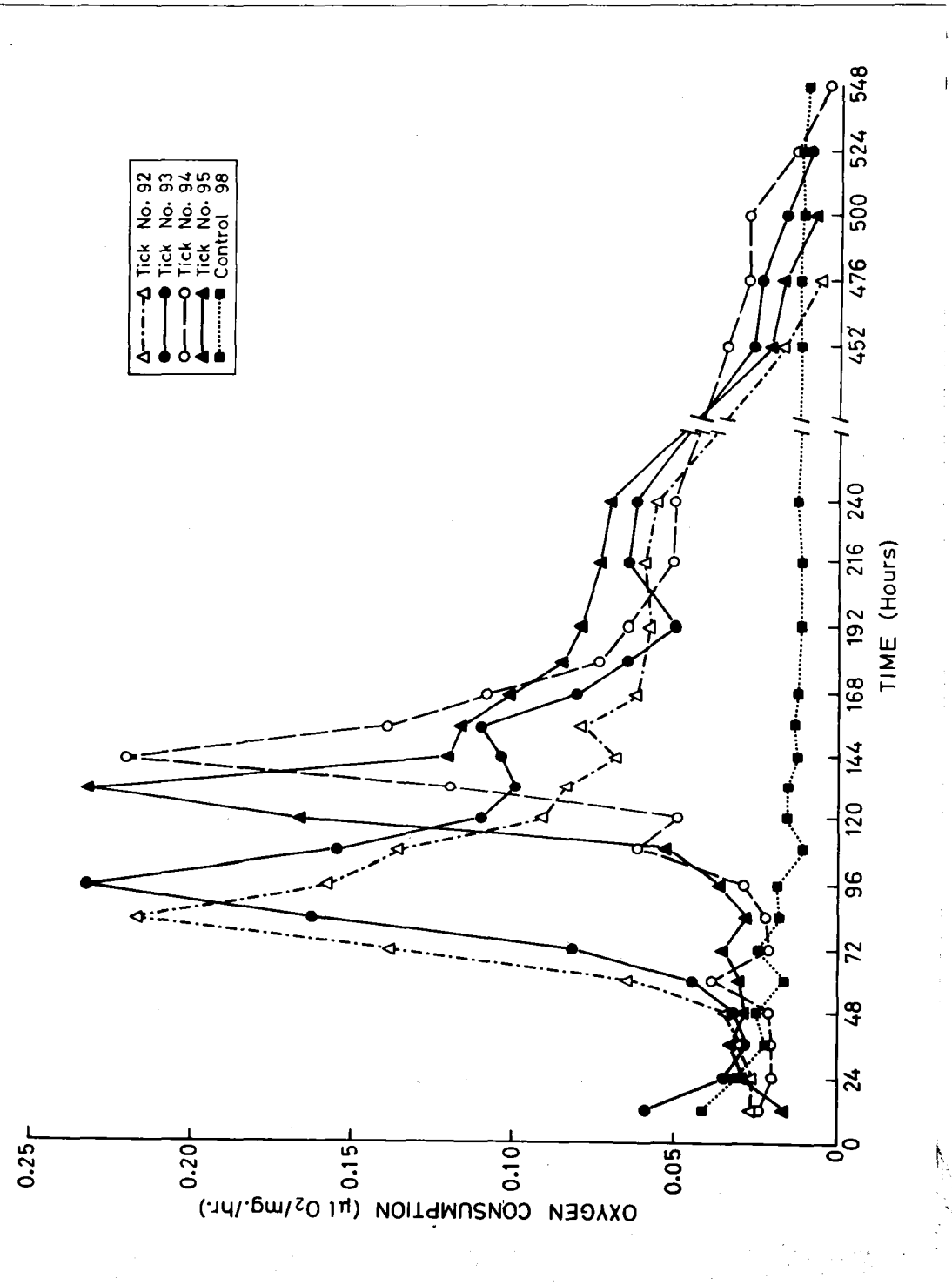


Figure 6. Rate of oxygen consumption of O. savignyi dermally treated with 200 ug ronnel at 25° C

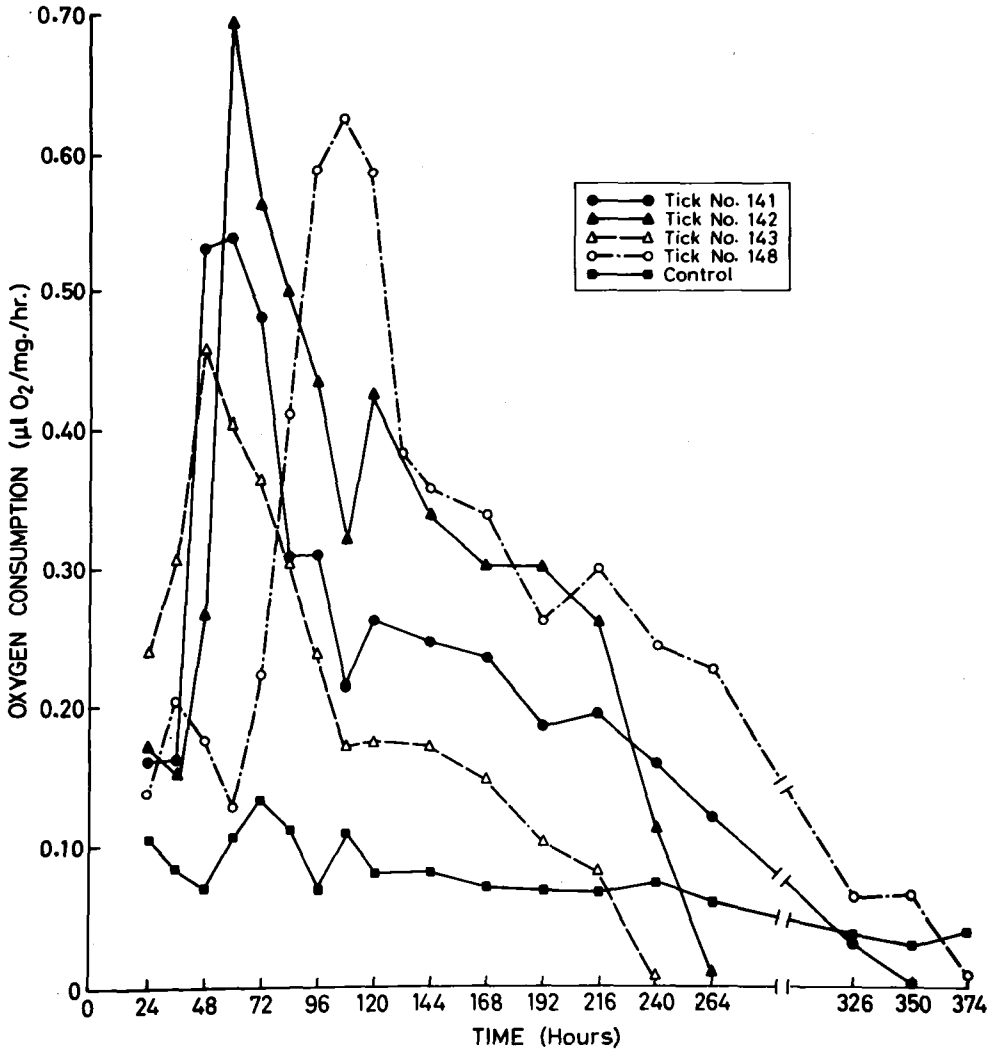


Figure 7. Rate of oxygen consumption of *O. savignyi* dermally treated with 200 ug ronnel at 35°C.

