RESPONSES OF *ARGAS PERSICUS* (OKEN 1818) TO COMPOUNDS OF CADMIUM AND ANTIMONY

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PREFACE

With man's mushrooming population demanding more and more food while at the same time utilizing more and more of the available land area for industrial and housing needs, it is imperative that man must strive untiringly to increase his agricultural efficiency. A major priority in increasing agricultural efficiency is controlling other biological species, namely arthropods, that devastate our food plants and debilitate our food animals by feeding upon them and serving as transmitters of bacterial, protozoan, and viral pathogens.

The traditional methods of controlling noxious arthropods by releasing broad spectrum poisons into our already dangerously polluted environment are no longer acceptable. Man's recent arousal to the esthetic value of wildlife and increasing awareness of the complexities of biological and ecological webs has demanded that new, species-specific control techniques be developed and employed, thereby preventing widespread destruction to non-target species and the upsetting of the so-called "balance of nature." The outstanding successes of recent species-specific control techniques, such as the various sterilization and biological control techniques, have started a revolution in arthropod control that has the potential of controlling the noxious species while avoiding the large scale damage to non-target species that has occurred in the past. Unfortunately, or fortunately, depending upon one's particular interest at a given moment, until the newly developed and undreamed-of control techniques become fully effective, we must

continue to test and rely upon chemicals to control man's chief competitor for food; striving to use those chemicals that do minimal ecological damage.

The author gratefully acknowledges the assistance and sacrifices of the following contributors to this investigation: Dr. D. E. Howell, Head, Department of Entomology, who served as academic adviser and research committee chairman; Dr. J. A. Hair, Assistant Professor, Department of Entomology, who served on the research committee and offered helpful suggestions throughout the study; Dr. G. W. Newell, Associate Professor, Poultry Science Department, who served on the research committee and generously contributed the needed resources of that department; and J. R. Bolte, Graduate Research Assistant, Department of Entomology, who aided with the photography.

The author wishes to point out that while the tick used in these experiments is referred to as Argas persicus, recent reconsideration of the genus Argas by Kohls et al. indicates that A. persicus is a complex of several species. The species used here will be designated Argas radiatus Railleit 1893 in a paper soon to appear in the Ann. Entomol. Soc. Amer. (Howell 1970, Personal Communication).

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INTRODUCTION

For many years cadmium salts have been known to alter biological activities in vertebrates. Much effort has been expended in trying to identify the specific physiological and pathological effects of cadmium in these animals. For some time it appeared that cadmium may prove to be useful in sterilizing male mammals, as it produces the castration effect without seriously impairing the hormonal secretory functions of the testes. More recently, however, it has been demonstrated that cadmium tends to become carcinogenic within a few months after introduction into an animal.

The literature contains very little information concerning the effects of cadmium upon free-living invertebrates. What there is deals almost exclusively with marine forms (Maruyama 1957, Deschiens and Tahiri 1961, and Gaujolle et al. 1965). During the past six years, students under the direction of Dr. D. E. Howell have been evaluating cadmium salts and related compounds as reproductive inhibitors and toxicants to various insects. Reported here are responses of the common fowl tick, Argas persicus (Oken), to treatments with cadmium chloride, antimony potassium tartrate, and triphenylantimony. Included also are results of preliminary studies dealing with the effects of cadmium chloride upon chicks and the replete female lone star tick, Amblyomma americanum (Linnaeus).

LITERATURE REVIEW

Argas persicus

The common fowl tick, Argas persicus (Oken 1818), is a cosmopolitan parasite of the domestic fowl (Robinson and Davidson 1913a). This parasite may be a serious pest to poultry in the semi-arid and desert areas of the United States, especially on farms where poultry run free (Medley and Ahrens 1968). In some parts of the world the presence of A. persicus is the determining factor in successful poultry rearing. The fowl tick is a known transmitter of the pathogens of fowl spirochetosis, Borrellia gallinarum, fowl piroplasmosis, Aegyptianella pullorum, and chicken cholera, Pasteurella avicida (Kaiser 1966a). The tick was experimentally infected with Mycobacterium tuberculosis by permitting it to feed on diseased hens. There is one authenticated case where A. persicus transmitted Bacillus anthracis to man (Steinhaus 1967). All members of the subgenus Persicargas are suspect as vectors of viruses that may infect lower animals and man (Hoogstraal and McCarthy 1965 and Hoogstraal et al. 1968a).

The literature contains numerous reports that A. persicus parasitizes many animals other than poultry. Nuttall and Warburton (1908) stated that in Persia it commonly attacks man and quail. In Canada, Gregson (1942) reported it from sparrows. Prescott and Clinton (1945) credited it with attacking vultures, ostriches, canaries, ducks, geese, and cattle. Adbussalam and Sarwar (1953), in Pakistan, found the tick in 16 species of trees frequented by herons and vultures. Arthur (1962)

stated that the tick has been found in Africa on herons, guinea fowl, secretary birds, wattled cranes, ibises, and pelicans, and in the United States on quail, wild doves, wild turkey, vultures, sparrows, and screech owls. The tendency has been to assume that any Argas tick found on birds and poultry is likely to be A. persicus. Recently, however, Hoogstraal and colleagues of the United States Naval Medical Research Center, Cairo, Egypt, have shown that many of the recordings from species of wild birds and mammals represent a different species. Kaiser (1966b) pointed out that many biological and microbiological studies on "A. persicus" should be suspect owing to the possibility of species misidentification.

Based upon distinguishable morphological and biological characteristics and the pattern of virus and Wolbachia infections, Hoogstraal (1957), Kaiser et al. (1964), Taylor et al. (1966), Hoogstraal and Kohls (1960 and 1967), and Hoogstraal et al. (1968a, b) have divided the genus Argas into the subgenera Chiroptargas, Secretargas, Argas, and Persicargas. Members of Chiroptargas are parasites of bats. Secretargas contains one known species, Argas (Secretargas) transgariepinus White 1846, parasitizing bats and possibly lizards. The Argas reflexus group was placed into the subgenus Argas, which is primarily a parasite of swallows, martins, and pigeons in localized areas of each continent. The Argas persicus complex was grouped into the subgenus Persicargas and contains numerous species from many parts of the world. Examples of subgenus Persicargas include: Argas (Persicargas) zumpti, South Africa; A. (P.) arboreus, Egypt, South Africa, and the Cameroons; A. (P.) abdussalami, Pakistan; A. (P.) robertsi, Australia; and A. (P.) persicus, world wide between latitudes 40° North and 40° South.

Morphologically, A. (P.) persicus can be distinguished from other members of Persicargas by the presence of large, lateral, setae-bearing integumental cells and post palpal bristles (Hoogstraal et al. 1964). Biologically, it is set apart by its natural infectivity by and transmission of the microorganisms mentioned above, and its apparent inability under natural conditions to become infected with and transmit Salmonella typhimurium, Wolbachia persica, and the Quaranfil and Nyamanini viruses (Kaiser 1966a, b, c). The synonymy of the fowl tick revised from Nuttall and Warburton (1908) is:

Argas (Persicargas) persicus (Oken 1818)

Rhynchoprion persicum Oken 1818

Argas persicus Fischer de Waldheim 1823

Argas (Persicargas) persicus (Oken 1818).

As an argasid tick, A. persicus lacks the scutum that is found in the ixodid, or hard ticks, and has an integument that is leathery. The capitulum is inferior in all but the larval stage, resulting in a body that appears flattened and somewhat ovate.

The eggs of A. persicus are spherical, yellowish-brown, 0.6 - 0.8 mm in diameter, and in warm weather may hatch in 8-11 days (Arthur 1962).

The hexapod larvae are nearly spherical, have a protruding capitulum, and range in size from 0.6 x 0.8 mm unfed to 2.5 x 2.0 mm engorged (Hooker et al. 1912). Engorged larvae molt about 8 days after feeding, or live up to 8 weeks in an unfed condition. Sexual differentiation is not observed in the gonads of larvae (Balashov and Goroshchenko 1962).

The two nymphal stages are designated as neonymph (N_1) and deuteronymph (N_2). Neonymphs range in size from 2.0 x 1.5 mm unfed to 4.0 x

3.0 mm engorged. About two weeks following a meal the neonymphs molt into deuteronymphs. Deuteronymphs range from 3.0 x 2.0 mm unfed to 8.5 x 5.0 mm engorged, and molt into adults about 15 days following a meal. Glukhov (1966) reported that both nymphal stages go into winter diapause in North Caucasus which lasts up to six months. Although gametogenesis commences during the nymphal stages, the nymphs have no genital aperture and sexual differentiation cannot be readily determined visually (Robinson and Davidson 1913a).

In adults the sexes are distinguishable by differences in the shape and position of the genital orifice, and the much larger size of the females (Robinson and Davidson 1914). The adult size ranges from about 4.5 x 3.5 mm in the unfed to 7.0 x 4.0 mm in the engorged males, and from 5.0 x 3.0 mm in the unfed to 7.3 x 12.0 mm in the engorged females (Hooker et al. 1912). Unfed adults may live up to 18 months or longer (Glukhov 1966). Nikitina (1960) observed that starving males in the spring often become cannibalistic, feeding upon large nymphs and females. While one meal appears to satisfy the nutritional requirements for continued spermatogenesis in the males, females must feed intermittently for continued oogenesis to occur (Arthur 1962). Following each blood meal a female deposits a batch of 50-150 eggs (Drummond 1960). Roveda (1940) reported ova mortality to be 4.8% under laboratory conditions of 22-38 C and 72-100% relative humidity. In the laboratory maintained at 23-26.5 C and 72-80% humidity, Loomis (1961) found the life cycle to average about seven weeks.

The males have no external genitalia and during "copulation" the capitulum is used to transfer spermatozoa to the females (Feldman-Muhsam 1967). The large, non-flagellated but motile sperm (Bedi 1962)

are transferred to the female in an immature condition enclosed within a packet, or spermatophore. After being placed into the female genital orifice the external covering of the packet ruptures, releasing two separate packets of sperm into the bifurcated uterus. The sperm mature in the uterus and remain viable there for many months (Feldman-Muhsam 1967). A fertilized female may be identified by the presence of the ectospermatophore adhering to the genital orifice following insemination.

At least the nymphs and adults of A. persicus are negatively phototactic and positively thigmotactic and remain hidden in cracks and crevices of roosting areas during the day (Hindle and Merriman 1912), emerging at night to feed on the legs of their host. Thus a heavy infestation may go unnoticed by a poultry farmer until he experiences loss of birds. The nymphs and adults become engorged in 5-30 minutes and return to a secluded place until the next meal. The larvae, however, remain attached to the body of their host for a period of 4-6 days and are referred to by the layman as "blue bugs" (Nuttall and Warburton 1908). The host is thought to be detected primarily through thermoreceptors of Haller's organ on the foretarsi, and the attachment site secondarily through the chemoreceptors of Haller's organ and palpi (Arthur 1962).

While engorging, the ticks secrete an anticoagulant that retards blood clotting and partially digests the imbibed cellular material (Arthur 1962). The initial stage of the meal may be mostly extravascular, later becoming primarily blood. Some meals may be entirely extravascular, in which case the imbibed tissue fluids produce the "white tick" appearance of some engorged specimens (Arthur 1962). The

secreted anticoagulant produces severe hemorrhage, followed by internal clotting around the puncture wound of the host. This combined with exsanguination of the host when ticks are abundant may severely debilitate a poultry flock.

As in the diet of other blood-sucking arthropods, the diet of fowl ticks contains an excess of water and chlorides (Lees 1946). These excess constituents of the blood are excreted by the coxal glands of a tick as it feeds (Robinson and Davidson 1913b and Tatchell 1967). By weight, the secretion of the coxal glands is approximately one half of the total blood meal, resulting in nearly pure haematin granules being stored in the gut (Tatchell 1964). Under the conditions in the tick gut, such as freedom from bacteria, high protein content, protection from light and oxygen, the concentrated blood can remain stable for the lifetime of the tick (Tatchell 1964).

Cadmium

Discovered in 1817 by Stromeyer (Prodan 1932a), cadmium is a white metal with a bluish tinge that does not occur naturally in an uncombined state. It is nearly always found associated with zinc ores, where the cadmium content varies from 0.10 - 0.50% (Prodan 1932a). It is a malleable metal that can be drawn into sheets and wires. It is insoluble in water and soluble in acids in pure form. When combined with other elements it becomes slightly soluble to soluble in water. In the periodic table cadmium is placed in the second group of elements with zinc and mercury, and as a heavy metal it has the tendency to form complex albumin compounds when coming into contact with organic material Prodan (1932b).

Cadmium has a history of many and varied uses. Industrially, it has been used as a constituent of alloys, in electroplating, photography, plating for rust prevention, storage batteries, vapor lamps, paints, etc. (Prodan 1932a). Biologically, cadmium has been used in germicides (Prodan 1932a), insecticides for insects with chewing mouth parts (Ginsberg 1934), research-wise in toxicity studies (Wilson et al. 1941) and physiological activities of heavy metals (Meek 1959), ascaracides in swine (Bunde et al. 1954, Guthrie 1954, and Burch and Blair 1955), nematicides (Levine and Ives 1954, and Feldmesser and Rebois 1966), molluscacides (Deschiens and Tahiri 1961), sterilizing male mammals (Kar 1961), treatment for human cutaneous fungus infections (Watkins 1964), polarographic studies (Lapanje 1964), and in studying zinc metabolism and nutrition (Powell et al. 1964a, b).

Cadmium poisoning in man dates back to 1857 (Prodan 1932a). During the five-year period of 1941-1946, there were at least 689 cases of cadmium poisoning by ingestation with food or drink (Fairhall 1957). More recently, cadmium poisoning in man has been limited primarily to inhalation of fumes by industrial employees involved in plating metals (Smith et al. 1960) and cutting and welding plated metals (Beton et al. 1960 and Blejer 1966). Although sufferers from acute cadmium poisoning may experience chest pains, pulmonary congestion and edema (Smith et al. 1960), survivors appear to suffer no permanent damage. Chronic cadmium poisoning is the more common form, and results from continued exposure to the fumes and an accumulative effect, primarily in the kidneys and liver (Schroeder et al. 1967). Patients suffering from chronic cadmium poisoning exhibit such symptoms as anemia (Tsuchiya 1967), cerebral hemorrhage (Schroeder 1966), rhinitis accompanied by bleeding from small

ulcerations in the nasopharynx (Baader 1952), yellowing pigmentation of the teeth (Princi 1947), emphysema (Friberg 1948), and renal damage leading to proteinuria, glycosuria, and aminoaciduria (Kench et al. 1965). Kennedy (1966) found renal stones, hypercalcuria, and radiological bone changes in workers exposed to compounds containing cadmium. Men exposed to cadmium fumes over a period of years may become sterile, and when autopsied at death reveal no, or occasional, spermatids present in the testes (Parizek 1960). Cadmium is toxic to human spermatozoa at concentrations of 2.0 mM (White 1955) and increases the death rate in mice at tissue levels less than those found in man (Schroeder et al. 1963). The maximum safe concentration of cadmium fumes in an industrial plant ranges from 0.10 - 0.50 mg/m^3 of air (Shabalina 1968). Industrial workers who are subjected to cadmium poisoning have a mortality rate of about 15% (Spolyar et al. 1944).

Carroll (1966) and Schroeder (1967) have associated hypertension and cardiovascular death rates in humans with the amount of cadmium contamination in our environment. Schroeder (1967) claimed that man is taking in 200 - 400 ug of cadmium per day, of which 3 ug per day is retained by the body. Over a period of 30 years this amounts to 10 mg. A single 0.50 ug/ml dose of cadmium in water injected subcutaneously into rats caused hypertension that increased with age and continued until death (Schroeder 1967). Forny et al. (1955) reported that humans with no known exposure to cadmium had at autopsy a mean concentration of 3.31 mg of cadmium per 100 g of liver. Part of man's source of cadmium is in his food. Market milk contains from 0.017 to 0.030 ppm cadmium (Murthy 1968). Sea foods, such as oysters and scallops, may contain up to 55 ppm cadmium (Brooks and Rumsby 1967). This may be one

explanation why states bordering sea coasts have a higher death rate than interior states (Schroeder 1967). Despite the above indications that environmental cadmium may be a potential hazard to man, Perry et al. (1967) reported that there has been no biological effect of accumulated cadmium in man demonstrated. Likewise, Kubota et al. (1968) found cadmium in less than half of the blood samples taken from humans, and that the samples containing cadmium showed no consistent distribution pattern in the United States.

Wilson et al. (1941) found that rats eating a diet containing 0.125% cadmium had hearts nearly twice as large as those on non-cadmium diets. They also observed that the pancreas showed marked atrophy. Drinking water containing 5 ppm cadmium for several months made rats hypertensive, while an intra-arterial injection of 50 ug produced acute but transient increase in diastolic pressure (Perry et al. 1965). Thirty to 40 gammas of cadmium per gram of tissue completely inhibited contractility of ventricle muscles in frog hearts (Turpaev and Mamedova 1956). Complete heart arrest was obtained in pigeon and turtle hearts within one and one-half minutes after injecting them with a Ringer solution containing 1:1,000,000 cadmium acetate (Salvant and Connet 1920).