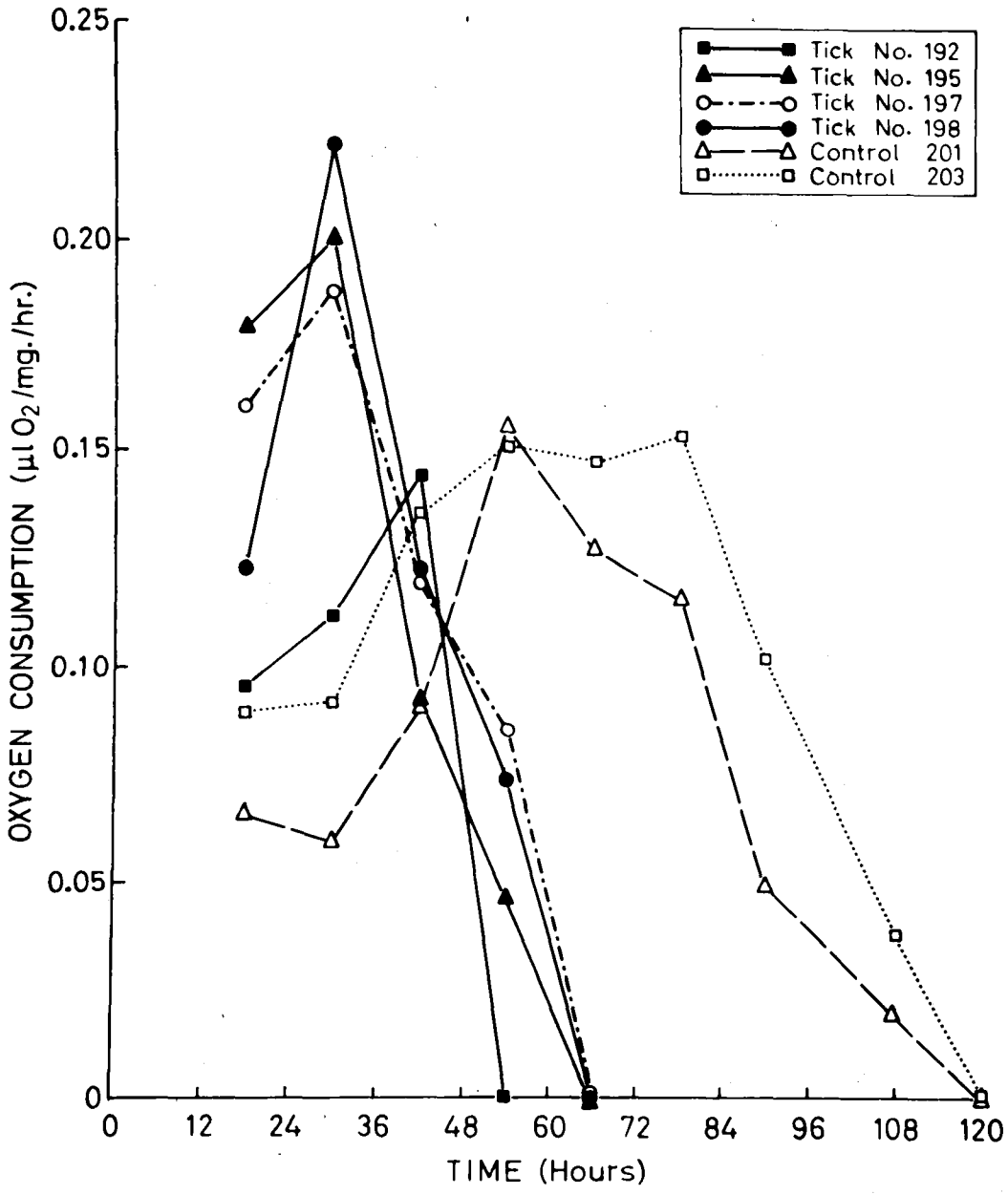


Figure 8. **Rate of oxygen consumption of** O. savignyi **dermally treated with 200 ug ronnel at 45°C.**