Nervous tissue is sensitive to minute concentrations of cadmium. A subcutaneous injection of 3.50 mg of cadmium chloride in one ml of water into the back of rats produced 100% ganglionic necrosis and death, whereas 0.50 mg produced 100% ganglionic necrosis without mortality (Gabbiani 1966). Parenteral injection of cadmium chloride into rats produced hemorrhagic lesions in the sensory ganglia that healed in 15 days (Gabbiani et al. 1967a), after which the animals were tolerant

to a second injection. Pretreatment with cobalt chloride inhibited ganglia damage. Oral dosages of 3 mg $CdCl_2$/kg body wt three times weekly for 3 weeks caused a sharp fall in the thiol groups in the brain of rats (Bologov and Uzbekov 1966). The same dosage introduced intragastrically by Bologov and Kalinin (1968) caused damage to the cerebral cortex, subcortical ganglia, and cerebellum. Simultaneous administration of 250 ug of Vitamin B_{12}/kg body wt markedly decreased the damage. Gabbiani et al. (1967b) destroyed the cerebral cortex fibers and cells in young rabbits, and the trigeminal and spinal ganglia of young hamsters, guinea pigs, and mice by subcutaneously injecting them with 0.05 mM $CdCl_2$/kg body wt prior to day 20 of life.

Male rats receiving an intrabronchial dose of 15 mg of cadmium sulfate, cadmium selenite, or cadmium stearate developed chronic pneumonia, necrosis of cartilage, purulent-necrotic bronchitis, emphysema, and diffuse sclerosis four to six months following injection (Shabalina 1967a). A single 1.0 mg $CdCl_2$/100 g body wt subcutaneous injection in rats produced sarcomas six months later that metastasized via the blood stream (Favino and Nazari 1967). Ten months later neoplastic cell emboli were found in the lungs, and 12 months later in the liver, myocardium, lungs, kidneys, and peritoneum. Forty per cent of the rats injected by Kazantzis and Hanburg (1966) developed sarcomata 6-12 months later. One year following a subcutaneous injection of cadmium, 70-80% of the rats and mice had interstitial cell tumors (Gunn et al. 1963). A single 0.17 - 0.30 mg subcutaneous injection of cadmium chloride into rats by Gunn et al. (1967) produced pleomorphic sarcomas in mesodermal tissue 10-12 months later.

Even though 82% of dietary cadmium is eliminated via the feces in

bovines (Miller et al. 1967) and 90% in goats (Miller et al. 1968), enough is absorbed to interfere with zinc absorption and metabolism (Cotzias et al. 1961a, and Powell et al. 1967). Dietary cadmium and zinc given to rats in a 1:1 ratio produced typical zinc deficiency symptoms (Petering et al. 1969). Gastrointestinal absorption of cadmium leads to accumulation and storage in the kidneys, liver, and spleen in cats (Wilson et al. 1941), dogs (Byerrum et al. 1960), mice (Shabalina 1967b), goats (Miller et al. 1968), and rats (Maekaeva et al. 1968). These animals also experienced other pathological conditions, such as desquamation of gastric epithelium and gastrointestinal wall necrosis, up to 30% decrease in testicular size accompanied by sterility, incorporation of cadmium into the milk of females, impaired sight, swollen joints, general unthriftiness, mortality, and in rabbits, anemia (Friberg 1952). In chicks, dietary cadmium replaced both iron and zinc at metabolic sites and rendered them inactive at concentrations of 25-40 ppm (Hill et al. 1963). Gunn et al. (1964) reported that cadmium inhibited keto acid oxidation, and when 1.35 mg was injected into chicks, tumors formed at the site of injection. Specific relationships between cadmium toxicity and utilization of dietary zinc were also demonstrated in turkeys (Supple 1961). Lease (1968) stated that dietary cadmium interferes with the absorption of zinc through occupation of some of the binding sites of a blood transport system, thus reducing zinc transport and producing zinc deficiency.

Physiologically active elements are excreted in proportion to metabolic loading (Cotzias et al. 1961b). In mice, Cd^{109} has a negligible turnover regardless of body load and continues to be absorbed and stored in non-osseous tissues (Cotzias et al. 1961b). Zinc is the most

abundant essential trace element in mammals (Schroeder et al. 1967b), especially in the prostate gland, semen, spermatozoa, and to a lesser extent in striated muscles (Timm and Schulz 1966). Several enzymes depend upon zinc as a cofactor, while no enzyme known utilizes cadmium. Since cadmium has no physiological function, it does not obey homeostatic control. Thus it competes for binding sites on protein, where it has a stronger affinity for SH groups than zinc. Mitochondria and chromosomes do not appear to discriminate between cadmium and zinc, permitting cadmium to serve as an antimetabolite to zinc on the subcellular level (Cotzias et al. 1961a).

Along with the more chronic pathology discussed above, cadmium has an immediate affinity for the testes and other oestrogen producing organs of all mammals and birds studied (Parizek 1964 and Chiquoine and Duncan 1965). A single 0.10 ml muscular injection of 0.04 molar solution of cadmium chloride caused localized necrosis and generalized disruption of germ cell coordination in the wood pigeon, Columba palumbus, (Lofts and Murton 1967). A 0.20 ml injection caused retardation of the photoperiodic responses in the testes. A single intraovarian injection of 0.50 mg of $CdCl_2$/kg body wt produced sterility in female bovines and goats by causing fibrosis and encapsulation of the ovaries in the bovines and complete oocyte destruction in the goats (Chatterjee and Kar 1965 and Chatterjee 1966). Kar (1962) produced sterility in male goats with an injection of cadmium chloride. Complete eradication of spermatogenesis in rabbits was produced by 0.05 mM $CdCl_2$/kg body wt subcutaneously injected (Paufler and Foote 1967). In a similar study with rabbits, Cameron (1965) observed no regeneration of germinal cells after 333 days. Male Rhesus monkeys were sterilized

with a single injection of 1.0% cadmium chloride on a mg/kg basis (Kar 1961 and Kar and Das 1962). Biopsy of cadmium poisoned monkeys showed definite degeneration in the proximal tubules, with occasional necrosis (Timme 1965). Kench and Sutherland (1967) found that serum albumin in cadmium poisoned monkeys had 3.0% less tryptophan than normal animals, a condition similar to the minialbumin isolated from chronically cadmium poisoned man (Kench et al. 1965). An injection of 0.56 mg $CdCl_2$/100 g body wt given subcutaneously to gerbils upset the regular succession of cells in the seminiferous tubules, disrupted spermatogenesis, and histochemically, inhibited adenosine triphosphate, 5-nucleotide, and other enzyme activities to render the cellular components functionless (Singh and Mathur 1968). In this case the cadmium inhibition was thought to be due to its combination with sulfhydryl groups of the protein moiety of enzymes to form mercaptides. Many enzymes contain sulfhydryl groups, which play some role in the nuclear division of spermatogenesis, that are destroyed by cadmium (Singh and Mathur 1968). Hodgen et al. (1969) demonstrated that cadmium also interferes with carbonic anhydrase activity in the testes of rats when administered both in vivo and in vitro. An in vitro injection of the testes with 10^{-3} M cadmium chloride decreased the carbonic anhydrase activity by 50%. An in vivo subcutaneous injection of 0.03 mM/kg body wt cadmium chloride first decreased the enzyme activity, then produced a sustained increase in the activity. The increased activity was thought to be due to hemorrhage of the testes, allowing the CA-rich erythrocytes to invade the interstitial spaces.

Rats have been utilized extensively in studying the physiological and pathological effects of cadmium on the reproductive system of male

mammals. Male rats injected with cadmium showed sustained sub-normal temperatures for both the body and testicles (Johnson and VanDenmark 1969). Bouissou and Fabre (1966) thought that the lower temperature of scrotal testes played a part in localizing the lesions produced by sub-acute doses of cadmium. Kar and Kandoj (1963) caused irreversible destruction to the seminiferous epithelium of rats by inuncting the scrotum with 20-40% cadmium chloride in an aqueous or glycerine medium. A subcutaneous injection of 150 ug $CdCl_2$/kg body wt caused fragmentation of nuclear material and separation of head and tail of sperm (Kar and Das 1962). A 200 ug $CdCl_2$/kg body wt injection into the scrotum caused up to 30% loss of weight in the testes (Davis and Coniglio 1967). Gupta et al. (1967) injected rats subcutaneously with 0.02 mM $CdCl_2$/kg body wt to produce extensive vascular damage to the testes, followed in two weeks by atrophy of the prostate gland and seminal vesicles to one half their normal size. The testicular pathology resembled the castration effect, starting in the interstitial tissues and characterized by capillary congestion, thrombosis, interstial edema, and finally by necrosis and fibrosis. Gunn et al. (1968a) found that only 0.012 mM $CdCl_2$/kg body wt injected subcutaneously was enough to produce complete testicular necrosis within seven days. Ten mg Cd/kg body wt caused irreversible calcification of the epithelium in the seminiferous tubules but not in the interstitial tissues (Takkar et al. 1968), perhaps causing the 2-4 mg/100 ml drop in the blood calcium observed in cadmium poisoned rabbits by Kennedy (1966). Although Dimov and Knorre (1967) felt that cadmium acted as a direct toxin to germinal epithelium, Waites and Setchell (1966) stated that the primary action site of cadmium was the testicular capillary epithelium, with

intertubular edema and spermatogenic damage resulting from the vascular destruction. Subsequent to cadmium poisoning, the capillaries regenerate but do not have properties inherent to the original vasculature (Gunn et al. 1966a). Repair of the interstitial tissues is associated with the return of hydrolytic and oxidative enzymes in the proliferating tissues (Knorre 1968). Parizek (1957) stated that endocrine activity of the testes returns. However, the germinal epithelium does not regenerate (Parizek 1957 and Knorre 1968). Cadmium damage to the testes can be blocked by simultaneous, or nearly simultaneous, administration of compounds of zinc (Gunn et al. 1961a), selenium (Kar and Das 1963), thiols (Gunn et al. 1966b), cobalt, BAL (British anti-Lewisite III), and cysteine (Gunn et al. 1968b). The protectors do not act by preventing cadmium from reaching the testes, but by binding with cadmium and transporting it away from the site of injury (Gunn et al. 1968a).

For many years cadmium was thought not to interfere with the reproductive capacity of females (Kar et al. 1959 and Gunn et al. 1961b). Recently, however, it was shown that cadmium administration to pregnant rats caused destruction of the pars foetalis of the placenta, resulting in maternal death in 76% of the cases (Parizek 1965 and Chiquoine 1965). A single injection of 0.03 mM cadmium acetate/kg body wt in pregnant rats caused complete foetal death, and during the last four days of pregnancy, total maternal mortality (Parizek et al. 1968). Simultaneously administered selenite prevented cadmium toxicity to both the foetuses and maternal organisms. Two mg $CdSO_4$/kg body wt injected into pregnant hamsters during early gestation caused severe facial abnormalities in the embryos by acting on the first brancial arch, probably via inhibition of the sulfhydryl enzyme succinoxidase (Virgil

and Carpenter 1968). Simultaneous injection with $ZnSO_4$ prevented the cleft palates and lip and facial fissures. Ferm and Carpenter (1968) found that about 66% of the embryos from poisoned female hamsters showed the teratogenic effect, while the maternal organism showed no pathological changes.

Arthropods have been used sparingly in studying the biological effects of cadmium salts. The pioneer in this area was Ginsberg, who in 1934 studied the toxicity of cadmium to chewing insects. He found that cadmium hydroxide, cadmium oxide, and cadmium phosphate were highly toxic to larvae of the silk moth, Bombyx mori, tent caterpillar, Malacosoma americana, and confused flour beetle, Tribolium confusum. One, two, three, and four pounds of cadmium hydroxide per 100 gallons of water, respectively, produced 70, 80, 100, and 100% mortality to the larvae. Five per cent cadmium hydroxide in 95% talc killed 82% of the silk moth larvae in two days. Fifteen per cent cadmium hydroxide in flour caused 100% mortality to T. confusum in ten days.

The toxicity of cadmium salts to the bollworm, Heliothis zea (Boddie), was determined by Keathley (1966). Cadmium chloride and cadmium acetate inhibited larval growth at all concentrations, while 2.0 mg/sq inch of diet surface caused 100% mortality. Cadmium sulfate also inhibited larval growth and caused 100% mortality at a concentration of 2.0 mg/sq inch of diet surface. Some larvae feeding on 0.02 mg/sq inch of diet surface survived to adults but exhibited abnormal wing development. Viable eggs from these adults hatched into apparently normal larvae. Incorporation of cadmium sulfate into the diet at a concentration of 0.60% caused 100% mortality, while a concentration of 0.06% inhibited larval growth.

Abdel-Razig (1966) demonstrated that in Drosophila melanogaster the effectiveness of cadmium as a reproductive inhibitor was dependent upon the method of treatment. Incorporated into a semi-solid food medium at concentrations of 0.10 - 1.0%, all cadmium compounds tested were highly repellent to both feeding and ovipositing activities of adults, resulting in death of the flies from a combination of starvation and toxic effects of the compounds. Dipping the pupae in 5.0-10.0% concentrations of cadmium chloride and cadmium nitrate in acetone-water caused a 10% reduction in emergence, while dipping the larvae in the same concentrations caused 90-100% mortality. Dusting newly emerged adults with cadminate, cadmium 2-hydroxyethyl mercaptide, cadmium n-octyl mercaptide, and cadmium tert-octyl mercaptide delayed oviposition and reduced the number of ova deposited at all concentrations.

The American cockroach, Periplaneta americana, was highly repelled by semi-solid food containing 0.001 - 0.05% cadmium chloride, cadmium nitrate, and cadmium acetate (Abdel-Razig 1966). The 0.05% concentration may have been toxic to them. The cockroaches were also repelled by concentrations of 0.10 - 1.0% cadmium chloride, cadmium acetate, and cadmium laurate in a liquid food. Inuncting the cockroaches on the meso- and metathorax with 2.50 and 5.0% cadmium mercaptides in a lanolin carrier caused alteration of the integument at the inunction sites two to three weeks following the treatment in 40-80% of the treated specimens. The inunction site sub-integumental tissue exhibited hyperplasia with excessive connective tissue formation. Thirty per cent of the cockroaches died during ecdysis, and those that survived had deformed wings and integument beneath the wings. Four of six cadmium mercaptides given to the German cockroach, Blatella germanica, at

concentrations of 2.50 and 5.0% in solid food delayed and reduced oothecal production. Cadmium 2-hydroxyethyl mercaptide prevented oothecal development but caused high mortality (Abdel-Razig 1966). The male German cockroaches suffered a 10-15% higher mortality rate than the females. Cadmium laurate fed to late nymphal instars inhibited reproduction and induced a high mortality.

Abdel-Razig (1966) also found that continuous feeding of cadmium compounds to the house fly, Musca domestica, delayed oviposition and caused a great reduction in the number of eggs deposited. Cadminate at concentrations of 0.25 and 0.50% in solid food reduced significantly both the number of eggs deposited and the percentage hatch. The house flies did not oviposit when maintained on food containing 2.0% cadmium chloride or 2.0% $CdCl_2$:10%$ZnCl_2$. Two per cent cadmium chloride treated food caused 100% mortality by the tenth day, while 10.0% zinc chloride caused 80% mortality during the same period. Cadmium acetate shortened the house fly life span and reduced its reproductive potential.

Feeding the stable fly, Stomoxys calcitrans, on blood pads containing cadmium chloride, cadmium succinate, cadmium iodide, and cadmium 2-hydroxyethyl mercaptide caused egg reduction, but also caused high mortality rates (Kunz 1967). Females feeding on pads containing 0.006% cadmium acetate oviposited a mean of 0.80 ova/day, compared with a mean of 21.20 ova/day for untreated females. The mortality rate of the treated females was 57% by the day egg deposition began. At this concentration, cadmium acetate inhibited ovarian growth so that the ovaries of 20 day treated flies had not developed beyond the ovarian stage normally found in four day untreated females. A cadmium chloride concentration of 0.0045% reduced egg production by 51.7% and caused 53%

mortality. At a concentration of 0.009%, cadmium succinate produced 95% mortality in the females by day six after treatment. Cadmium iodide and cadmium 2-hydroxyethyl mercaptide both caused more than 50% mortality at concentrations of 0.006 and 0.018%, respectively, and reduced egg production. No repellency was noticed for any of the compounds, and hatchability of eggs from treated females was similar to that of the untreated females. When added to the larval diet, cadmium acetate at a concentration of 0.033%, and cadmium acetate and cadmium succinate at 0.008% prevented emergence of adults. Larval growth was also retarded by these treatments. Dipping of two to three day pupae for 10-20 minutes in 0.05 - 1.0% concentrations of cadmium chloride and cadmium acetate did not affect the egg production of resulting females.

Working with the southern house mosquito, Culex quinquefasciatus Say, Bosworth (1969) found that 1.0 ppm cadmium acetate, cadmium chloride, and cadmium iodide in the rearing water of first instar larvae caused mortalities of 51, 64, and 45%, respectively, while increasing the median pupation time to 13, 14, and 15 days, respectively. The median pupation time for untreated larvae was approximately ten days. Females reared from the 1.0 ppm cadmium chloride treated larvae exhibited distorted ovaries. The testes of companion males apparently escaped damage. A larval diet of 1:100 cadminate to diet caused 64% mortality to first instars and increased pupation time to 13 days. Pupae reared in water containing 10.0 ppt cadmium chloride, cadmium acetate, and zinc chloride died before or during emergence. Adults feeding on sugar pads containing 3.0% cadmium chloride at 0.10 - 1.0 ppt were killed.