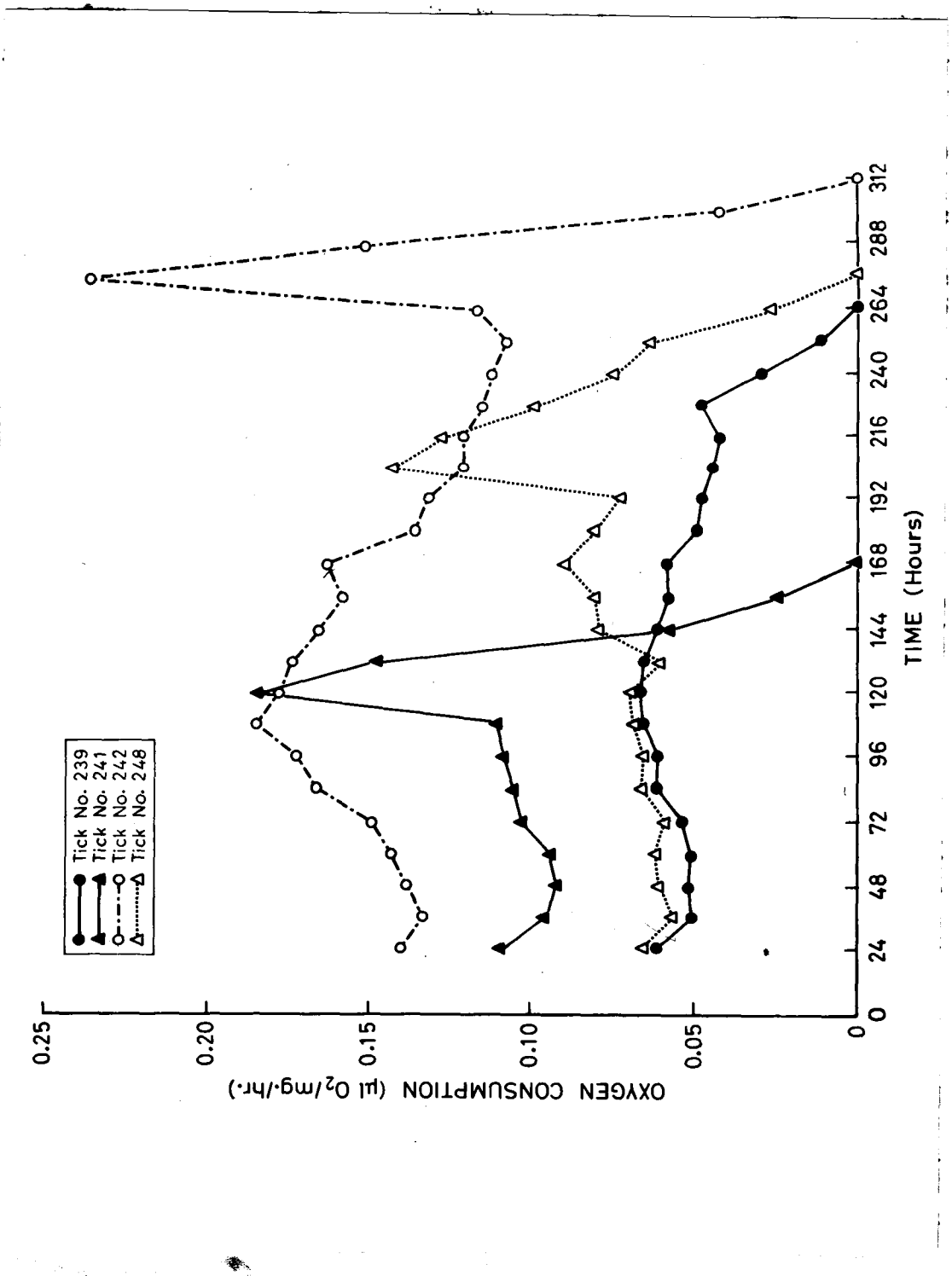


Figure 9. Rate of oxygen consumption of non-treated O. savignyi at 40°C.

ticks kept at detrimental conditions, may indicate that the tick is forced to increase its metabolic rate to cope with the critical situation it is facing.

An observation worth noting is that dermally-treated ticks demonstrated a marked decrease in weight corresponding to the oxygen uptake peak hours (Figure 10), except at 15°C where the appreciable loss in weight was observed only with the 40% group of ticks exhibiting enhanced metabolic rates. Nevertheless, when placed at 35°C, the remaining 60% group had an increased weight loss corresponding to their increased oxygen consumption rates at that temperature. The bodies of the affected ticks were shrivelled especially at the posterior region. Sorptive dusts such as Almicide and sodium fluoride cause insects to lose water at an abnormal rate (Lord, 1950; Ebeling and Pence, 1957). Lord (1950) demonstrated that water vapor was rapidly evolved from the body of T. castaneum treated with the inert dust Almicide at low humidities, and that this was associated with an increase in respiration. He also noted that a possible explanation of the increased oxygen uptake of the affected insects was that the animals attempted to replace water lost through the cuticle by water derived from metabolic sources. Ebeling and Pence (1957) reported that parathion, when applied dermally, caused the mite Tetranychus telarius to become shrunken and dark in appearance and shrivelled at death, while with Aramite or chlorobenzilate, the mites

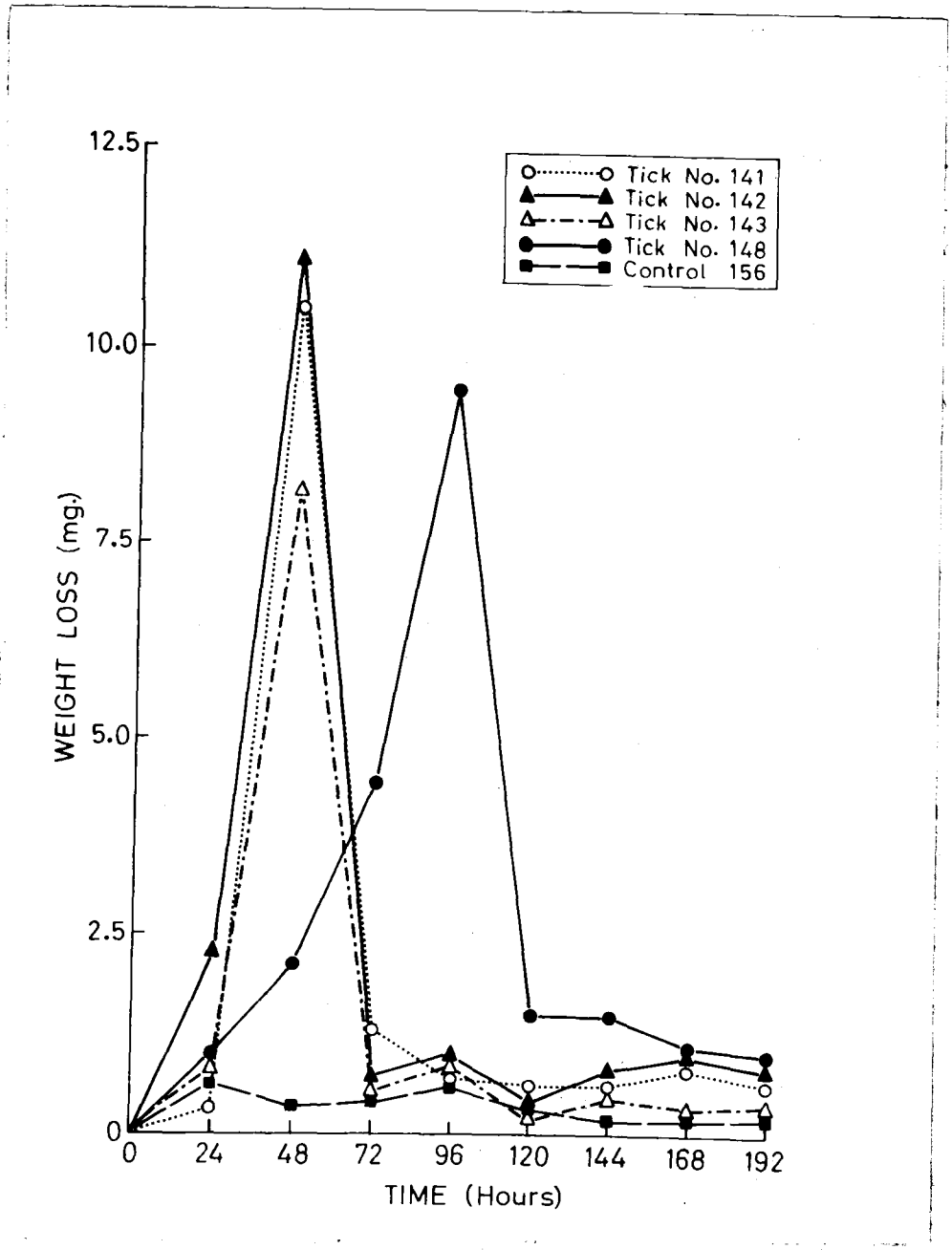


Figure 10. Weight loss (mg) of O. savignyi dermally treated with 200 ug ronnel at 35°C.

retained a remarkably lifelike appearance and posture. Ebeling and Wagner (1959) commented that a number of insecticides and other compounds can cause insects to lose water by means not always involving the removal of the lipoid protective layer, such as parathion, which was observed to cause desiccation and shrivelling of mites and termites. The facts that (1) ronnel did not cause an abnormal decrease in weight of treated ticks held at 15°C for 20 days except with the 40% group exhibiting higher oxygen uptakes at that temperature, (2) the immediate loss in the weight of the remaining 60% group showing instant increase in oxygen consumption when transferred to 35°C, (3) the latent period needed for the ticks to lose appreciable weights in all other topical application experiments, and (4) the variation of this latent period with different temperatures and its correspondence with the enhanced oxygen uptake peaks, indicate that this loss is associated, to a certain extent, with the high metabolic activity during the peak hours of oxygen consumption.

Absorption and Degradation of Ronnel

The results presented in Table 4 show that the amount of ronnel absorbed at 35°C during the first four days was not appreciably higher than that at 25°C indicating that the tick cuticle acts as a barrier against the rapid penetration of ronnel even at relatively high temperatures. The sharp

Table 4. Gas chromatographic determination of external residues of ronnel on *O. savignyi* dermally treated with 200 ug.

Temperature °C	Hours after treatment	Replicates* I	II	III	IV	Mean (ug)
25	0	162	160	168	159	162
	24	136	152	150	144	146
	48	102	92	110	105	102
	72	90	110	81	102	98
	96	110	127	80	95	103
	120	93	110	130	85	105
35	24	140	153	150	132	144
	48	90	114	100	104	102
	72	94	72	90	84	85
	96	110	80	90	87	92
	120	23	19	24	23	22

* Each figure under the replicates is the mean of three determinations.

decrease in the external residue of ronnel at 35°C after the fourth day is probably due to the breakdown of the pesticide rather than absorption since there was no corresponding increase in the internal concentration (Table 5). The data in the same table also indicate that internal degradation of the pesticide is directly related to temperature, being more at 35°C than at 25°C.

Coxal Fluid

The excretion of coxal fluid from the ticks was observed to commence about 15 minutes from the start of feeding and intermittently continue after engorgement. Most of the fluid was excreted in the first hour although some ticks continued excretion for longer periods but almost exclusively within the first day after feeding. Similar observations have been reported by Christophers (1906) and Lavoipierre and Riek (1955). Gas chromatographic analyses of coxal fluid collected from ticks artificially fed on blood containing ronnel are shown in Figures 11, 12, 13, 14, and 15. The pesticide was detected in the fluid collected from eight ticks one hour after engorgement. After 20 hours, ronnel showed a distinct peak in the fluid collected from the three ticks that continued excretion up to that time. Coxal fluid collected from one tick 16 days after feeding showed no detectable ronnel (lower limit of sensitivity 0.05 ppm), indicating that the pesticide may have been

Table 5. Gas chromatographic determination of internal residues of ronnel in O. savignyi dermally treated with 200 ug.

Temperature °C	Hours after treatment	Replicates* I	II	III	IV	Mean (ug)
25	0	34	23	31	4	23
	24	24	28	49	31	33
	48	37	38	15	33	31
	72	21	10	24	21	19
	96	12	30	17	19	20
	120	21	17	18	18	19
35	24	9	12	11	22	14
	48	15	11	6	4	9
	72	4	3	4	3	4
	96	5	2	4	2	3
	120	3	3	4	1	3

* Each figure under the replicates is the mean of three determinations.

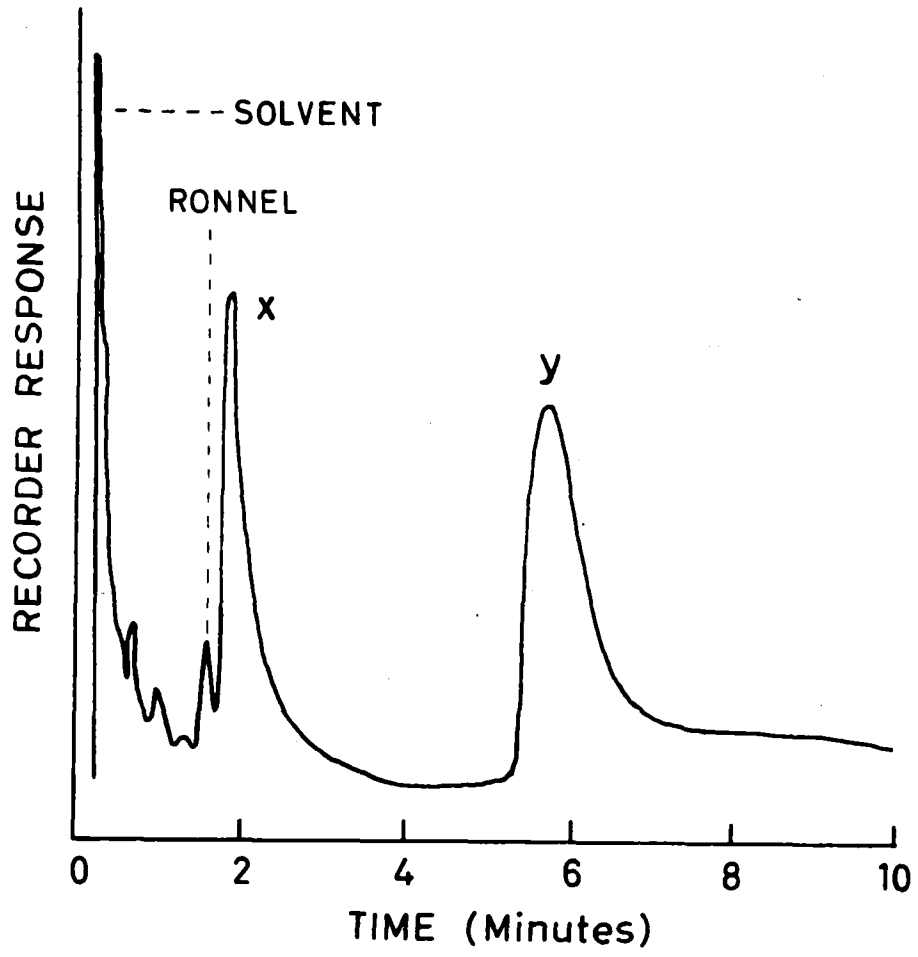


Figure 11. Chromatogram of coxal fluid collected from O. savignyi within the first hour after feeding.