Antimony

Antimony is a naturally occurring element that was known as a metal before the beginning of the 17th century (Weast 1969). It is a bluish-white metal that is common but not abundant. More often than not, it is combined with oxygen to produce the so-called "White Antimony Blende" (Hey 1966). Antimony may also be recovered from flue dusts. Industrially, antimony is widely used in making type metals, alloys, batteries, cable sheathing, coloring glass, vulcanizing rubber, paints, striking surface for safety matches, etc. (Hey 1966 and Weast 1969). Medicinally, trivalent antimony compounds have been used for more than 40 years in treating human schistosomiasis (DeWitt 1965), bilharziasis (Mansour 1967), leishmaniasis (Maleki 1967), trypanosomiasis (Ercoli 1968), and filarial lymphomas (El-Toraei 1968). Hydrated antimony potassium tartrate has also been used as a medicinal emetic (Weast 1969). The biological half-life of antimony in the human liver is 38 days. However, patients may retain an abnormally high antimony level in the blood for more than a year following treatment (Mansour 1967). The maximum safe concentration of antimony in dusts for humans is recommended to be 0.50 ug/m^3 of air (Weast 1969).

Belyaeva (1967) reported that female workers in an antimony plant suffer significantly more gynecological afflictions than those who do not (77.5% vs 56%). He stated that female workers are particularly susceptible to spontaneous late abortions (12.5% vs 4.1%). By the age of one year, infants of working mothers were lagging noticeably behind those of non-workers. Antimony was demonstrated in the blood, urine, milk, placenta, and amniotic fluid of working mothers. In comparing these observations with laboratory studies, Belyaeva (1967) found that

exposing female rats to antimony trioxide caused sterility and reduction in the number of young born by deranging ovogenesis.

Drinking water containing 5 ppm antimony exhibited inherent toxicity to mice (Schroeder et al. 1968). Evidence of this included shortened median life span, suppression of growth in older animals, and loss of weight in animals after 18 months of age. The life span of females was shortened by 49-86 days, whereas the males were little affected. The mice appeared to accumulate antimony in the lungs and liver.

Chronic oral poisoning of guinea pigs with trivalent antimony in doses of 0.25 mg/kg body wt caused functional disturbances in the liver and thyroid gland (Osintseva et al. 1966). The guinea pigs also experienced a slight increase in hemosiderin content in the spleen, slight changes in the glomerula apparatus of the kidneys, and an interstitial reaction in the lungs.

Daily injections of antimony potassium tartrate into conscious dogs had little effect upon the diastolic blood pressure (Cotten and Logan 1966). However, the dogs died of other symptoms three to five days after injections began. Geometrically increasing the injection dosages in anesthetized dogs lowered the blood pressure and increased the heart rate progressively.

Bosworth (1969) reported that antimony potassium tartrate at concentrations of 0.10 ppt and 10.0 ppm in the rearing water of larvae appeared to have no effect upon the reproductive organs of the mosquito, Culex quinquefasciatus Say. Triphenylantimony was very toxic to the German cockroach, Blatella germanica, when incorporated into a diet at concentrations of 1.0, 2.0, and 5.0% (Abdel-Razig 1966). On the other

hand, Abdel-Razig (1966) reported that the above concentrations of triphenylantimony increased both the fecundity and fertility of the house fly, Musca domestica. Flies feeding on a diet containing 2.0% triphenylantimony produced 30% more eggs and larvae. He also stated that flies feeding on 5.0% triphenylantimony suffered no mortality.

The reader who is interested in the subject of insect sterilization is referred to the literature reviews of Kunz (1967) and Bosworth (1969), and "Induced Sterilization and Control of Insects," by Proverbs (1969).

MATERIALS AND METHODS

Argas persicus

A parent tick colony was established from specimens furnished by Livestock Insects Investigations, Entomology Research Division, Agriculture Research Service, USDA, Kerrville, Texas. Maintenance of the colony was accomplished by using a modification of Micks' (1951) technique. The colony was housed in a heavy cardboard container having inside dimensions of 9½ x 13 inches (Figure 1). Two four-inch square windows were cut near the bottom on opposite sides of the container to enable observation of the colony without removing the lid. The inside of the container was lined with a transparent sheet of cellulose nitrate to prevent the nymphs and adults from climbing the wall. The larvae could climb the cellulose nitrate but were prevented from escaping by smearing a band of vaseline near the upper margin. Those larvae not repelled by the vaseline became entrapped while trying to crawl through it. Interior ventilation of the container was permitted by a 4 x 6 inch opening in the lid, covered with screen mesh and a double layer of black muslin cloth. A double layer of black muslin cloth was also wrapped around the container to simulate nocturnal conditions for the enclosed ticks. The floor of the container was covered with wood shavings to a depth of approximately two inches. The shavings served as an absorbent for excretions from both the ticks and introduced chicks, as hiding places for the ticks, and as an anchor for the center piece on which most of the ticks remained.

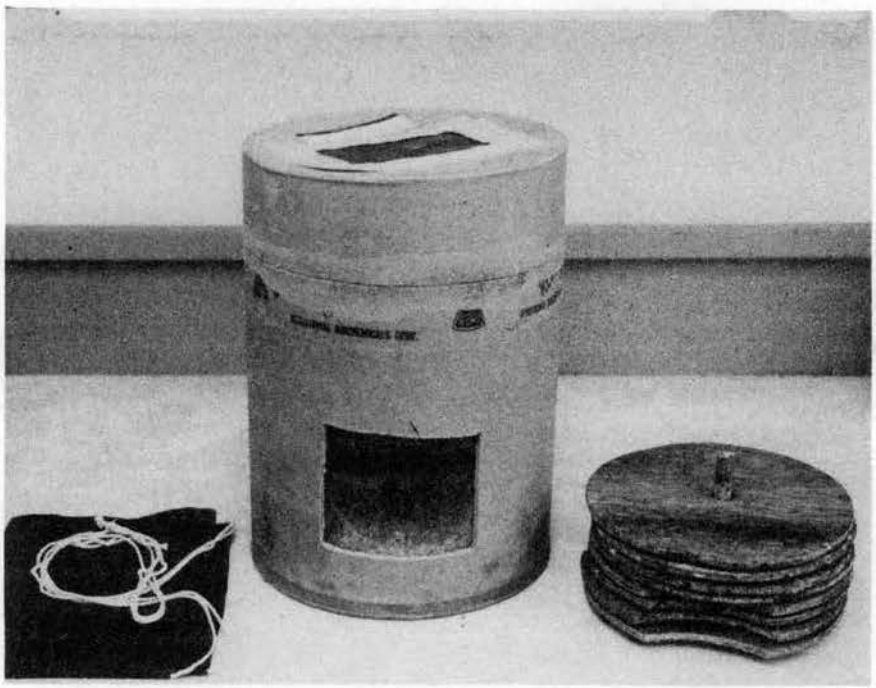

Fig. 1. Container used for housing the parent colony of A. persicus. Left, black muslin wrapping; right, center piece of container.

The center piece consisted of eight ¼ x 8 inch plywood disks stacked with ¼ inch separating adjacent disks (Fig. 1.). The separation between adjacent disks was maintained by four evenly spaced, radiating ¼ x ¼ x 2 inch wood strips. Each plywood disk contained a center hole one inch in diameter, through which a 3/4 inch dowel was passed. A nail extended through the base of the dowel perpendicular to its long axis, permitting removal of the center piece as a unit by lifting on the top of the dowel. Or individual plywood disks could be removed from the top without disturbing the lower disks or the dowel shaft. This arrangement furnished a compact resting site exposing a large surface area capable of accommodating a large population of ticks.

The tick colony was maintained under laboratory conditions of 80 F ± 5 F and relative humidity ranging from about 40 - 70%.

Feeding of the ticks was accomplished by placing chicks on the top shelf of the center piece and leaving them overnight. The nymphs and adults were fed at weekly intervals after the larvae had been removed from the colony. The chicks were prevented from falling off the center piece by a ½ inch wire mesh enclosure 3½ inches high and seven inches in diameter placed on the top shelf of the center piece. Since feeding nymphs and adults do considerable damage to the chicks and since the larvae remain attached for four to six days while engorging, it was necessary to feed the larvae without allowing the nymphs and adults to reach the chicks. This was accomplished by placing a circular strip of cellulose nitrate two inches high and 7½ inches in diameter between the two top shelves of the center piece while permitting the larvae to become attached to the chicks (Fig. 2). If all of the larvae failed to become attached in one night, additional chicks were introduced into

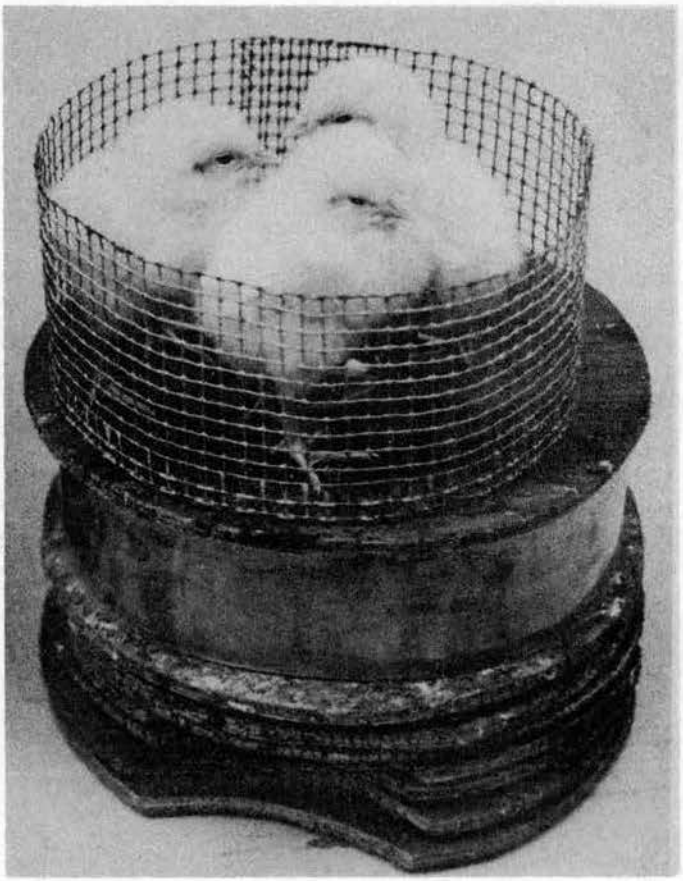

Fig. 2. Colony container center piece prepared to prevent A. persicus nymphs and adults from feeding on chicks while the larvae become attached.

the colony the following night. This periodic feeding schedule produced relatively homogeneous age groups for testing purposes.

The chicks harboring larvae were segregated into a cage (Fig. 3) and held until the beginning of the fourth day following larval attachment. They were then transferred to a five gallon bucket (Fig. 4) and left until the engorged larvae had dropped.

The five gallon bucket contained a center piece with two shelves. The top shelf was 5½ inches from the floor and consisted of ½ inch wire mesh 10½ inches in diameter stapled over a ¼ x 1 inch plywood rim. The second shelf was half way between the top shelf and the floor and was of ¼ inch plywood 9½ inches in diameter to accommodate for the sloping bucket wall. The bottom shelf was covered with paper towels and served to catch feces and debris falling through the wire bottom of the top shelf. The engorged larvae which dropped from the chicks gathered beneath pieces of paper toweling provided for that purpose on the bucket floor. This arrangement permitted rapid removal of the larvae from the bucket without having to sift through the materials that collected on the bottom shelf of the center piece. The few larvae that tried to escape from the bucket were prevented from doing so by a band of vaseline smeared around the upper wall margin.

Collected larvae not being used for tests were returned to the parent colony. The larvae to be used for tests were transferred to 1 x 6 inch test tubes and held in a dark bioclimatic chamber at 80 F and 45-65% relative humidity (Fig. 5). The test tubes contained a piece of paper towel to serve as a resting surface and as an absorbent for excretions. Black muslin cloth secured by a rubber band served as a cover for the test tubes.

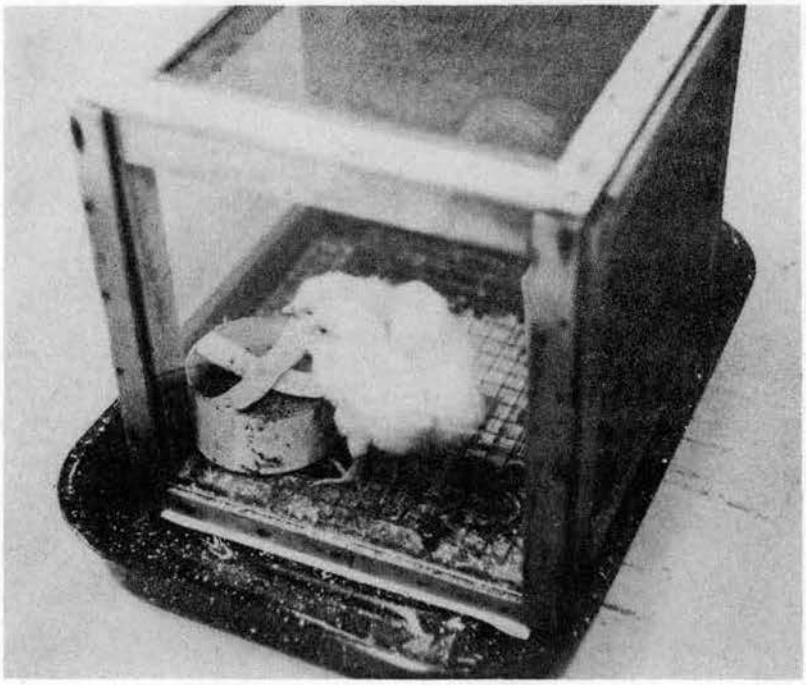

Fig. 3. A modified insect cage used for housing chicks harboring A. persicus larvae.

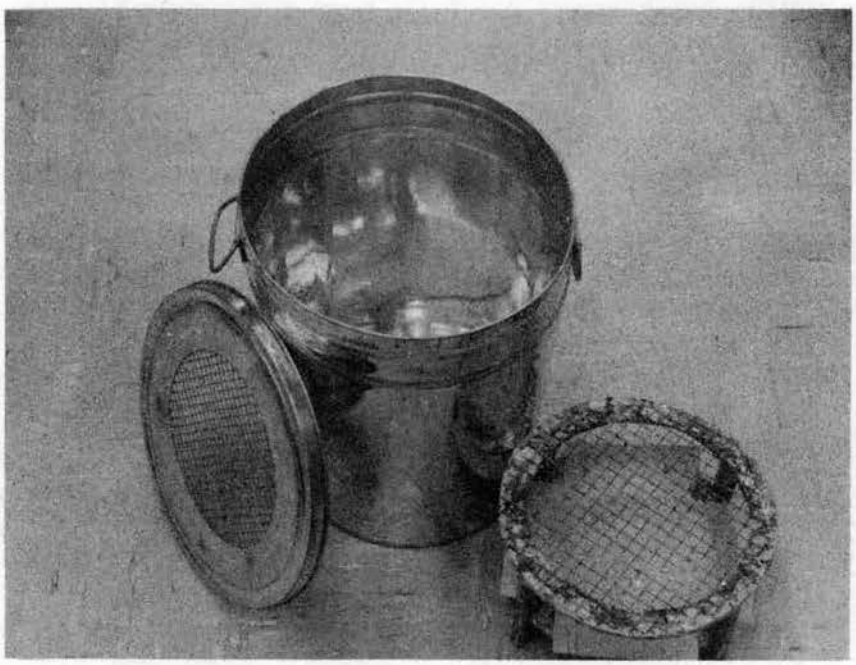

Fig. 4. Five gallon bucket used to maintain chicks while A. persicus larvae were dropping. Right, center piece constructed to keep chicks, wastes, and larvae separated.

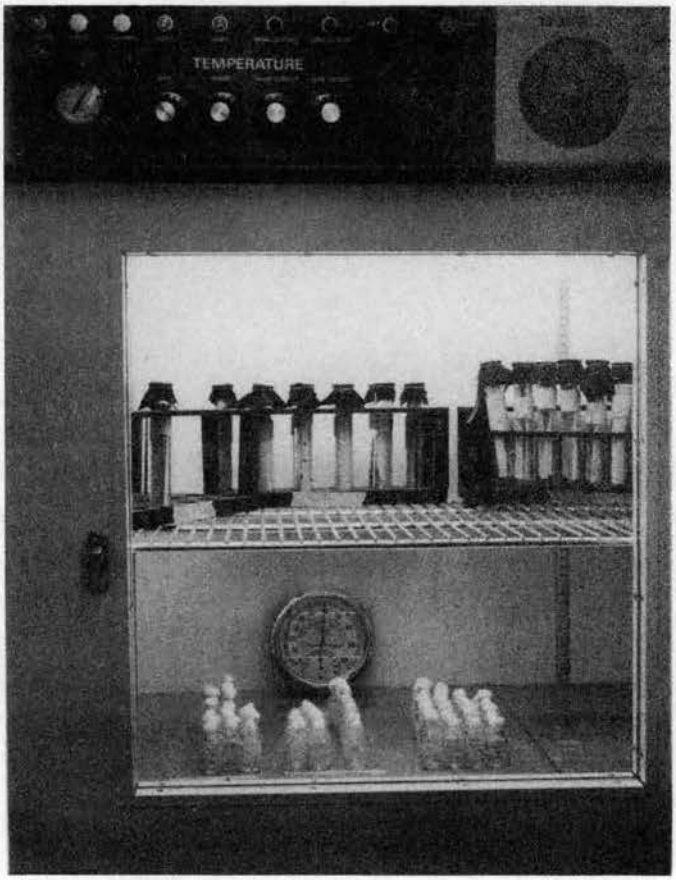

Fig. 5. Bioclimatic chamber containing A. persicus. Top shelf, test tubes containing nymphs and adults; bottom shelf, vials containing eggs and larvae.