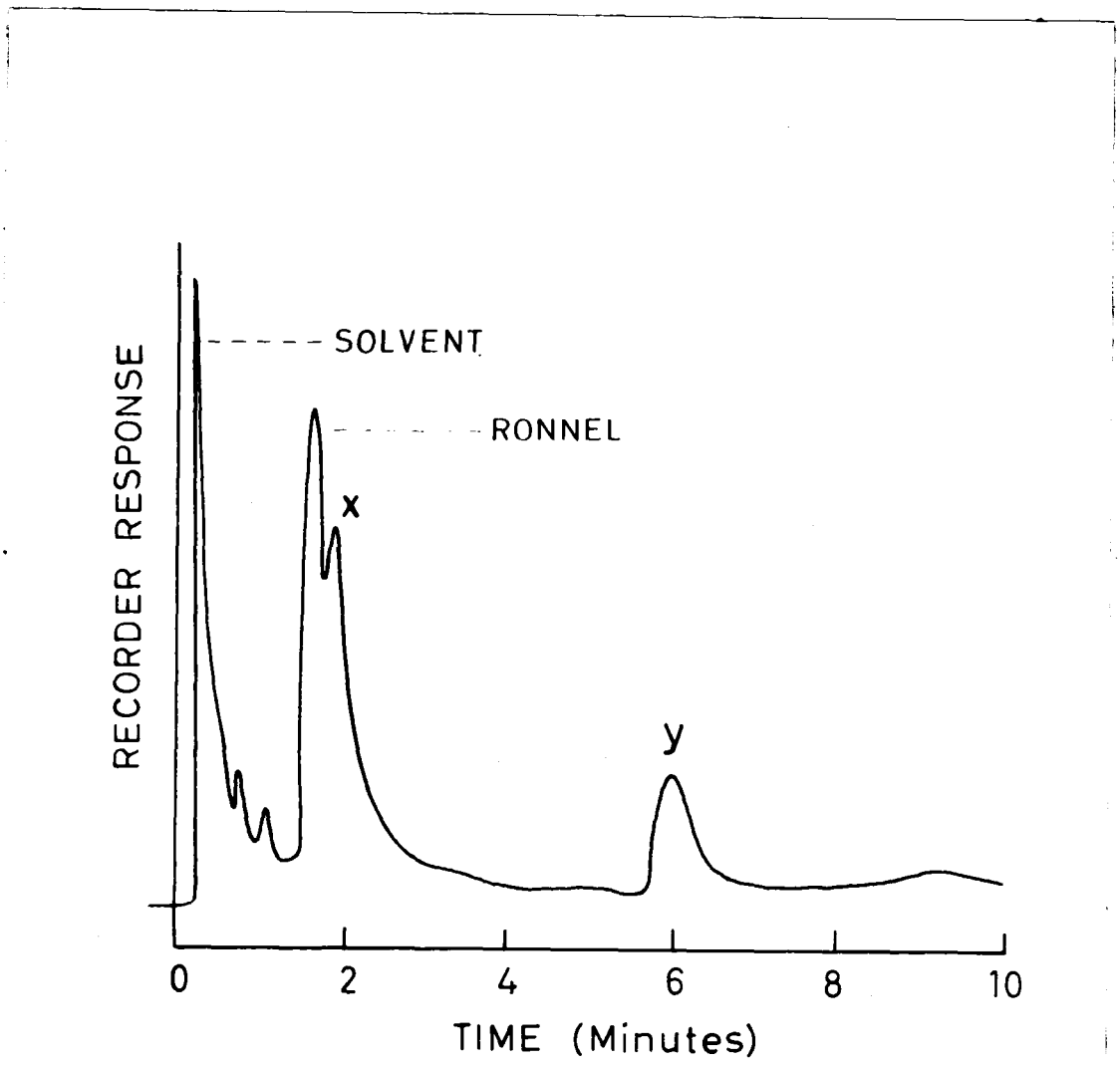


Figure 12. Chromatogram of coxal fluid collected from O. savignyi 20 hours after feeding.

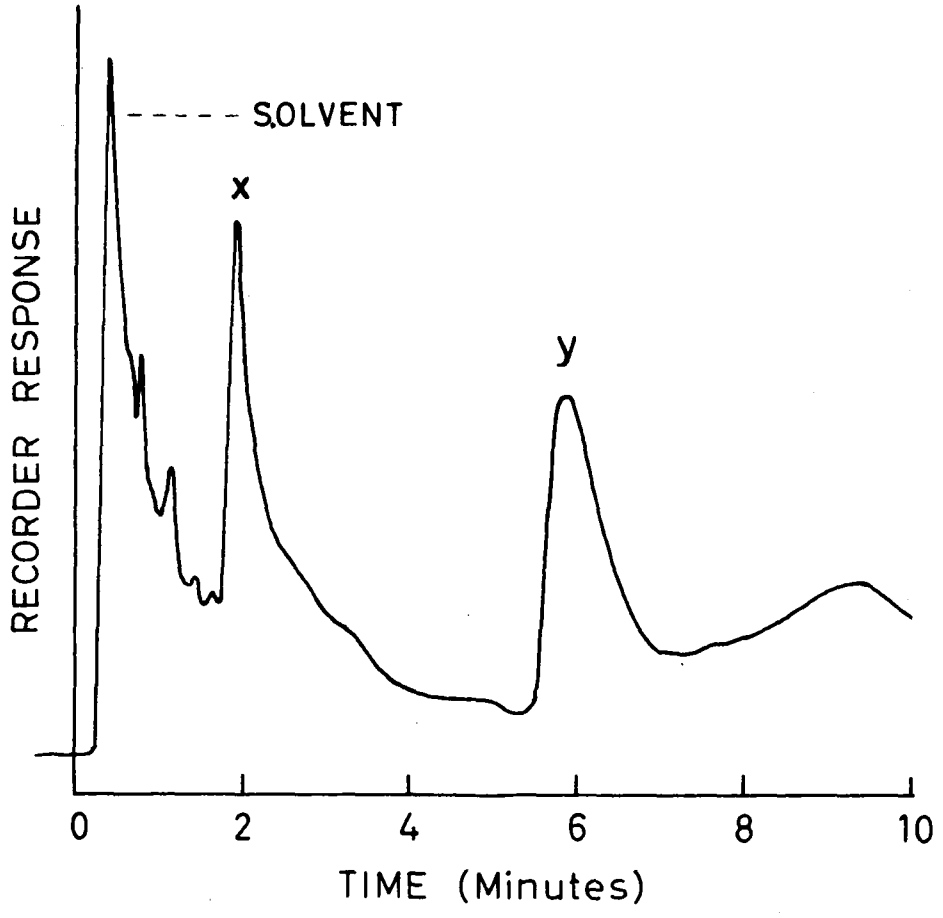


Figure 13. Chromatogram of coxal fluid collected from O. savignyi 16 days after feeding.