Treatments

The compounds tested were $CdCl_2 \cdot 2\frac{1}{2}H_2O$, $K(SbO)C_4H_4O_6 \cdot \frac{1}{2}H_2O$, $(C_6H_5)_3Sb$, and $ZnCl_2$. These compounds were administered to the ticks by: (1) in vivo feeding of larvae and neonymphs on cadmium chloride poisoned chicks; (2) in vitro feeding of neonymphs on chicken blood containing cadmium chloride, cadmium chloride-zinc chloride (50:50), antimony potassium tartrate, and triphenylantimony; (3) inuncting neonymphs with lanolin containing cadmium chloride, and deuteronymphs and adults with lanolin containing triphenylantimony; and (4) dipping of ova in solutions of cadmium chloride and antimony potassium tartrate.

In Vivo Feeding

This treatment method necessitated a preliminary study to determine the relative toxicity of cadmium to chicks. This was determined by adding cadmium chloride and cadmium chloride-zinc chloride to the drinking water of the chicks at concentrations of 0.025 - 1.0% on a weight/volume basis and recording the survival duration and percentage mortality of the chicks for each treatment level. From this determination, cadmium chloride concentrations of 0.075 and 0.15% were selected as the most appropriate for the in vivo tests. Because of the toxicity of the cadmium compounds, larvae were allowed to attach to the chicks three days prior to giving the chicks water containing cadmium chloride. This enabled the larvae to feed on cadmium poisoned chicks for the last two or three days of their engorgement period. If the chicks died before the larvae became completely engorged, they were replaced with others receiving the same dosage of cadmium chloride. Larvae engorged only on chicks poisoned with the 0.15% concentration, whereas neonymphs were permitted to feed on chicks poisoned with both the 0.075 and 0.15%

concentrations. The neonymphs were treated by leaving them overnight in one pound coffee cans with chicks that had been on the cadmium chloride water for two or three days. The coffee cans contained an elevated ½ inch wire mesh floor to prevent active chicks from injuring the ticks. Beneath the elevated floor was a double layer of paper toweling to absorb the moisture from chick feces.

In Vitro Feeding

Blood for the in vitro treatments was obtained by bleeding roosters into a jar containing heparin sodium as a blood preservative. Heparin was used at a concentration of 20 mg/100 ml of blood. Immediately after bleeding, the blood was placed into a refrigerator and maintained at 4 C until it was needed. Skin to serve as feeding membranes was removed from the apteria regions beneath the wings of the roosters at the time of bleeding. The skin was put into a freezer immediately after removal and left there until thawed for use.

A search of the literature produced no reference of Argas persicus having been fed previously in vitro. Therefore a feeding technique had to be developed prior to using this method of treatment. The apparatus used consisted of a specially designed water bath and a Chemical Rubber Company immersion heater having a magnetically controlled thermostat that maintained the water bath temperature within ± 0.5 C of the desired temperature (Fig. 6). The water bath was constructed from a two-piece plastic freezer dish, with the lid soldered to the base and the top cut away. Four 7/8 inch holes, one at each end and two in front, were cut through the dish sides near the base. A 7/8 inch rubber washer was then soldered around each hole on both the inside and

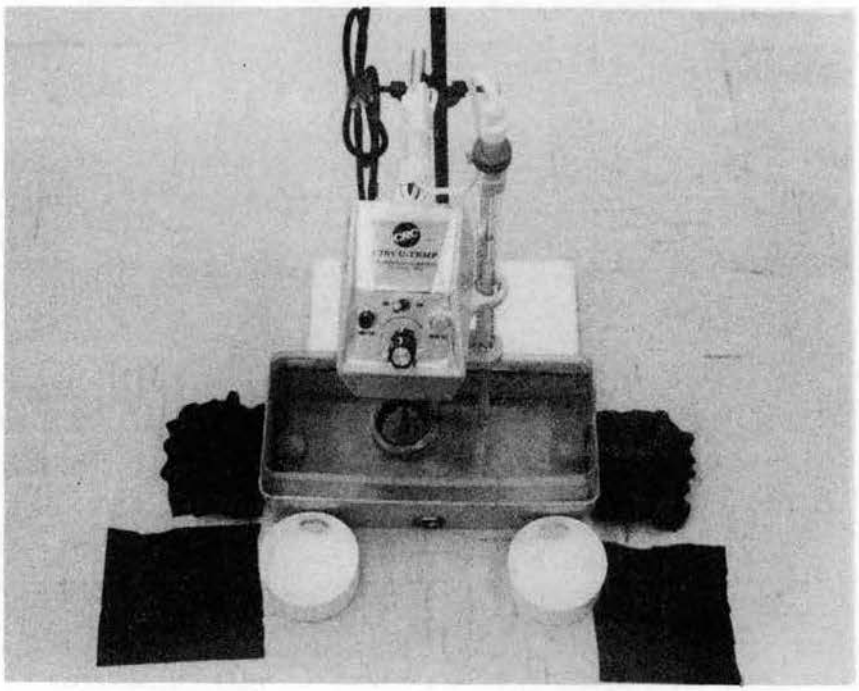

Fig. 6. Immersion heater and water bath apparatus used for in vitro feeding of A. persicus neonymphs.

outside surfaces. The washers fitted snugly around inserted vials and prevented water leakage from the water bath during use.

In setting up the feeding stations, 10 ml vials were filled with refrigerated chicken blood, the respective pre-measured test compound, and 0.00307 g glutathione as a feeding inducer (Galun and Kindler 1965). A piece of thin chicken skin was then stretched over the mouth of a vial and secured against the vial lip with a small rubber band. Excess skin was trimmed as close as possible to the vial lip. After the skin membrane had been warmed, the vial was inserted through a hole in the side of a ½ pint ice cream cup and positioned so that the vial's lip fitted snugly against the interior wall of the cup (Fig. 7). The distal end of the vial was then inserted through a hole into the water bath. Approximately 3/4 of the vial extended into the water bath and served as a conducting surface to heat the enclosed chicken blood. The ticks that were to be fed were placed into the ½ pint cup, which was then covered with a piece of black muslin that was secured with a rubber band. The water bath was filled with water that was heated and maintained at 40 C ± 0.5 C, approximating the normal body temperature of chickens (Barger and Card 1949). Feeding stations were set up in the evening and left overnight in a dark laboratory. The ticks that survived the treatments were transferred to the test tubes described above and kept in a dark bioclimatic chamber at 80 F and 45-65% relative humidity.

Neonymphs were fed in vitro on chicken blood containing cadmium chloride at concentrations of 0.0001, 0.001, 0.005, 0.01, 0.25, and 0.50%; cadmium chloride-zinc chloride each at concentrations of 0.0001 and 0.001%; antimony potassium tartrate at concentrations of 0.0001,

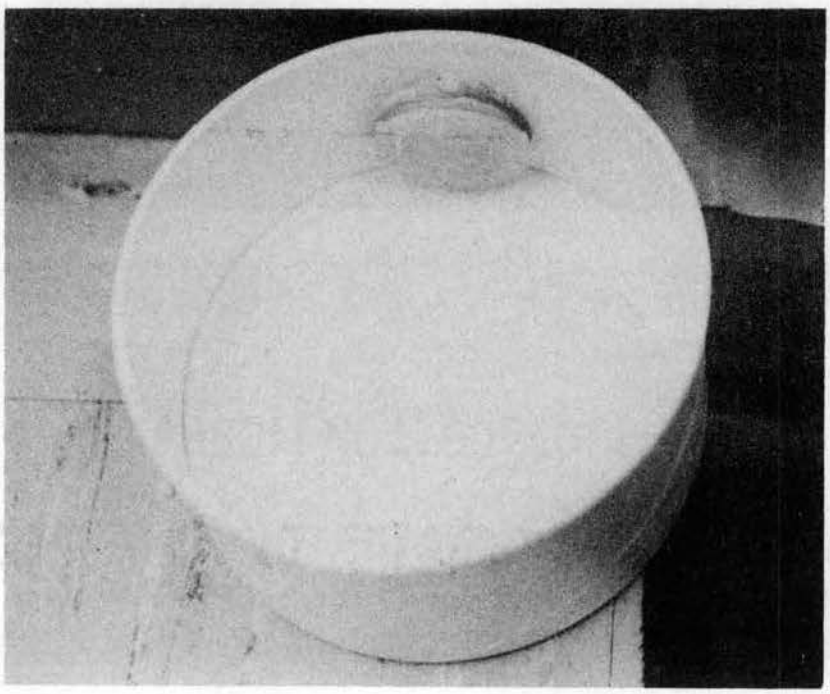

Fig. 7. A feeding station prepared for in vitro feeding of A. persicus neonymphs. The chicken skin membrane covering the proximal end of a vial is exposed. The distal end of the vial extends into the water bath.

0.001, 0.01, 0.075, 0.133%; and triphenylantimony at a concentration of 0.001%. A control station was maintained where the neonymphs engorged on chicken blood minus the test chemicals.

Inuncting

The ticks that were used for inuncting studies were treated the day following engorgement on chicks. Neonymphs were inuncted with cadmium chloride at concentrations of 0.05, 0.10, 0.25, 0.50, 1.25, and 2.50%. Deuteronymphs and adults were inuncted with triphenylantimony at concentrations of 0.05, 0.10, 0.50, and 1.25%. A check and control group were used for each compound tested. Ticks of the control groups were inuncted with lanolin only, while those of the check groups were not inuncted.

Lanolin was used as a solvent and carrier for the test compounds. The appropriate concentration of a test compound was thoroughly mixed with 2.0 g of lanolin and stored until it had dissolved. After the test compound had dissolved the inunctum was thoroughly stirred again prior to being used. A toothpick was used to transfer a sample of the mixture to the dorsum of a tick. The treated neonymphs had a mean weight of 0.83 mg. The quantity of cadmium chloride applied to each tick averaged 0.24 mg. The ticks receiving the 2.50% concentration of cadmium chloride received an average of 0.006 mg of cadmium chloride, with the ticks of the other groups receiving proportionately less as the treatment concentration decreased. Although these data were not obtained for the deuteronymphs and the adults receiving the triphenylantimony treatments, these ticks were much larger and the tendency would be to apply a larger quantity of the lanolin-triphenylantimony mixture. Inuncted ticks were maintained in the bioclimatic chamber as

described above. After all the ticks of a treatment group had undergone ecdysis, they were transferred to a clean test tube to prevent the resulting ova from becoming contaminated with the test compound.

Egg Dipping

The toxicity of cadmium chloride and antimony potassium tartrate to A. persicus ova was studied by dipping the ova in physiological saline containing the compounds for periods ranging from 30 seconds to 30 minutes. All ova had been deposited for less than 24 hours at the time of dipping. Ova were dipped in cadmium chloride concentrations of 0.01, 0.10, 0.50, 1.0, 5.0, and 10.0% and in antimony potassium tartrate at concentrations of 0.10, 0.20, 0.30, and 0.40%. One group of ova was dipped in physiological saline only to serve as a control. Another group was not dipped and served as a check against the saline. The dipped ova were air dried and then put into one-ounce vials. The vials were stoppered lightly with cotton and maintained in the bioclimatic chamber until all resulting larvae had died, allowing sufficient time for all ova to hatch prior to collecting the toxicity data.

Histological Studies

Histological preparations of the adult reproductive system were made for microscopic detection of morphological damage resulting from the treatments. When the females of a test or control group had ceased ovipositing their first batch of eggs, a sample of each sex was removed from the test tube and fixed for four hours in Carnoy's fluid (Humason 1962). After washing the ticks in absolute ethyl alcohol, the reproductive systems were dissected from them under 70% ethyl alcohol, and after dehydrating, they were embedded in paraffin (Paraplast).

The embedded specimens were serially sectioned at ten microns and mounted on microscope slides. The mounted sections were: (1) mordanted with Heidenhain's iron alum for ten minutes; (2) stained with Heidenhain's hematoxylin for ten minutes; (3) destained in picric acid for 30 minutes; (4) washed in tap water for 15 minutes; (5) dehydrated through the alcohol series; and (6) mounted under a cover glass with Permount (Fisher Scientific Co.). This procedure produced sections with dark blue or black nuclei contrasted against a light yellowish cytoplasmic background.

Amblyomma americanum

Concurrent with studying the effects of cadmium chloride on the soft tick, A. persicus, limited observations were made on the effects of cadmium chloride on a hard tick, A. americanum. Replete A. americanum females were removed from cattle or from the ground of holding pens and transported to the laboratory for treatment. In the laboratory each tick was placed into individual one-ounce plastic cups for holding throughout the study. Each test female received a 0.05 ml subcuticular injection of physiological saline containing a cadmium chloride concentration of 0.0001, 0.001, 0.01, 1.0, or 5.0%. The injections were administered on the dorsum of the abdomen with a 3/8 inch, 26 gauge hypodermic needle. The control ticks received 0.05 ml of physiological saline only, while the check ticks were not injected at all. When a female began ovipositing it was moved into a constant temperature chamber, where the resulting larvae were held at 80 F and 70% relative humidity until hatching occurred. Upon hatching, the larvae were transferred to conditions of 80 F and 45-65% relative humidity and held until the study terminated. Data were collected on

toxicity of cadmium chloride to the ticks, number of ova deposited per treatment, ova deposited per female per treatment, percentage hatch per treatment, and longevity of hatched larvae.

Because of the numbers involved, only estimates were made on the total number of eggs obtained from each treatment. In assuming that the volume occupied by an unhatched ovum is approximately equal to that occupied by a hatched, starved larva, an estimate of ova number was made by using the volume method. Counting revealed that a syringe volume of 0.1 cc contained approximately 600 ova. An estimate of total ova per treatment was obtained by multiplying the total volume occupied by the pooled larvae and unhatched ova of a treatment by the 600 ova per 0.1 cc conversion factor. The percentage hatch was determined by pouring the syringe contents into a petri dish and counting the number of larvae and unhatched ova occupying a binocular grid square. A mean of three or four countings was used. The number of unhatched eggs per grid square multiplied by the total number of squares occupied furnished an estimate of unhatched ova. This was converted to a percentage of the estimated total ova to give percentage hatch. In cases where all the eggs of a particular female failed to hatch, the grid square method was used to estimate the total number of ova deposited.

RESULTS AND DISCUSSION

In Vivo Feeding

The toxicity of cadmium chloride and cadmium chloride-zinc chloride to chicks is illustrated in Table 1. Nearly 67% of the one week chicks died when receiving cadmium chloride in drinking water at a concentration of 0.025%. While the two week chicks survived the 0.025% cadmium chloride treatments, higher dosages were lethal to all chicks treated. A 0.01% concentration of cadmium chloride in the drinking water was not lethal to the chicks. However, chicks receiving the 0.01% treatment were Barred Plymouth Rock while those receiving the other treatments were White Leghorn. Thus chick survival at this concentration may have resulted from a difference in breed physiology. Aside from possible breed differences, when cadmium chloride alone was used, toxicity appeared to be more dependent upon treatment level than chick age. A 50:50 ratio by weight of cadmium chloride-zinc chloride in the drinking water at concentrations of 0.025% and 0.50%, respectively, was lethal to 16.67% and 100% of the chicks.

There are numerous reports that cadmium and zinc are antagonistic in biological systems, and that simultaneous administration of zinc chloride with cadmium chloride protects against cadmium induced injury (Parizek 1957 and 1960, Meek 1959, Gunn et al. 1961, Kar et al. 1962, and Mason et al. 1964). The above results, however, indicate that zinc failed to protect the chicks against cadmium injury at the concentrations used in these tests.

Table 1. Toxicity of cadmium chloride and cadmium chloride-zinc chloride to chicks when administered in the drinking water on a weight/volume percentage basis.

No. of Chicks	Age of Chicks	Treatment	Days Survival Mean	Days Survival Range	Per Cent Mortality
1	1 week	1.0% $CdCl_2$	5.0	---	100
1	2 weeks	1.0% "	1.0	---	"
1	1 week	0.5% "	1.0	---	"
3	1 week	0.25% "	6.5	6-7	"
3	3 weeks[a]	0.15% "	5.0	3-9	"
1	2 weeks	0.15%	3.0	---	"
6	1 week	0.15% "	3.6	3-4	"
3	1 week	0.075% "	2.0	---	"
1	1 week	0.05% "	2.0	---	"
6	1 week	0.025% "	12.6+	3-21+[b]	66.66
2	2 weeks	0.025% "	14.0+[c]	---	0
6	1 week	0.01%	14.0+	---	0
4	1 week	0.05% $CdCl_2$:$ZnCl_2$	5.0	4-6	100
6	1 week	0.025% "	12.66+	6-14+	16.67

[a]Larvae attached

[b]Chicks sacrificed after 21st day

[c]Chicks sacrificed after 14th day

The visual symptoms of cadmium poisoning in the chicks included anemia, followed by diminishing activity, constantly closed eyes, droopy stance with refusal to sit, dullness, frequent falling, diarrhea, and just before death a wrinkled, bluish appearance of the beak and legs.

The assumption that cadmium chloride was being transported in the circulatory system of the poisoned chicks is supported by Table 2. While there was essentially no difference between the percentage mortality of control ticks and neonymphs feeding on chicks utilizing drinking water containing 0.075% cadmium chloride, a null hypothesis of proportions (Freund 1960) showed that the percentage mortality of the ticks feeding on chicks receiving 0.15% cadmium chloride treated water was significantly higher. The differences among percentage mortalities of the control ticks and larvae and neonymphs feeding on 0.15% cadmium chloride poisoned chicks were statistically significant at the 5% and 1% levels, respectively.