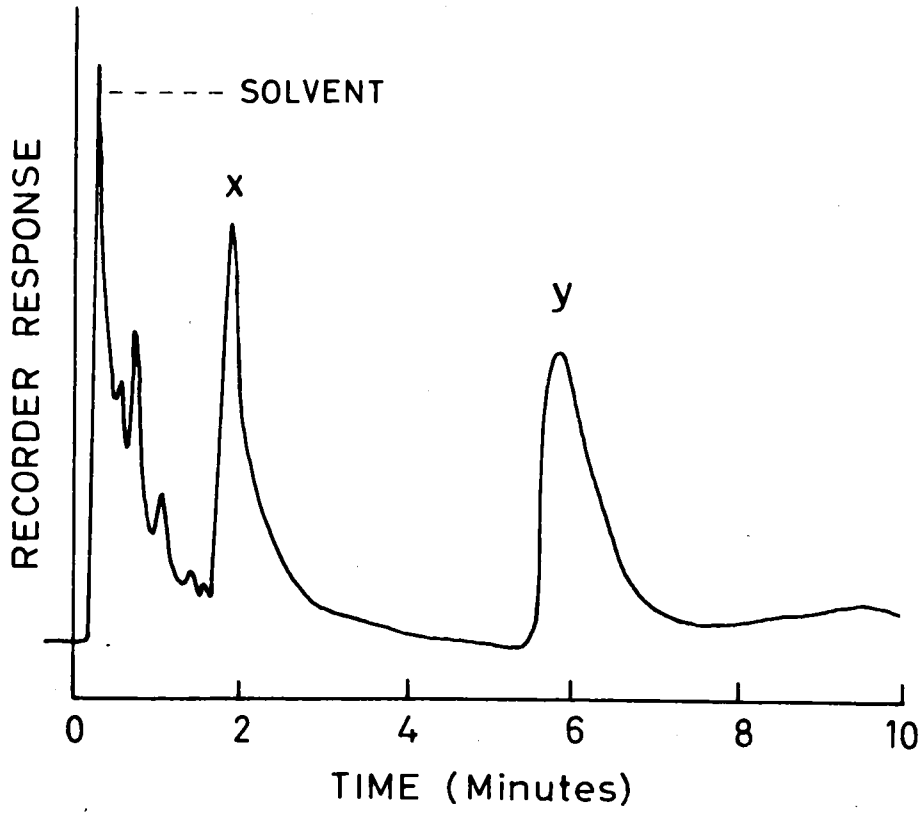


Figure 14. Chromatogram of coxal fluid collected from non-treated O. savignyi.

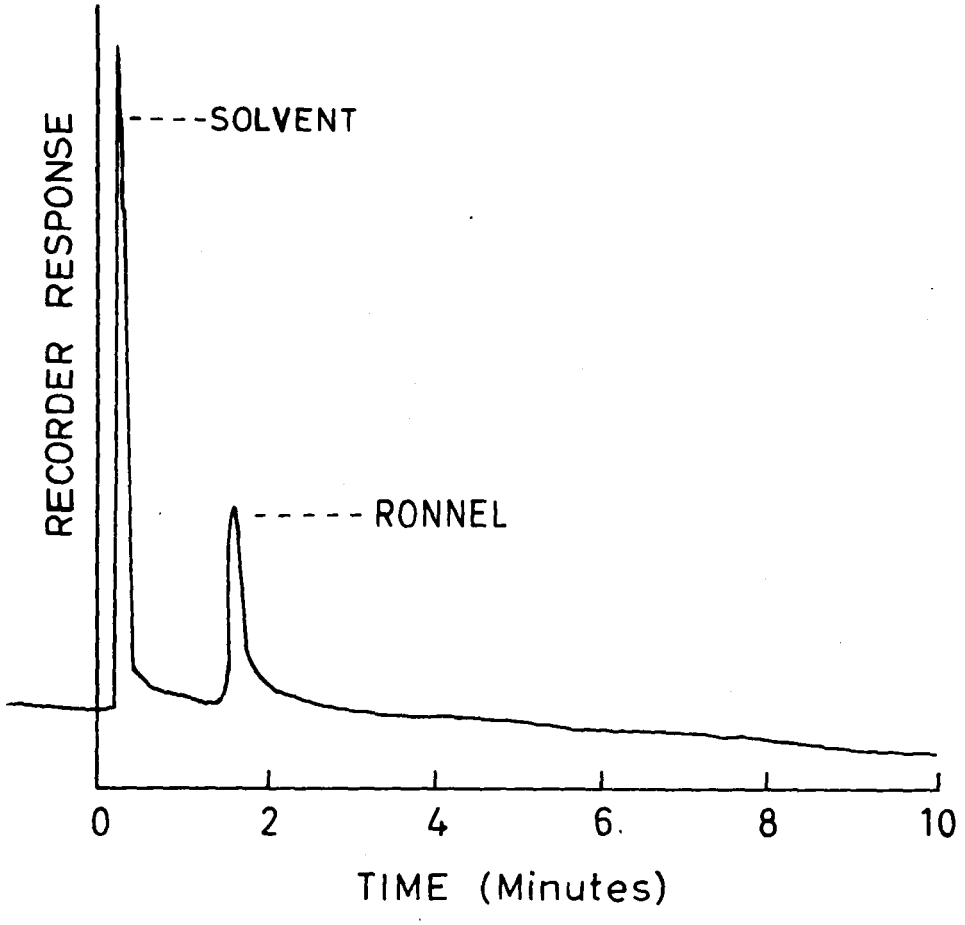


Figure 15. Chromatogram of standard ronnel.

metabolized during this period. The peaks X and Y in Figures 11-14 are those of unidentified materials present in the coxal fluid. They are not of insecticidal nature since the same peaks were present in the chromatogram of the non-treated tick. Topically-treated ticks were also observed to excrete coxal fluid at the hyperactivity stage and in some ticks, white crystals were formed around the openings of the coxal organs, mouth parts, and rectal region (Figure 16). However, the nature of these crystals was not determined. Christophers (1906) in describing the feeding process of O. savignyi stated that:

> "In as much as the volume of the blood meal is from two to six times the tick's original body weight and engorgement is usually completed in about half an hour, the tick must have a means of reducing the total volume and of preserving the internal medium while feeding. For this purpose coxal organs function as ionic (chloride) regulator and for ultra-rapid excretion of a large volume of water during ingestion of blood. About half the ingested water is excreted in coxal fluid".

He also noted that when O. savignyi is warmed or irritated it exudes a clear fluid from the coxal organs which may serve in part as a defensive mechanism. Examination of the results of gas chromatography shows that the coxal fluid excreted within the first day of feeding has an appreciable role in eliminating part of the pesticide ingested. The detection of ronnel further indicated that there is a rapid passage of fluids from the stomach to the coxal organs at the time of feeding.