Reproductive data for the surviving larvae and neonymphs are presented in Table 3. Although the control group produced nearly twice as many ova as the treated groups, the percentage hatch was much lower. Subjection of these data to a Chi-square test (Snedecor 1956) showed that the 63.08% hatch for the control group was not quite significantly different at the 5% level from the 82.33% hatch of the 0.075% cadmium chloride treated neonymphs. The explanation for the lower percentage hatch of the control ova is not known. Perhaps the chloride imbibed by the treated ticks contributed slightly to the viability of subsequent ova. This may also help to explain why a mean of 14.97 days was required for the control eggs to hatch, while the eggs from larvae

Table 2. Mortality of A. persicus following in vivo engorgement of larvae and neonymphs on chicks consuming cadmium chloride treated water.

Treatment	Number Engorged	Stage Treated	Deaths				Per Cent[a] Mortality
			Larvae	N_1	N_2	Total	
Control	751	Larva, N_1[b]	27	4	-	31	4.02
0.075%	203	N_1	-	7	3	10	4.92
0.15%	237	Larva	31	4	-	35	14.76
0.15%	77	N_1	-	30	2	32	41.55

[a]Prior to adult

[b]Neonymph

Table 3. Reproductive data for A. persicus following in vivo engorgement of larvae and neonymphs on cadmium chloride poisoned chicks.

Treatment	No. Females	No. Males	Eggs Laid	Eggs Hatched	% Hatch	Days (Mean)	Ova/[a] Female	Ova/[a] Male
Control	50	63	5729	3654	63.08	14.97	114.58	90.89
0.075% (N_1)	66	80	3846	3185	82.33	14.45	58.26	48.07
0.15% (Larva)	51	43	3704	2875	80.13	13.96	72.63	77.16
0.15% (N_1)	70	22	2401	1845	76.93	14.43	34.30	109.13
0.15% (Partly[b] engorged larvae)	18	17	1104	833	75.44	13.13	61.33	64.94

[a]Total eggs deposited/treatment divided by the number of females and males present during the oviposition period.

[b]Chicks died before larvae were completely engorged; larvae were transferred to non-poisoned chicks to complete engorgement.

partly engorged on the 0.15% cadmium chloride poisoned chicks required a mean hatching period of 13.13 days.

Despite the data suggesting that the treatments may have aided viability of ova from the treated ticks, Table 3 indicates that the treatments impaired gametogenesis, quantitatively speaking. The mean of 114.58 ova per female deposited by the control group is significantly different at the 5% level from the mean number of ova deposited by the females of the treated groups. The number of ova produced per female appeared to be influenced more by age at treatment than by treatment concentration. Thus the larvae receiving the 0.15% cadmium chloride treatment produced more ova than the neonymphs receiving 0.075% cadmium chloride. Females from the neonymphs receiving the 0.15% cadmium chloride treatment produced less than half the number of ova (34.30 vs 72.63) produced by females from the larvae receiving the same treatment. This should be expected if Balashov and Goroschchenko (1962) were correct in stating that gonad differentiation is not observed in the larva of A. persicus. Since gametogenesis does not occur until after gonadal differentiation, treatment of larvae with reproductive toxicants would be less likely to impair reproduction than treating the nymphs during the time of gonadal differentiation. The "Eggs/Male" column in Table 3 (and following tables) is included for the reader's interpretation. I could not find reference to the number of females capable of being inseminated by one male tick. Since this is not reported, an abnormally low male to female ratio in a test group may present false reproductive data for the males.

These tests indicated that ticks could be treated with cadmium chloride by permitting them to engorge upon cadmium treated chicks.

But the inherent toxicity of cadmium to the chicks made this treatment method impractical. Hence the in vivo studies were terminated.

In Vitro Feeding

The in vitro feeding of A. persicus met with limited success, yet repetition of feeding trials furnished a sufficient quantity of engorged ticks with which to conduct valid tests. Of the 2188 neonymphs offered chicken blood containing only heparin sodium and glutathione, 7.58% engorged (Table 4). When cadmium chloride at concentrations greater than 0.005% were added to the blood preparations the percentage engorgement decreased. The percentage engorgement on chicken blood containing cadmium chloride concentrations of 0.005% and 0.001% exceeded that of the controls (Table 4). This is confusing in that when blood containing 0.0001% cadmium chloride was tested, only 3.54% of the neonymphs engorged. Addition of equal weights of zinc chloride to the 0.001% and 0.0001% cadmium chloride blood solutions appeared not to alter percentage engorgement. If cadmium chloride inhibited feeding of A. persicus, as it was reported to have done with Drosophila melanogaster and Periplaneta americana (Abdel-Razig 1965), it did so only at concentrations of 0.0001, 0.25, and 0.50%.

Gaun and Kindler (1965) reported that glutathione is specific in activating the sucking response in ticks. These authors obtained 78% engorgement of Ornithodorus tholozani when feeding them through "an artificial parafilm membrane" on whole blood containing 10^{-2} M glutathione warmed to 38 C. In this study A. persicus did not feed through either a Parafilm M membrane (American Can Co.) or a Parafilm M membrane smeared with chicken skin oils extracted in acetone. Nor did

Table 4. Responses of A. persicus to in vitro engorgement of neonymphs on chicken blood containing cadmium chloride and cadmium chloride-zinc chloride.

Treatment	Attempted Feedings	Number Engorged	Per Cent Engorged	Mortality[a] N_1	Mortality[a] N_2	% Survival To Adult
Control	2188	166	7.58	33	-	80.12
0.50% $CdCl_2$	451	16	3.58	16	-	0
0.25% "	1155	37	3.20	37	-	0
0.01% "	2940	198	6.74	194	-	2.01
0.005% "	4038	374	9.21	370	-	1.07
0.001% "	1708	228	13.00	223	1	2.01
0.0001%	4177	148	3.54	108	-	27.02
0.001% $CdCl_2$: $ZnCl_2$ (50:50)	3798	236	6.26	233	-	1.18
0.0001% "	2992	189	6.30	170	-	10.05

[a] N_1, N_2; neonymphs, deuteronymphs, respectively.

0.00307 g/10 ml (10^{-2} M) glutathione increase percentage engorgement by more than 50%. A major obstacle to engorgement in this study may have been the high temperature requirement of the blood necessary to simulate the natural body temperature of fowl. At 40 C the blood began to coagulate and putrify within two to three hours after heating. Glutathione retarded the rate of putrification of the blood, and this may have contributed more to the increased engorgement rate than its capacity to act as a feeding inducer. The thickness of the chicken skin membranes may also have been a hindrance to in vitro engorgement of A. persicus, considering that neonymphs normally engorge upon the feet and lower legs of their host.

Table 4 indicates that cadmium chloride was extremely toxic to the ticks engorging in vitro. All of the ticks that engorged on blood containing 0.25 and 0.50% cadmium chloride expired. From 1.0 - 2.0% of those engorging on blood containing 0.005, 0.001, and 0.01% cadmium chloride survived to become adults. The neonymphs engorging on the 0.0001% cadmium chloride blood solution experienced a moderate survival of 27.02%. Even this highest survival rate was significantly lower at the 1% level from those engorging on blood lacking cadmium chloride. With one exception, the neonymphs died prior to molting into deuteronymphs. Usually death occurred during the night of engorgement. Addition of equal proportions by weight of zinc chloride to cadmium chloride increased the mortality of engorging ticks. These results were contrary to the reports stated above that simultaneous administration of zinc chloride with cadmium chloride masks the damaging effects of cadmium chloride to organisms. When equal weights of zinc chloride were added to the 0.001 and 0.0001% cadmium chloride blood solutions,

the percentage survival decreased from 2.01 to 1.18% and 27.02 to 10.05%, respectively. Perhaps equimolar concentrations of zinc chloride-cadmium chloride may have been more effective in preventing cadmium chloride damage to A. persicus.

Comparing the results of these tests with similar studies utilizing insects indicates that equivalent concentrations of cadmium chloride may be more toxic to A. persicus than to insects. For example, Kunz (1967) reported that a cadmium chloride concentration of 0.0045% in bovine blood produced 53% mortality to the stable fly, Stomoxys calcitrans, compared with 98.93% mortality to A. persicus at a concentration of 0.005%. Likewise, Bosworth (1969) found that adding 1.0 ppm cadmium chloride to the rearing pans of first instar C. quinquefasciatus caused 64% mortality, compared with 73.98% mortality for A. persicus at the same concentration. This higher toxicity of cadmium chloride to A. persicus may result from the tick's habit of excreting water and chlorides through the coxal glands as it engorges (Lees 1946), resulting in highly concentrated haematin granules being stored in the gut (Tatchell 1964). Sulfhydryl groups of blood proteins have a high affinity for cadmium (Singh and Mathur (1968), and concentration of the blood proteins in the gut undoubtedly leads to cadmium concentration as well. Cadmium also replaces iron and zinc at metabolic sites (Hill et al. 1963). Hence the high concentration of cadmium in the gut constituents may impede metabolism, if not prevent it.

Apparently the in vitro blood preparations per se contained inherent toxic properties to the engorging ticks, for in comparing the 80.12% survival for the controls of Table 4 with the interpolated 95.98% survival for the controls of Table 2, it is seen that there was

approximately 15.86% greater survival for the ticks engorging on the chicks. This is to be expected in that the compounded effects of the blood preservative heparin, feeding inducer glutathione, and mechanical manipulation of the blood in preparing the feeding stations probably produced a sub-optimal diet compared with that taken from the living chicks.

The reproductive data for ticks engorged in vitro on blood containing cadmium chloride and cadmium chloride-zinc chloride are tabulated in Table 5. The percentages of ova hatching in the treated groups were not significantly different from those in the control. It appears that the addition of zinc chloride to the 0.001 and 0.0001% cadmium chloride treatments slightly increased the fertility of the eggs in ticks surviving these treatments, as 86.50 and 85.10% hatch, respectively, occurred in these groups, whereas only 74.75% of the ova in the control group hatched. The higher percentage hatch in the treated groups may be a result of sampling error, for the number of females ovipositing in these groups was much smaller than in the control group. The mean number of days required for the ova to hatch ranged from 13 to 15 days for all groups and did not appear to be dependent upon treatment concentrations.

From Table 3 it was noticed that females resulting from larva and neonymphs engorging on chicks oviposited a mean of 114.58 ova. In vitro engorgement of neonymphs on blood lacking the test compounds reduced the mean number of ova deposited by the resulting females to 56.37 (Table 5). The indications are that the prepared in vitro diet of preserved blood was detrimental to oogenesis and egg production. The only in vitro treatment causing a significantly lower egg

Table 5. Reproductive data for A. persicus engorged in vitro as neonymphs on chicken blood containing cadmium chloride and cadmium chloride-zinc chloride.

Treatment	No. F	No. M	No. Ova Deposited	No. Ova Hatched	Per Cent Hatch	Days (Mean)	Ova/F	Ova/M
Control	47	48	2559	1914	74.75	14.10	56.37	53.20
0.50% $CdCl_2$	0[a]	-	-	-	-	-	-	-
0.25% "	0[a]	-	-	-	-	-	-	-
0.01% "	1	3	23	19	82.60	13.00	23.00	7.67
0.005% "	3	1	195	125	64.10	14.00	65.00	195.00
0.001% "	4	1	213	163	76.45	15.00	53.25	213.00
0.0001%	7	4	910	738	81.00	13.64	130.00	227.75
0.001% ($CdCl_2$:$ZnCl_2$)	3	2	178	154	86.50	13.00	59.33	89.00
0.0001% "	8	8	1135	966	85.10	14.79	141.86	141.86

[a]Treatments caused 100% mortality (Table 4)

production than the in vitro control group was the 0.01% cadmium chloride treatment, where the lone surviving female deposited only 23.00 ova. The females resulting from neonymphs engorging on blood containing 0.0001% cadmium chloride and 0.0001% cadmium chloride-zinc chloride produced a mean of 130.00 and 141.86 ova, respectively. These means were much greater than that for the ticks engorging on living chicks. This would suggest the possibility that the very low concentrations of cadmium and zinc salts imbibed by the ticks actually aided oogenesis and fertility. While cadmium supposedly has no known biological function (Cotzias et al. 1961a), zinc is an essential trace element utilized as a cofactor in many enzymes of mammals (Timm and Schulz 1966).

Histological preparations of the reproductive systems of untreated ticks are presented in Figures 8-10. Figure 8 shows all stages of spermatogenesis and a large bundle of mycetomes (microsymbiotes associated with fat bodies and gonads of many arthropods, Steinhaus 1967). The sub-circular morphology of the spermatids indicates that maturation (spermatelosis, Bedi 1962) is occurring. During spermatelosis the spermatids elongate to become spermatozoa. The odd shape of the spermatids may also be partly a result of aging in the tick, for both the male in Figure 8 and the female in Figure 9 were older than the treated ticks when fixed. Aging is also indicated in Figure 9, where some previous ootid stalks are evident. These stalks are the sites where ova have descended into the lumen of the ovary prior to oviposition. Figure 10 shows essentially the same thing as Figure 8 except that most of the spermatids are well rounded, indicating that they have not begun

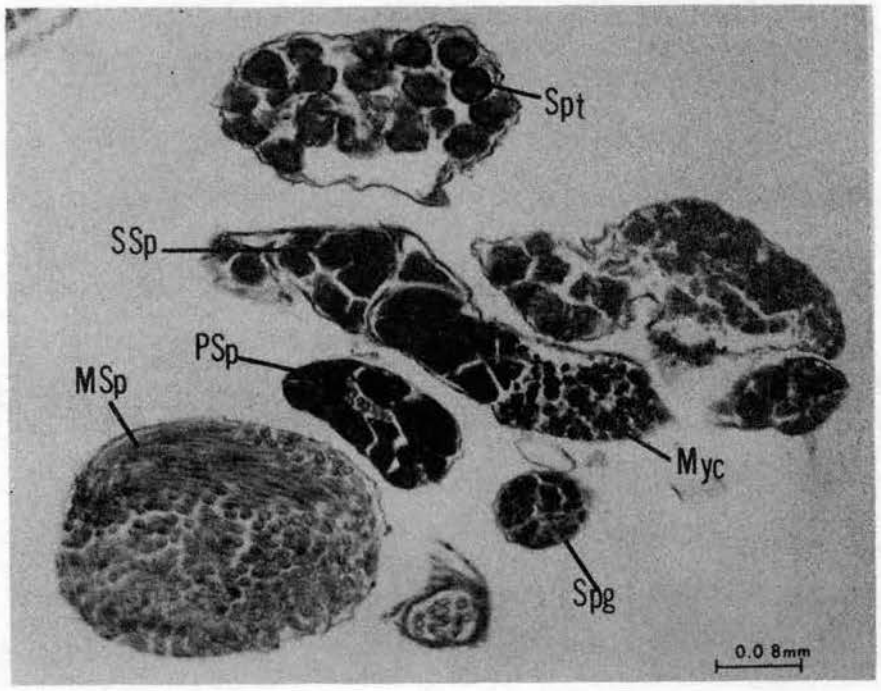

Fig. 8. Section through the reproductive system of an untreated male A. persicus showing all stages of spermatogenesis and associated mycetomes. Spg, Spermatogonium; PSp, Primary Spermatocyte; SSp, Secondary Spermatocyte; Spt, Spermatid; MSp, Mature Spermatozoa; Myc, Mycetomes.

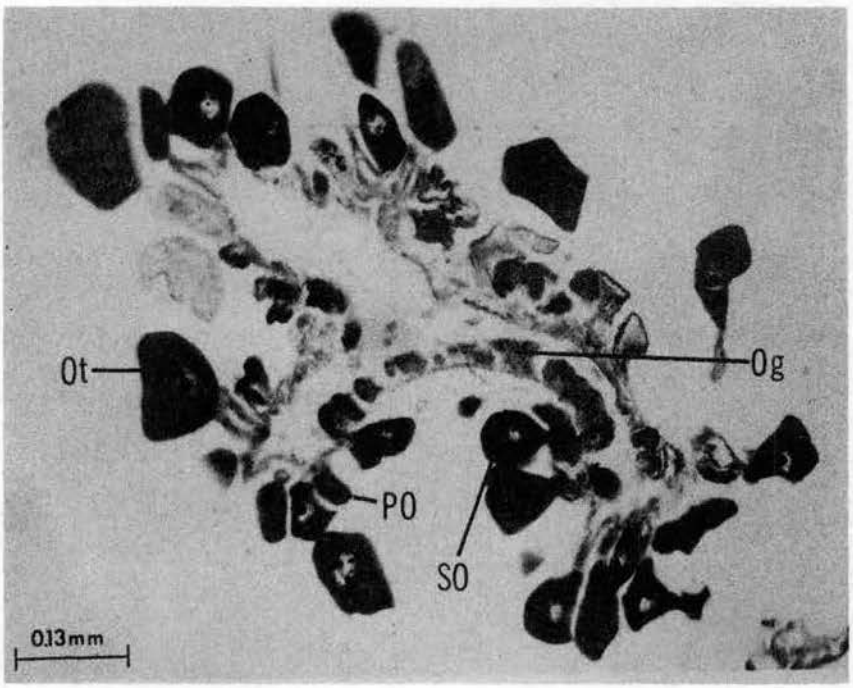

Fig. 9. Section through the ovary of an untreated female A. persicus illustrating the stages of gametogenesis. Og, Oogonium; PO, Primary Oocyte; SO, Secondary Oocyte; Ot, Ootid.