Figure 16. Ventral view of poisoned *O. savignyi* showing crystals around coxal organs, mouth parts and rectal region.

V. SUMMARY AND CONCLUSIONS

The soft tick O. savignyi is widely distributed in the Middle East, and although its role as a disease vector is minimal, it can cause animals to lose blood in appreciable amounts leading to their death.

The effect of ronnel applied dermally or as a feed additive on the metabolism of O. savignyi was evaluated by estimating the amount of oxygen consumed per mg body weight per minute. Since the body temperature of this poikilothermic animal fluctuates with that of the surrounding, the effect of changes in temperature on the metabolic processes of this tick when dermally treated was also evaluated.

The results revealed that the metabolic rate of ticks artificially fed blood containing ronnel at the rate of 100 mg/kg was partially affected, while that of ticks fed on rats receiving 100 mg/kg body weight was not. The concentrations of ronnel ingested in both experiments were not lethal to the ticks. Dermally-applied ronnel showed an enhanced oxygen uptake rate of this tick prior to its death with a latent period that was directly temperature related. The pesticide also caused the tick to lose weight at a time corresponding to the oxygen uptake peak hours. It was also shown that the tick cuticle played an important role

in hindering the absorption of ronnel. The investigation showed that coxal fluid excreted within the first day of feeding contained detectable amounts of ronnel indicating a rapid passage of fluids from the tick stomach to the coxal organs immediately after feeding.

LITERATURE CITED

Bennett, H.J., and B.F. Dowden. 1966. Some effects of chlordane on the respiration of Procambarus clarki. WHO/EBL/66.58. 1-16.

Bennington, E. 1960. A systemic insecticide and bait for flea and rat control. J. Econ. Entomol. 53(1): 169-170.

Christophers, S.R. 1906. The anatomy and histology of ticks. Sci. Mem. med. sanit. Dept. India, n.s. (23), 55 pp. In: Hoogstraal, H. African Ixodoidea. I. Ticks of the Sudan. Washington, D.C., Navy Department.

Diggle, W.M., and J.C. Gage. 1951. Cholinesterase inhibition by parathion in vivo. Nature. 168: 998.

Dobson, R.C., and R.C. Peterson. 1963. Horn fly control on beef cattle by the use of cable rubbers. J. Econ. Entomol. 56(2): 230-234.

Dorough, H.W., and B.W. Arthur. 1961. Toxicity of several organophosphates administered in the diet of broilers to house fly larvae in the feces. J. Econ. Entomol. 54(6): 1117-1121.

Dorsey, C.K., J.O. Heishman, and C.H. Taylor. 1962. Horn fly control-using spray, dust, and pour-on formulations. J. Econ. Entomol. 55(4): 425-432.

Drummond, R.O. 1958. Laboratory screening tests of animal systemic insecticides. J. Econ. Entomol. 51(4): 425-427.

Drummond, R.O., B. Moore, and M.J. Wrich. 1960. Field tests with insecticides for control of lone star ticks on cattle. J. Econ. Entomol. 53(5): 935-955.

Drummond, R.O., O.H. Graham, W.P. Meleney, and G. Diamont. 1964. Field tests in Mexico with new insecticides and Arsenic for the control of Boophilus ticks on cattle. J. Econ. Entomol. 57(3): 340-346.

Drummond, R.O., and O.H. Graham. 1964. Insecticide tests against the tropical horse tick, Dermacentor nitens, on horses. J. Econ. Entomol. 57(4): 549-553.

Drummond, R.O. 1965. Further evaluation of animal systemic insecticides. J. Econ. Entomol. 58(4): 776.

Drummond, R.O., T.M. Whelstone, and S.E. Ernst. 1967. Insecticidal control of the ear tick in the ears of cattle. J. Econ. Entomol. 60(4): 1021-1025.

Ebeling, W., and R.J. Pence. 1957. The use of pesticides in the preparation of insects and mites for photography. Ann. Ent. Soc. Amer. 50: 637-638.

Ebeling, W., and R.E. Wagner. 1959. Rapid desiccation of drywood termites with inert sorptive dusts and other substances. J. Econ. Entomol. 52(2): 190-207.

Eddy, G.W. 1961. Laboratory tests of residues of organophosphorus compounds against house flies. J. Econ. Entomol. 54(2): 386-388.

Flynn, A.D., and W.G. Eden. 1960. Systemic and tropical insecticidal activity of some substituted phosphates and phosphorothioates. J. Econ. Entomol. 53(4): 692-693.

Fnapp, F.W., and G.F. Krause. 1960. Control of the northern fowl mite, Ornithonyssus sylviarum (C. & F.) with ronnel, Bayer L 13/59 and Bayer 21/199. J. Econ. Entomol. 53(1): 4-5.

Frear, D.E.H. 1965. Pesticide Index. College Science Publishers, State College, PA. 295 pp.

Harvey, T.L., and J.R. Brethour. 1961. Effectiveness of ruelene and ronnel for ear tick compared with cattle grub control. J. Econ. Entomol. 54(4): 814-815.

Harvey, G.T., and A.W.A. Brown. 1951. The effect of insecticides on the rate of oxygen consumption in Blattella. Canad. J. Zool. 29: 42-53.

Hoffman, R.A., and R.H. Roberts. 1963. Horn fly control studies in Mississippi. J. Econ. Entomol. 56(3): 258-261.

Hoogstraal, H. 1956. African Ixodoidea. I. Ticks of the Sudan (with special reference to Equatoria Province and with preliminary reviews of the genera Boophilus, Margaropus and Hyalomma). Washington, D.C., Navy Department. 1101 pp.

Hopkins, L.T. 1961. Functions of the madeira cockroach (Leucophaea maderae) alimentary tract in the absorption, metabolism, and excretion of ronnel. J. Econ. Entomol. 54(2): 224-230.

Hopkins, L.T. 1962. In vivo oxidation of ronnel in the madeira cockroach. J. Econ. Entomol. 55(3): 334-336.

Hurlbut, H.S. 1956. West Nile virus infection in arthropods. Am. J. Trop. Med. Hyg. 5: 76-85.

Jackson, J.B., R.O. Drummond, W.B. Buck, and L.M. Hunt. 1960. Toxicity of organic phosphorus insecticides to horses. J. Econ. Entomol. 53(4): 602-604.

Johnson, W.T., and G.S. Langford. 1960. Residual effectiveness of certain insecticides in horn fly control. J. Econ. Entomol. 53(3): 477-478.

Kawar, N.S., N.J. Bostanian, and S.M. Badawi. 1968. Insecticide residues in the milk of dairy cows treated for control of ectoparasites. J. Dairy Sci. 51(7): 1023-1025.

Keister, M., and J. Buck. 1964. Respiration: some exogenous and endogenous effects on rate of respiration. pp 617-658. In: Rockstein, M. (Ed). The Physiology of Insecta. Vol. III. Acad. Press, N.Y.

Kilpatrick, J.W., and H.F. Schoof. 1959a. The effectiveness of ronnel as a cord impregnant for house fly control. J. Econ. Entomol. 52(4): 779-780.

Knapp, F.W., C.J. Terbaar, and C.C. Roan. 1960. Systemic insecticides for cattle grub control. J. Econ. Entomol. 53(4):

Knowles, C.O., and B.W. Arthur. 1966. Residue associated with backrubber application of ronnel to dairy cows. J. Econ. Entomol. 59(3): 752-753.

Lavoipierre, M.M.J., and R.F. Riek. 1955. Observations on the feeding habits of Argasid ticks and on the effect of their bites on laboratory animals, together with a note on the production of coxal fluid by several of the species studied. Ann. Trop. Med. and Parasitol. 49 (1): 96-113.

Linkfield, R.L., and W.M. Reid. 1958. Newer acaricides and insecticides in the control of ectoparasites of poultry. J. Econ. Entomol. 51(2): 188-190.

Lord, K.A. 1950. The effects of insecticides on respiration. II. The effects of a number of insecticides on the oxygen of adult Tribolium castaneum Hbst. at 25°C. Ann. App. Biol. 37(1): 105-122.

Lovell, J.B. 1963. The relationship of anticholinesterase activity, penetration, and insect and mammalian toxicity of certain organophosphorus insecticides. J. Econ. Entomol. 56(3): 310-317.

March, R.B., R.L. Metcalf, T.R. Fukuto, and M.G. Maxon. 1955. Metabolism of Systox in the white mouse and American cockroach. J. Econ. Entomol. 48(4): 355-363.

McGregor, W.S., and R.C. Bushland. 1957. Test with Dow ET-57 against two species of cattle grubs. J. Econ. Entomol. 50(3): 246-249.

Metcalf, R.L., and R.B. March. 1949. Studies on the mode of action of parathion and its derivatives and their toxicity to insects. J. Econ. Entomol. 42: 721-728.

Metcalf, R.L. 1955. Organic Insecticides. Interscience Publishers, Inc., N.Y. 392 pp.

Micks, D.W., and M.R.V. Murthy. 1961. The effects of DDT on oxidative metabolism in susceptible and DDT-resistant Aedes aegypti. J. Econ. Entomol. 54(3): 461-465.

Nevill, E.M. 1964. The role of carbon dioxide as stimulant and attractant to the sand tampan, Ornithodoros savignyi (Audouin). Onderstepoort J. Vet. Res. 31(1): 59-68 (In Biol. Abst. 46, 695, 1965).

O'Brien, R.D. 1956. The inhibition of cholinesterase and succinoxidase by malathion and its isomer. J. Econ. Entomol. 49(4): 484-490.

O'Brien, R.D. 1957. Properties and metabolism in the cockroach and mouse of malathion and malaoxon. J. Econ. Entomol. 50: 159-164.

O'Brien, R.D. 1960. Toxic Phosphorus Esters. Acad. Press. N.Y. 434 pp.

O'Brien, R.D. 1966. Mode of action of insecticides. Ann. Rev. Entomol. 11: 369-402.

O'Brien, R.D. 1967. Insecticides, Action and Metabolism. Acad. Press, N.Y. 332 pp.

Ode, P.E., and J.G. Matthysse. 1964. Feed additive larviciding to control face fly. J. Econ. Entomol. 57(5): 637-640.

Plapp, F.W., and J.E. Casida. 1958. Hydrolysis of the alkylphosphate bond in certain dialkyl aryl phosphorothioate insecticides by rats, cockroaches, and alkali. J. Econ. Entomol. 51(6): 800-803.

Radeleff, R.D., and G.T. Woodard. 1957. Toxicological studies of Dow ET-57 in cattle and sheep. J. Econ. Entomol. 50: 249-251.

Radeleff, R.D., J.B. Jackson, L.M. Hunt, W.B. Buck, and M. Wrigh. 1963. The acute toxicity of ronnel applied dermally to cattle and sheep. J. Econ. Entomol. 56(3): 272-274.

Roth, A.R., and G.W. Eddy. 1957. Test with Dow ET-57 against cattle grubs in Oregon. J. Econ. Entomol. 50(3): 244-246.

Sherman, M., and E. Ross. 1960. Toxicity to house fly larvae of dropping from chickens fed insecticide-treated rations. J. Econ. Entomol. 53(3): 429-432.

Sherman, M., E. Ross, and G.H. Komatsu. 1962. Differential susceptibility of maggots of several species to droppings from chickens fed insecticide-treated rations. J. Econ. Entomol. 55(6): 990-993.

Tarshis, B. 1958. A preliminary study on feeding Ornithodoros savignyi (Audouin) on human blood through animal-derived membranes (Acarina: Argasidae). Ann. Ent. Soc. Amer. 51: 294-299.

Taylor, R.M., H.S. Hurlbut, T.H. Work, J.R. Kingston, and T.E. Frothingham. 1955. Sindbis virus: a newly recognized arthropod-transmitted virus. Am. J. Trop. Med. Hyg. 4(5): 844-862.

Thurber, H.E., and G.D. Peterson, Jr. 1960. Feed lot tests with ronnel for control of cattle grubs. J. Econ. Entomol. 53(3): 339-341.

Treece, R.E. 1962. Feed additive for control of face fly larvae in cattle dung. J. Econ. Entomol. 55(5): 765-768.

Umbriet, W.W., R.H. Burris, and J.F. Stanffer. 1964. Manometric Techniques. Burgess Publishing Co., Minneapolis 15, Minnesota. 4th ed. 305 pp.

van Asperen, K. 1958. Mode of action of organophosphorus insecticides. Nature. 81: 355-356.

Vandekar, M. 1968. Mammalian toxicity of acaricides. WHO/VBC/68.57. 163-174.

Walton, G.S. 1963. The use of O, O-dimethyl O-2, 4, 5, trichlorophenyl phosphorothiate in the treatment of demodectic mange in the dog. The Veter. Record 75(13): 355-357.

Weinburgh, H.B., J.W. Kilpatrick, and H.F. Schoof. 1961. Field studies on the control of resistant house flies. J. Econ. Entomol. 54(1): 114-116.

West, T.F., and J.E. Hardy. 1961. Chemical Control of Insects. Chapman and Hall Ltd., London. 2nd ed. pp 206.

Winteringham, F.P.W., and A. Harrison. 1956. Study of anticholinesterase action in insects by a labelled pool technique. Nature. 178: 81-83.

Winteringham, F.P.W., and S.E. Lewis. 1959. On the mode of action of insecticides. Ann. Review Ent. 4: 303-318.