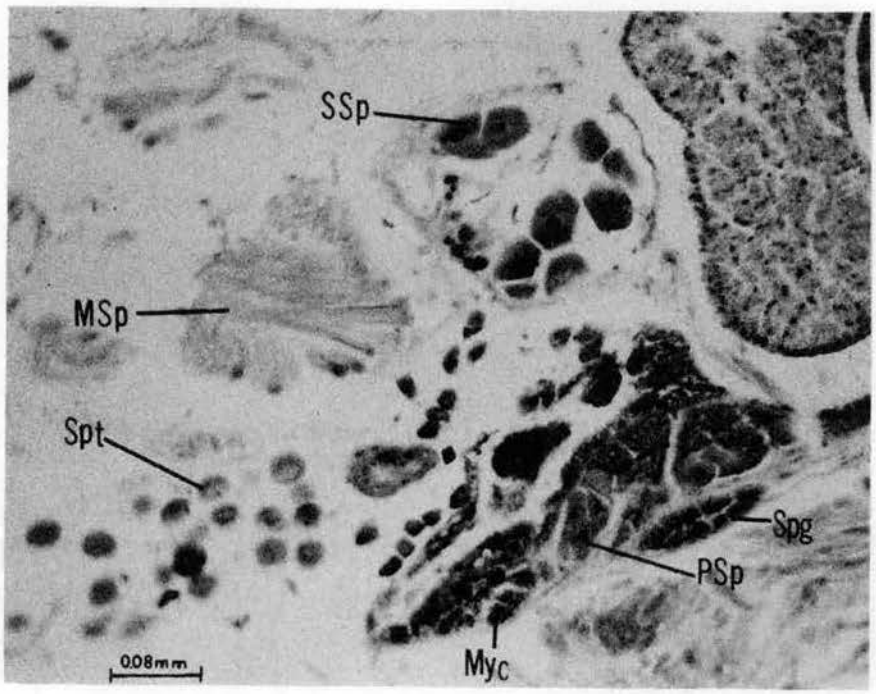

Fig. 10. Section through the reproductive system of an in vitro engorged (untreated blood) male A. persicus. Spg, Spermatogonium; PSp, Primary Spermatocyte; SSp, Secondary Spermatocyte; Spt, Spermatid; MSp, Mature Spermatozoa; Myc, Mycetomes.

spermatelosis. Numerous sperm are also present, suggesting that the in vitro treatments per se did not adversely affect spermatogenesis.

Figures 11-15 show representative histological sections of the reproductive systems of ticks that engorged in vitro on chicken blood containing cadmium chloride and cadmium chloride-zinc chloride. From the appearance of the spermatids in Figure 11, it seems that the 0.01% cadmium chloride treatment had no damaging effect upon spermatogenesis in the surviving males. The one surviving female of this treatment died after depositing only 23 eggs and prior to fixing. Hence it appears that the significantly lower reproduction subsequent to this treatment resulted more from toxicity to the ticks than from reproductive inhibition. Figure 12 illustrates all stages of oogenesis in a female treated with 0.001% cadmium chloride. Although the survival rate for this treatment was only 2.01% (Table 4), the survivors seemed to suffer no gametic damage. Various stages of gametogenesis in ticks receiving 0.0001% cadmium chloride and 0.0001% cadmium chloride-zinc chloride in vitro are shown in Figures 13-15. The mean number of ova deposited per female and male and the percentage hatch of ova from ticks receiving these concentrations were much higher than for the control group (Table 5). While mortality was high at the 0.0001% concentrations, survivors exhibited an increase in both fecundity and fertility over the controls. These differences may not be statistically significant, however, because of the small sample sizes resulting from the treatments.

In 1962 Bedi published that A. persicus spermatozoa are transferred to females in an immature state, and that spermatelosis occurs in the uterus. The illustrations above do not support his statement, for

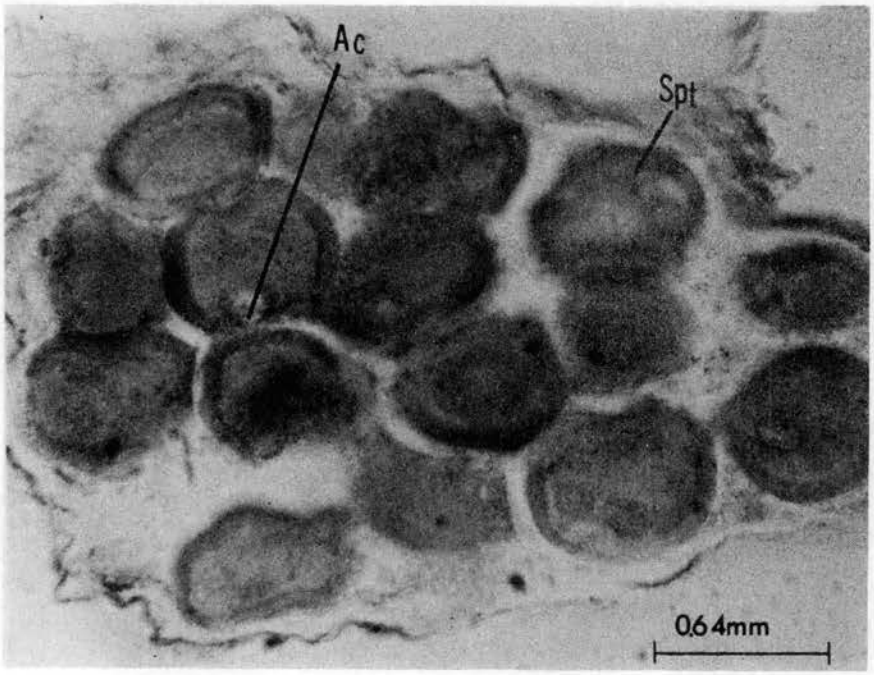

Fig. 11. A sectioned packet of spermatids from a male A. persicus that engorged in vitro on chicken blood containing 0.01% cadmium chloride. Spt, Spermatid; AC, Acrosomal Cap.

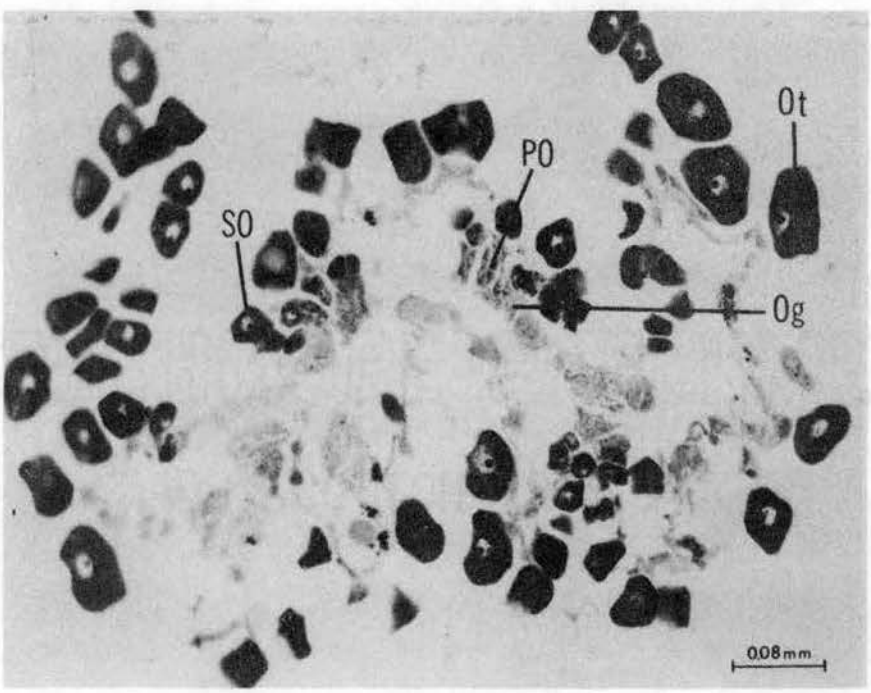

Fig. 12. Sectioned ovary illustrating all stages of oogenesis in a female A. persicus that engorged in vitro on chicken blood containing 0.001% cadmium chloride. Og, Oogonium; PO, Primary Oocyte; SO, Secondary Oocyte; Ot, Ootid.

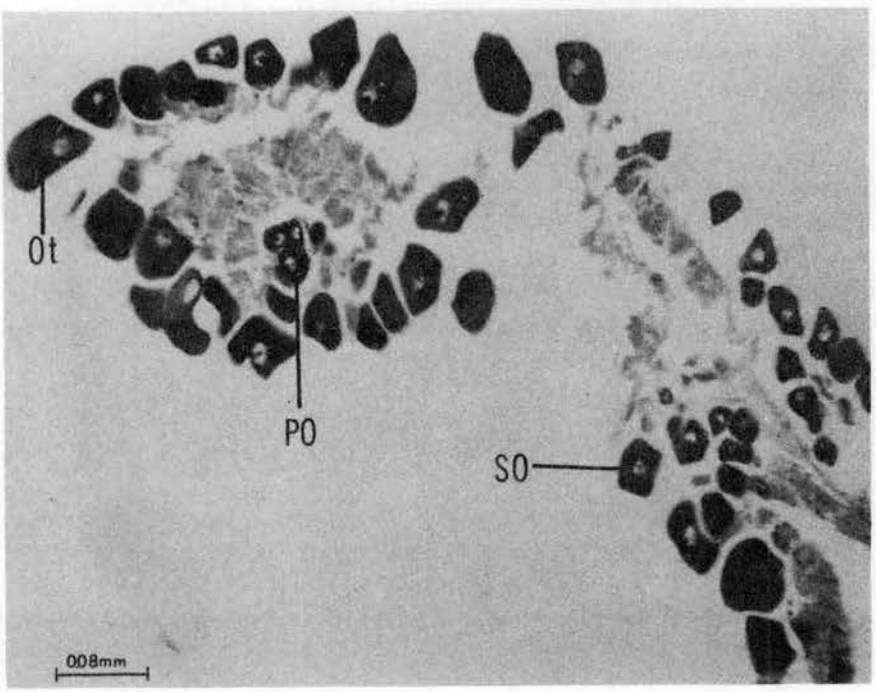

Fig. 13. Sectioned ovary showing oocytes and ootids of a female A. persicus that engorged in vitro on chicken blood containing 0.0001% cadmium chloride. PO, Primary Oocyte; SO, Secondary Oocyte; Ot, Ootid.

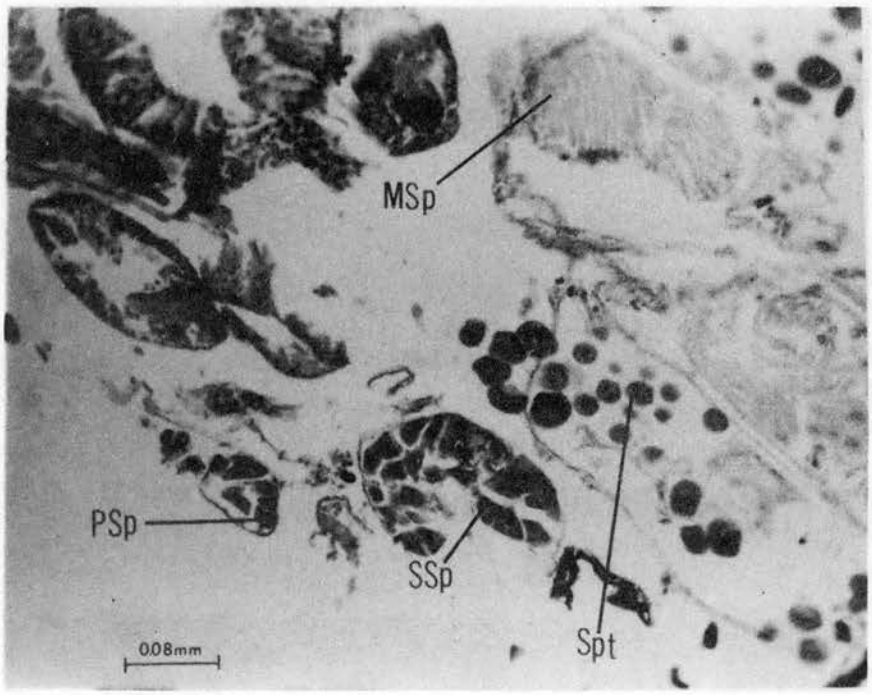

Fig. 14. A section through the accessory glands of a male A. persicus that engorged in vitro on chicken blood containing 0.0001% cadmium chloride-zinc chloride. PSp, Primary Spermatocyte; SSp, Secondary Spermatocyte; Spt, Spermatid; MSp, Mature Spermatozoa.

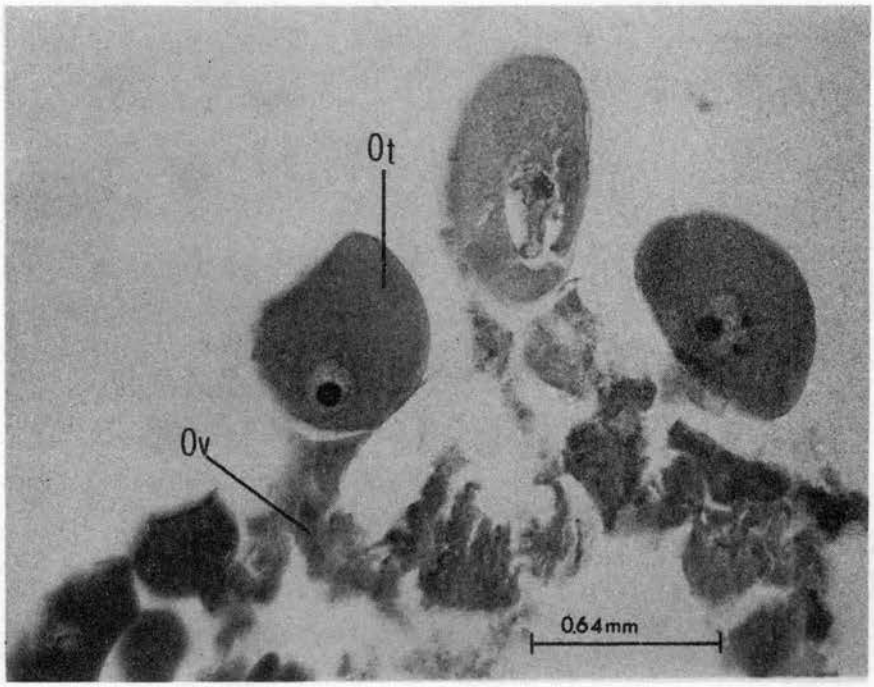

Fig. 15. Sectioned ootids from a female A. persicus that engorged in vitro on chicken blood containing 0.0001% cadmium chloride-zinc chloride. Ot, Ootid; Ov, Ovary.

sections of mature spermatozoa can be seen in the figures displaying male reproductive systems. In no instance has the author recognized immature spermatozoa in the uterus of a sectioned female. In this study, however, females were not sectioned immediately following insemination. Such a technique might have revealed females harboring immature spermatozoa.

Lower concentrations of antimony potassium tartrate fed in vitro to A. persicus neonymphs were not nearly as toxic to the ticks as were comparable concentrations of cadmium chloride (Table 6). For example, approximately 70% of the neonymphs engorging on the 0.001 and 0.0001% antimony potassium tartrate solutions survived to become adults, compared with 2.01% and 27.02% for respective concentrations of cadmium chloride. The 70% survival rate for the antimony potassium tartrate engorged ticks was still significantly lower at the 5% level than that for the in vitro control group, indicating that the survival rate was treatment dependent. Concentrations of antimony potassium tartrate greater than 0.01% appeared to be as toxic to the ticks as comparable concentrations of cadmium chloride. As in the cadmium chloride tests, a majority of the deaths (all but 5) occurred in the instar treated. Usually death occurred within a day following engorgement on the test compound.

The mean number of ova deposited per female subsequent to neonymph engorgement on antimony potassium tartrate at concentrations of 0.001 and 0.0001% was much lower than it was for comparable cadmium chloride concentrations (Table 7). At these concentrations, females of the antimony potassium tartrate treatments deposited approximately one half the mean number of ova as those in both the control and comparable

Table 6. Responses of A. persicus to in vitro engorgement of neonymphs on chicken blood containing antimony potassium tartrate and triphenylantimony. Controls are included in Table 4 data.

Treatment	Attempted Feedings	Number Engorged	Per Cent Engorged	Mortality[a] N_1	Mortality[a] N_2	% Survival to Adult
0.133% APT[b]	4,490	174	3.8	173	-	0.5
0.075% "	2,989	124	4.1	124	-	0
0.01% "	8,340	104	1.2	97	-	6.7
0.001% "	3,471	211	6.0	62	-	70.6
0.0001% "	4,793	273	5.6	78	4	69.9
0.001% T[c]	1,184	64	5.4	18	1	65.2

[a] N_1, N_2; neonymphs, deuteronymphs, respectively

[b] Antimony Potassium Tartrate

[c] Triphenylantimony

Table 7. Reproductive data for A. persicus engorged in vitro as neonymphs on chicken blood containing antimony potassium tartrate and triphenylantimony. Controls are included in Table 5 data.

Treatment	No. F	No. M	Ova Deposited	Ova Hatched	% Hatch	Days (Mean)	Ova/ F	Ova/ M
0.133% APT[a]	1	1[b]	128	111	86.70	13.0	128	128
0.075% "	0	0	-	-	-	-	-	-
0.01% "	7	1	669	70	10.46	14.0	95.8	669
0.001% "	27	28	787	695	88.40	16.0	29.2	28.1
0.0001% "	18	16	701	600	85.40	14.3	38.9	43.8
0.001% T[c]	21	18	1,340	1,115	83.2	14.75	63.81	77.44

[a]Antimony Potassium Tartrate

[b]Introduced non-treated tick

[c]Triphenylantimony

cadmium chloride treatment groups. While the fecundity of these females was significantly lower at the 5% level, the percentage hatch of the resulting ova was not. In the 0.01 and 0.133% antimony potassium tartrate groups the mean number of ova deposited per female was 95.8 and 128, respectively. The reason for the larger number of ova deposited in these groups is not known. However, these sample sizes were much smaller, and it was noted that whenever a large number of ticks were present in the vials a much smaller mean number of ova was recorded, regardless of whether a vial contained a control or treated group. Perhaps there was population density inhibition upon the fecundity of females in crowded vials. The females from the 0.01% antimony potassium tartrate treatment produced ova having only 10.46% hatchability. This was significantly lower (1% level) than the hatchability of ova in any other group. Triphenylantimony is insoluble in water, yet data from Tables 6 and 7 indicate that at a concentration of 0.001%, the neonymphs picked it up during in vitro engorgement.

Figure 16 illustrates what appears to be definite damage to the spermatids of males that engorged as neonymphs on blood containing 0.01% antimony potassium tartrate. While it is normal for spermatids to be oddly shaped, depending upon the state of maturity, only males from this treatment exhibited wrinkled and ruptured spermatids. Females from the same treatment also experienced gametic damage. Figure 17 clearly shows ootid damage. One secondary oocyte in the figure also appears to be ruptured. The more mature stages of gametogenesis seemed to be more susceptible to damage than the earlier stages. Since the cytoplasm to nucleus ratio in developing gametes greatly increases with

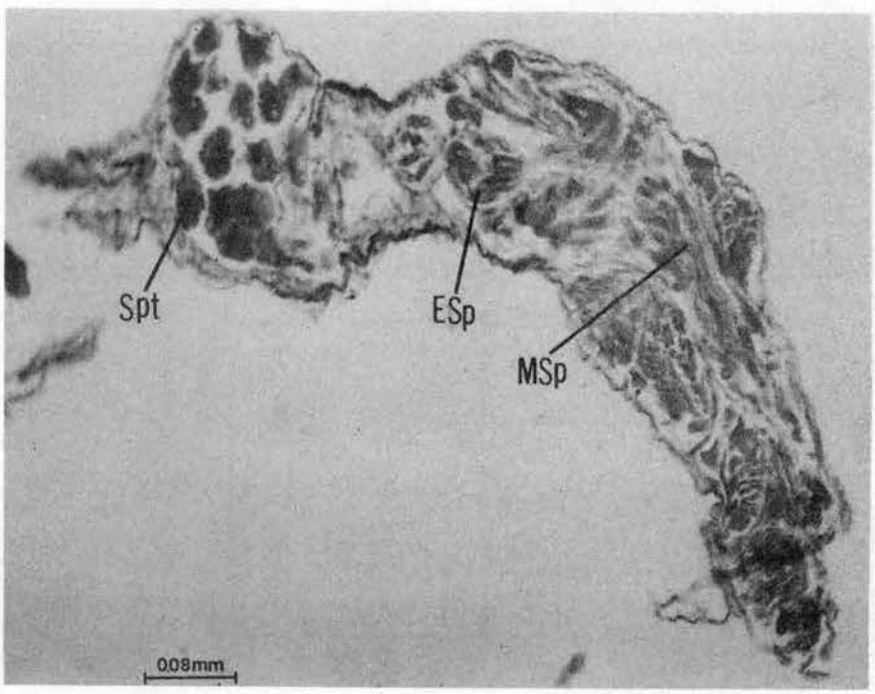

Fig. 16. A section showing damaged spermatids in the reproductive system of a male A. persicus that engorged in vitro on chicken blood containing 0.01% antimony potassium tartrate. Spt, Spermatid; ESp, Elongating Spermatid; MSp, Mature Spermatozoa.

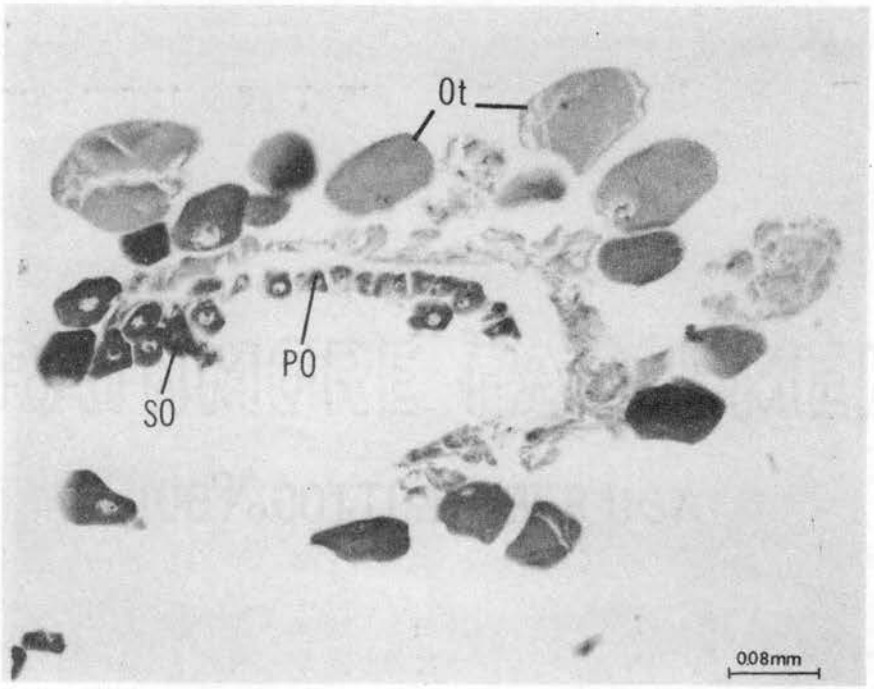

Fig. 17. Sectioned ovary illustrating damaged ootids in a female A. persicus that engorged in vitro on chicken blood containing 0.01% antimony potassium tartrate. PO, Primary Oocyte; SO, Secondary Oocyte; Ot, Ootid.

maturity, perhaps the primary sites of destruction were cytoplasmic rather than nuclear.

Fertility and histological evidence suggest that the ticks engorging in vitro on antimony potassium tartrate at concentrations less than 0.01% suffered no gametic damage. With the exception of one female, all the ticks engorging at concentrations greater than 0.01% died. The lone survivor appeared normal, and the author suggests that it may have failed to imbibe enough of the test compound to affect it.

Inuncting

Lanolin was used as a carrier for the test compounds in the inuncting studies for three reasons: (1) the test compounds are soluble in lanolin; (2) the tick cuticle has a high affinity for lanolin; and (3) lanolin has a very low evaporative potential, enabling the test compounds to maintain cuticular contact until the ticks undergo ecdysis.

A surprising result of the inuncting studies was that lanolin itself caused a high mortality to the inuncted neonymphs. It was observed that in most cases the lanolin migrated to completely envelop the cuticular surface of the ticks. Hence the mode of action of the lanolin is thought to have been physical blockage of the spiracles rather than chemical toxicity to the ticks. In determining the toxicity of the inuncted compounds, it is therefore necessary to consider the effect of the lanolin as well as that of the chemicals tested.

Table 8 shows that 69.06% of the neonymphs inuncted with lanolin only died prior to becoming adults, whereas only 2.65% of the untreated neonymphs died. In the control group, as well as in the treated groups, most of the deaths occurred to neonymphs prior to molting into deutonymphs. A null hypothesis of proportions showed that mortality in the

Table 8. Effects of inuncting A. persicus neonymphs with lanolin containing cadmium chloride.

Treatment	Nymphs Treated	Mortality[a]			Per Cent Mortality (Prior to Adult)
		N_1	N_2	Total	
Check[b]	264	7	-	7	2.65
Control[c]	288	191	18	209	69.06
2.50% $CdCl_2$	175	110	13	123	70.27
1.25% "	120	111	3	114	95.00
0.75% "	196	140	30	170	86.73
0.50% "	145	139	6	145	100.00
0.25% "	205	194	4	198	92.15
0.10% "	225	209	-	209	92.88
0.05% "	114	89	-	89	78.07

[a] N_1, N_2; neonymphs, deuteronymphs, respectively

[b] Not treated

[c] Inuncted with lanolin only

neonymphs inuncted with lanolin containing 0.05 and 2.50% cadmium chloride was not significantly higher at the 5% level than that of the control group. The mortality of neonymphs inuncted with cadmium chloride concentrations ranging from 0.10% to 1.25% was significantly higher at the 5% level than that of the control group which was inuncted with lanolin only. Thus it may be assumed that at these concentrations, enough cadmium chloride was absorbed through the cuticle to be toxic to the neonymphs. Why the 2.5% cadmium chloride treatment was not toxic to the neonymphs, when lower concentrations were, is not known.

Reproductive data for cadmium chloride inuncted neonymphs are tabulated in Table 9. The results obtained are difficult to comprehend. There was a direct relationship between treatment concentration and the mean number of ova deposited per female. Females from neonymphs inuncted with 2.5, 1.25, and 0.75% cadmium chloride concentrations, respectively, deposited a mean of 114.80, 110.33, and 79.00 ova. The control females deposited a mean of 56.40 ova. Females inuncted with 0.10 and 0.05% cadmium chloride concentrations, respectively, deposited a mean of 44.25 and 30.00 ova. An inverse relationship between treatment concentration and percentage hatch of ova was not clear cut, but the tendency was indicated. The percentage hatch of ova from treated females ranged from 72.40% in the 2.5% cadmium chloride treatment group, to 94.30% in the 0.10% cadmium chloride treatment group.

There was no evidence of microbial growth in any of the egg vials. Hence it is thought that interaction between the mycetomes and the absorbed chemicals could have influenced the results for both the mortality data and the reproductive data in the inuncting studies. Even though mycetomes are usually associated with the gonads of

Table 9. Reproductive data for A. persicus neonymphs inuncted with lanolin containing cadmium chloride.

Treatment	No. F	No. M	Ova Deposited	Ova Hatched	% Hatch	Days (Mean)	Ova/F	Ova/M
Control[a]	72	19	1,523	1,143	75.04	14.17	56.40	31.08
2.50% $CdCl_2$	15	26	1,722	1,248	72.41	13.82	114.80	66.23
1.25% "	3	5	331	275	83.00	13.44	110.33	66.20
0.75% "	3	3	237	187	78.00	13.60	79.00	79.00
0.50% "	0	0[b]	-	-	-	-	-	-
0.25% "	0	1[c]	-	-	-	-	-	-
0.10% "	4	6	177	167	94.30	14.75	44.25	29.50
0.05% "	3	13	90	82	91.10	13.50	30.00	6.92

[a]Inuncted with lanolin only

[b]Treatment caused 100% mortality (Table 8)

[c]Six of seven treatment survivors apparently eaten by chicks prior to oviposition

arthropods, their functions are still largely a mystery. At this time it is not known how biological or chemical alteration of the mycetomes would affect the biology and reproduction of ticks.

Examination of histological preparations of adult reproductive systems resulting from cadmium chloride inuncted neonymphs revealed no apparent gametic damage.

Inuncting of deuteronymphs and adults with triphenylantimony was conducted on a much smaller scale than similar tests with neonymphs and cadmium chloride, thus the results may not be as valid. Nevertheless, Table 10 shows that neither lanolin nor triphenylantimony treatments caused as high a mortality rate in deuteronymphs and adults as the lanolin and cadmium chloride did in the neonymphs. The adults inuncted with lanolin only suffered 23.10% mortality, compared with 69.06% mortality for the neonymphs. Maturity of the ticks may have been a factor in the survival differences between the neonymphs and adults. The surface area of the mature ticks was greater, hence the proportion of lanolin to cuticle surface area may have been smaller. This would reduce the chances of spiracle blockage, and if this were a factor causing mortality, reduce the mortality rate.

By the null hypothesis, 65.0% mortality was the 5% level of significance for adults inuncted with triphenylantimony. From Table 10 it is seen that only the adults inuncted with the 0.05% concentration of triphenylantimony suffered a percentage mortality significantly higher than that of the control group. On the other hand, all groups of deuteronymphs inuncted with triphenylantimony had a percentage mortality equal to or exceeding the adult level of significance, with the percentage mortality increasing proportionately with treatment

Table 10. Effects of inuncting A. persicus deuteronymphs and adults with lanolin containing triphenylantimony.

Treatment	Instar Treated	Number Treated	Mortality[a]			Per Cent Mortality
			N_2	Adult	Total	
Check[b]	N_2, Adult	11	0	0	0	0
Control[c]	Adult	13	0	3	3	23.10
1.25% TPA[d]	N_2	20	18	-	18	90.00
" "	Adult	17	-	8	8	47.10
0.50% "	N_2	20	13	-	13	65.00
"	Adult	20	-	8	8	40.00
0.10% "	N_2	20	15	-	15	75.00
" "	Adult	20	-	7	7	35.00
0.05% "	N_2	20	13	-	13	65.00
" "	Adult	20	-	13	13	65.00

[a] N_2, deuteronymphs

[b] Not treated, served as checks for both N_2's and adults

[c] Treated with lanolin only

[d] Triphenylantimony

concentration. The mortality for triphenylantimony inuncted deuteronymphs ranged from 65.0% for those treated with a concentration of 0.05%, to 90.0% for those receiving the 1.25% concentration. As with the cadmium chloride tests, mortality in these tests usually occurred to the instar treated. However, three deuteronymphs inuncted with triphenylantimony survived an ecdysis and then died after oviposition had begun. Except for the 0.05% treatment, deuteronymph mortality exceeded adult mortality at a given treatment concentration.

Although lanolin was not as lethal to deuteronymphs and adults as it was to neonymphs, it drastically reduced fecundity and fertility and increased mean hatching time in these groups. Table 11 shows that females inuncted with lanolin as adults had a mean egg production of less than one half that of females from untreated deuteronymphs. Ova fertility in the lanolin inuncted group was 19.10% lower, and the mean time required for hatching of the fertile eggs was increased by 2.5 days. The lower egg production in the inuncted group may have resulted from physical obstruction of the genital pore or Gene's organ. Feldman-Muhsam (1964) reported that obstruction of the outlets of the accessory glands may prevent oviposition but does not affect the internal physiological processes of egg development.

With the exceptions of those deuteronymphs inuncted with 0.05% and 1.25% triphenylantimony, the mean number of ova deposited by the chemically treated females was much lower than the mean number deposited by the females inuncted with lanolin only. While some of the differences were statistically significant at the 5% level, they had no pattern and did not appear to be treatment dependent. In the case of the 1.25% triphenylantimony treatment, only one female survived to reproduction

Table 11. Reproductive data for A. persicus inuncted as deuteronymphs and adults with lanolin containing triphenylantimony.

Concentration and Instar Treated	No.[a] F	No.[b] M	Ova Deposited	Ova Hatched	% Hatch	Days (Means)	Ova/F	Ova/M
Check, N_2, A[c]	6	5	957	539	60.2	13.0	156.1	191.4
Control, A[d]	8	5	564	232	41.1	15.5	70.5	112.8
1.25% T[e], N_2	1	1	156	45	28.8	15.6	156.0	156.0
" " A	8	6	111	86	77.4	14.0	13.9	18.5
0.50% ", N_2	4	1	60	44	73.3	14.0	15.0	60.0
" ", A	6	11	351	103	29.1	17.0	58.5	31.9
0.10% ", N_2	3	1	165	141	85.5	13.6	55.0	165.0
" ", A	5	11	265	41	15.4	14.0	53.0	24.1
0.05% ", N_2	3	1[f]	262	197	75.2	16.2	87.3	262.0
" ", A	7	6	98	2	2.4	14.0	14.0	16.3

[a,b]Mean number of females and males present during oviposition

[c]Untreated, check for both N_2 (deuteronymphs) and A (adults)

[d]Adults inuncted with lanolin only

[e]Triphenylantimony

[f]Two apparently eaten by chicks

age and the abnormally high egg production may have resulted from failure to the tick to pick up the test chemical. It is also possible that the female did not receive as large a quantity of lanolin during treatment, thereby escaping the effects of accessory gland obstruction by the lanolin.

The increased mean number of days required for the ova to hatch seemed to be related more to the effects of the lanolin than to triphenylantimony. While ova from all inuncted groups required at least a mean of one day longer to hatch, ova from groups inuncted with lanolin plus triphenylantimony had a mean hatching period of 1.5 days shorter to 1.5 days longer than those from ticks inuncted with lanolin only.

In general, ova from the triphenylantimony inuncted females had a significantly lower percentage hatch, and ova resulting from surviving inuncted deuteronymphs had a higher percentage hatch than those of the control group. The exception to the generalization was the 1.25% triphenylantimony treated groups. At this concentration, inuncted females deposited ova having 77.4% hatchability, but only 28.8% hatching occurred in the ova deposited by females surviving from inuncted deuteronymphs. The percentage hatch of ova from both triphenylantimony inuncted deuteronymphs and adults was lower than for comparable concentrations of cadmium chloride inuncted neonymphs.

The inconsistencies in the reproductive data for triphenylantimony inuncted deuteronymphs and adults may be a result of interaction between the physical effects of lanolin and chemical effects of triphenylantimony. This is especially true for the inuncted adults, where the lanolin may have interfered with insemination, oviposition, and

respiration of the deposited ova. Inuncted females often deposited ova coated with lanolin, which in some cases contained dissolved triphenylantimony. Lanolin covering an ovum undoubtedly inhibits embryonic respiration, thus reducing the chances of survival to the hatching stage. Depending upon the quantity of lanolin enclosing an egg, the degree of physical exclusion of oxygen from an embryo would probably also affect its responses to different concentrations of a test chemical dissolved in the lanolin.

Examination of histological preparations revealed apparent gametic damage at all concentrations of triphenylantimony used. The inuncted adults were more susceptible to gametic damage than the adults resulting from inuncted deuteronymphs. The adults inuncted with 0.50% triphenylantimony displayed disruption of gametogenesis in both sexes. About 50% of the spermatids from males of this group exhibited a wrinkled, shrunken morphology similar to that illustrated in Figure 16. The females of this group had ootids characterized by cytoplasmic damage in the form of splitting, clumping, or uneven distribution within the cell. Figure 18 demonstrates damaged spermatids from a male inuncted with 0.10% triphenylantimony. Females inuncted with this concentration appeared to have suffered no gametic damage, histologically. Females inuncted with a concentration of 0.05% triphenylantimony displayed damaged ootids containing polymorphous cytoplasmic clumping (Fig. 19). The only group of inuncted deuteronymphs to reveal gametic damage as adults was the one inuncted with 1.25% triphenylantimony. The damage in this case appeared in the form of shrunken or oddly shaped ova (Fig. 20). Perhaps these were damaged ootids or ova that were being resorbed by the ovary of the females.

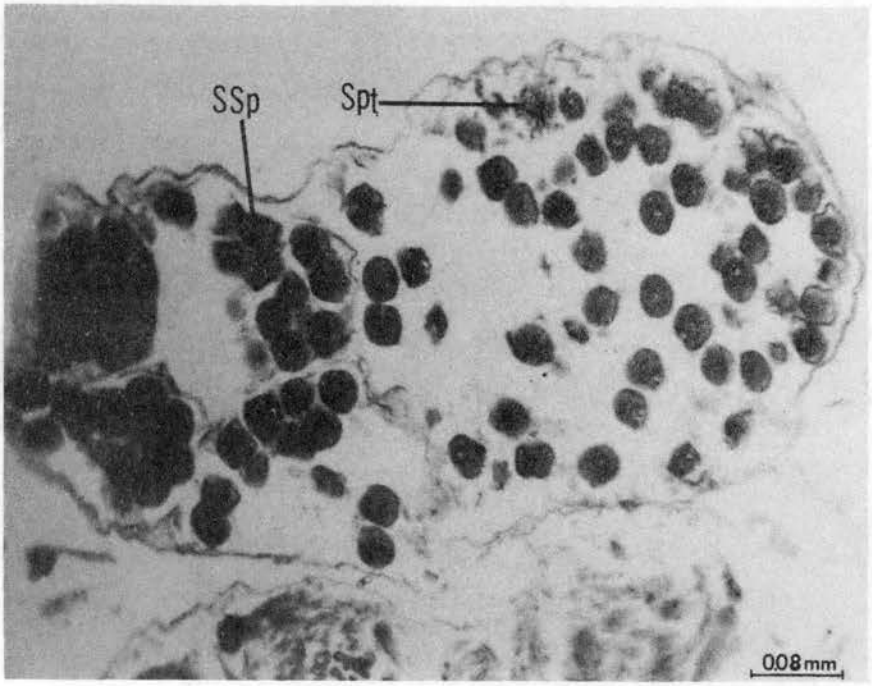

Fig. 18. A section displaying damaged spermatids in the reproductive system of a male A. persicus that was inuncted as an adult with 0.10% triphenylantimony. SSp, late Secondary Spermatocyte; Spt, early Spermatid.

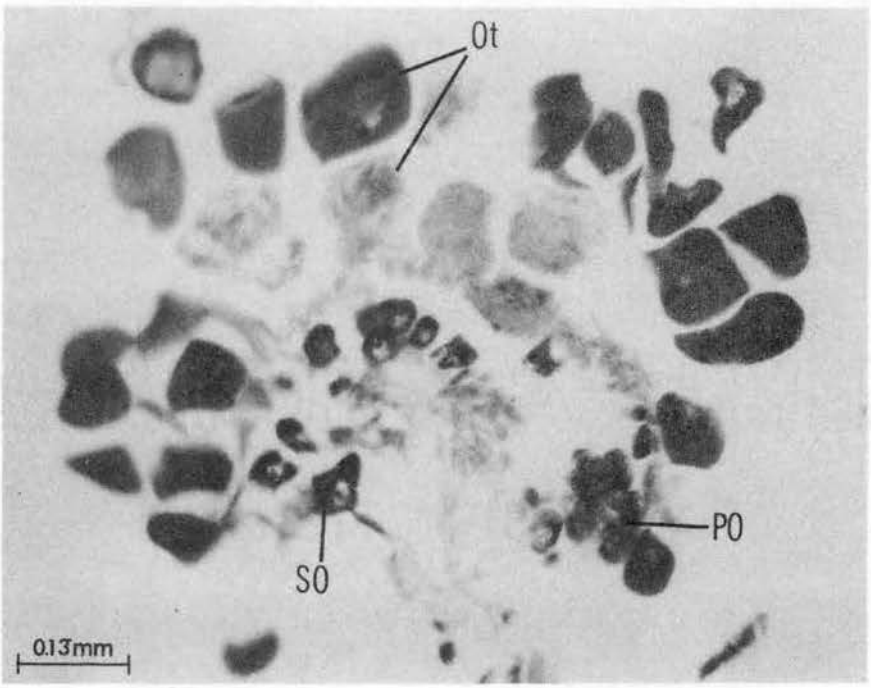

Fig. 19. Sectioned ovary showing damaged ootids from a female A. persicus that was inuncted as an adult with 0.05% triphenylantimony. PO, Primary Oocyte; SO, Secondary Oocyte; Ot, Ootid.

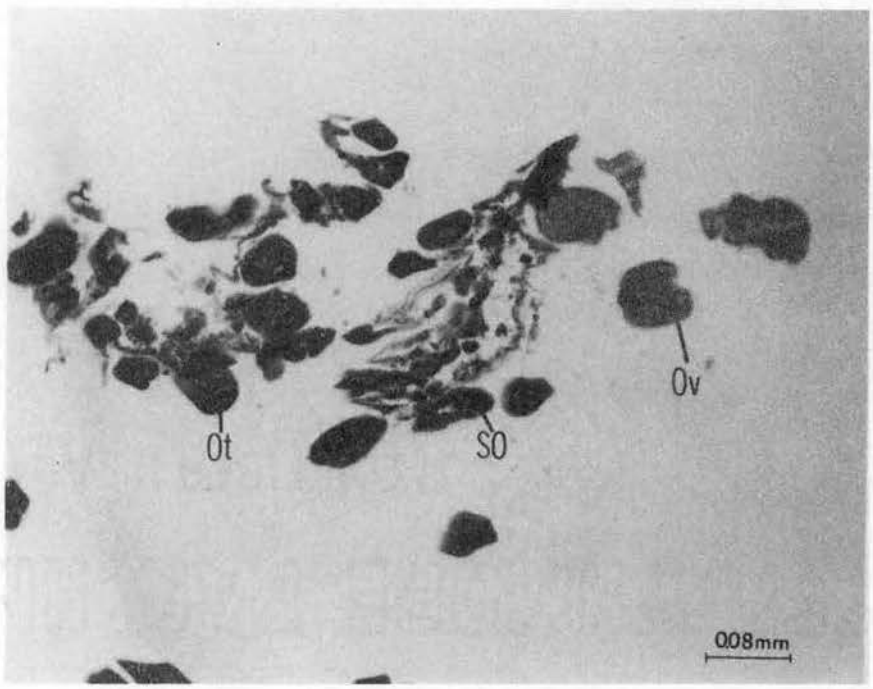

Fig. 20. Sectioned ovary illustrating three nearly mature, distorted ova in a female A. persicus that was inuncted as a deuteronymph with 1.25% triphenylantimony. SO, Secondary Oocyte; Ot, Ootid; Ov, Ovum.

Egg Dipping

An unexpected result in the egg dipping tests was that submergence of A. persicus ova in physiological saline for one minute reduced significantly the percentage hatch. From 87.98 to 96.66% of the untreated ova hatched, compared with only 50.80% for those submerged in saline. Dipping the ova in saline also resulted in reduced mean hatching time of one to two days.

Dipping the ova in cadmium chloride concentrations of 0.01 - 10.0% increased the percentage hatch over that of the control at all concentrations tested except the 5.0% level (Table 12). At this concentration a mean of only 46.87% of the ova hatched. This, however, was not significantly lower at the 5% level than the percentage hatch for the control group. These results indicate that saline containing cadmium chloride was actually more favorable to hatching of dipped ova than saline only. This indication is amplified by the fact that the mean time required for hatching was reduced in the ova dipped in cadmium chloride. Untreated ova required a mean of 15.0 days to hatch. Ova dipped in saline required a mean of 13.0 days to hatch, whereas those dipped in cadmium chloride solutions had a mean hatching period of 10.7 to 12.3 days.

Table 13 shows that dipping ova in saline containing 0.10 - 0.40% antimony potassium tartrate had the same general effects upon hatching as the cadmium chloride solutions. A higher percentage hatch occurred in ova dipped in saline-antimony potassium tartrate solutions than in saline only. At a given concentration of antimony potassium tartrate, the percentage hatch tended to increase with submergence duration. Dipping ova in antimony potassium tartrate did not alter the time

Table 12. Effects of dipping A. persicus ova in physiological saline containing cadmium chloride.

Concentration	Minutes Submerged	Ova Dipped	Ova Hatched	Per Cent Hatch	Days Required
Check	Not Dipped	60	58	96.66	15.0
Control[a]	1	187	95	50.80	13.0
10.0%	0.5	200	95	47.50	15.0
"	1	200	100	50.00	15.0
"	5	200	106	53.00	14.0
"	10	200	106	53.00	14.0
"	15	200	133	66.60	14.5
"	30	200	147	73.50	14.0
				57.52 ($\bar{x}$)	14.3 ($\bar{x}$)
5.0%	0.5	216	108	50.00	14.0
"	1	177	68	38.42	13.5
"	5	200	88	44.00	14.0
"	10	250	122	48.80	14.5
"	15	268	118	43.50	16.0
"	30	200	113	56.50	13.0
				46.87 ($\bar{x}$)	14.1 ($\bar{x}$)
1.0%	0.5	200	126	63.00	11.0
"	1	200	134	67.00	11.0
"	30	200	180	90.00	10.0
				73.3 ($\bar{x}$)	10.7 ($\bar{x}$)
0.5%	0.5	200	120	60.00	11.0
"	1	200	150	75.00	11.0
"	30	198	133	66.20	14.0
				67.06 ($\bar{x}$)	12.0 ($\bar{x}$)
0.1%	0.5	204	97	42.17	14.0
"	1	232	143	61.20	13.0
"	30	205	178	86.82	10.0
				63.40 ($\bar{x}$)	12.3 ($\bar{x}$)
0.01%	0.5	177	152	85.87	12.0
"	1	179	167	87.65	11.0
"	30	200	126	63.00	14.0
				78.84 ($\bar{x}$)	12.3 ($\bar{x}$)

[a]Submerged in saline only

Table 13. Effects of dipping A. persicus ova in physiological saline containing antimony potassium tartrate.

Concentration	Minutes Submerged	Ova Dipped	Ova Hatched	Per Cent Hatch	Days Required
Check	Not Dipped	174	152	87.98	14.0
0.4%	1	192	97	51.04	13.0
"	5	200	118	59.00	16.0
"	10	200	156	78.00	14.0
"	15	200	137	68.80	14.0
"	30	200	147	73.50	15.0
				66.07 ($\bar{x}$)	14.5 ($\bar{x}$)
0.3%	1	200	143	71.50	13.0
"	5	200	106	53.00	13.0
"	10	200	125	62.50	14.0
"	15	200	104	52.00	13.0
"	30	200	157	78.50	15.0
				63.50 ($\bar{x}$)	13.5 ($\bar{x}$)
0.2%	1	200	152	76.00	14.0
"	5	200	163	81.50	14.0
"	10	200	156	78.00	14.0
"	15	200	166	83.00	14.0
"	30	200	165	82.50	15.0
				78.20 ($\bar{x}$)	14.2 ($\bar{x}$)
0.1%	1	104	45	43.27	15.0
"	5	233	128	54.81	14.0
"	10	200	91	45.50	15.0
"	15	200	159	79.50	14.0
"	30	200	173	86.50	16.0
				61.92 ($\bar{x}$)	14.8 ($\bar{x}$)

required for hatching as drastically as did dipping them in cadmium chloride. Ova dipped in antimony potassium tartrate required a mean of 13.5 - 14.8 days to hatch, compared to 14.0 days for untreated ova.

Although the data indicate that addition of cadmium chloride and antimony potassium tartrate to the physiological saline was more favorable to hatching than saline alone, none of the treated egg groups experienced a hatching rate equalling that of untreated ova. As pointed out earlier, the two untreated groups of ova had hatchability rates of 96.66 (Table 12) and 87.98% (Table 13), which were significantly higher at the 5% level than those of all treated egg groups except the 0.01% cadmium chloride and 0.20% antimony potassium tartrate treatments.

Amblyomma americanum

The method used to estimate the number of ova deposited and percentage hatch is highly subjective. However, it is believed that since all the estimates were treated alike, any major variations in the data among the treatments would probably be due to the treatment effects. As a preliminary study, the results should be sound enough to indicate any treatment-toxicity trends that would suggest whether further study is worthwhile.

Table 14 contains a summary of the results of the study. All of the females injected with 1.0% and 5.0% cadmium chloride solutions died within a day and prior to ovipositing. Twenty per cent of the ticks receiving the 0.01% cadmium chloride injection died prior to ovipositing. This treatment reduced the number of ova deposited per female to nearly one half the number of ova deposited by the check group. The

Table 14. Effects of cadmium chloride injection into replete female lone star ticks, A. americanum.

Treatment	Number of Females			Est. Ova	Mean ova/ Female	Est. Hatch	Per Cent Hatch
	Treated	Died	Oviposited				
Check[a]	8	1	7	24,000	3,428.6	22,400[b]	93.33
Control[c]	7	1	6	17,800	2,833.3	16,500[d]	92.64
5.0% $CdCl_2$	15	15	-	-	-	-	-
1.0% "	10	10	-	-	-	-	-
0.01% "	20	4	16	29,000	1,812.5	25,550[e]	88.11
0.001%	11	2	9	29,400	3,266.7	26,400[f]	89.79
0.0001% "	10	0	10	33,000	3,300.0	29,700	90.00

[a]Not treated

[b]One egg batch failed to hatch; est. ova, 3,000

[c]Injected with physiological saline only

[d]One egg batch failed to hatch; est. ova, 1,350

[e]Two batches of eggs failed to hatch; est. ova, 2,000

[f]One egg batch failed to hatch; est. ova, 2,400

smaller number of ova per female deposited in the control group compared to other groups probably resulted from sampling error, considering that the sample size was much smaller. The percentage hatch appeared to be treatment dependent. The group receiving the 0.01% cadmium chloride injection experienced 88.11% hatching, and as the treatment concentration decreased, the percentage hatch increased to 93.33% in the check ticks. A null hypothesis of the proportion ($P_1 = P_2$) of ova hatching showed that the proportion of ova hatching in the groups receiving 0.01%, 0.001%, and 0.0001% cadmium chloride injections was significantly lower at the 5% level from the proportion of ova hatching in the check group. The proportion of ova hatching in the check group and the control group were not significantly different at the 5% level. This indicates that the reduction in both numbers and percentage hatch of ova was due to the cadmium chloride and not to the physiological saline in which the cadmium chloride was dissolved. All but one group of females deposited one or two batches of eggs in which none hatched. This may have resulted from mechanical injury during injection, blocking fertilization of the ova within the females. More than likely it was due to the females being removed from the host prior to being inseminated by a male.

The treatments did not appear to affect the longevity of hatched larvae. The larvae from all treated groups, checks, and controls began dying 21 to 22 days after hatching had begun.

SUMMARY AND CONCLUSIONS

Responses of the fowl tick, Argas persicus (Oken 1818), to compounds of cadmium and antimony were investigated in the laboratory. The ticks received test compounds via in vivo engorgement on poisoned chicks, in vitro engorgement on preserved chicken blood, inuncting with lanolin, and dipping of ova in physiological saline.

When engorging on chicks consuming drinking water containing 0.075% cadmium chloride, neonymphs experienced a percentage mortality essentially equal to that of untreated ticks. Larvae and neonymphs suffered 14.76 and 41.56% mortality, respectively, when engorging on chicks poisoned with 0.15% cadmium chloride. These values were significantly higher (5 and 1% level, respectively) than that of the control ticks. Females resulting from treated larvae and neonymphs deposited significantly fewer ova, on the average, than control females. However, the fecundity reduction of the treated ticks appeared to be more age dependent than treatment dependent, as oviposition was inhibited more in neonymphs receiving 0.075% cadmium chloride than in larvae receiving 0.15% cadmium chloride. Compared with the untreated ticks, percentage hatch of ova was higher, and the mean number of days required for hatching fewer, in the treated ticks.

Neonymphs engorging in vitro on chicken blood containing cadmium chloride concentrations of 0.0001 - 0.50% and cadmium chloride-zinc chloride concentrations of 0.001 and 0.0001% experienced an extremely low survival rate. The survival rate was 2.01% or less for neonymphs

engorging on cadmium chloride and cadmium chloride-zinc chloride concentrations exceeding 0.0001%. At the 0.0001% concentration, 27.02% of the neonymphs survived to become adults when engorging on blood containing cadmium chloride, versus 10.05% for those engorging on blood containing cadmium chloride-zinc chloride. Antimony potassium tartrate and triphenylantimony were less toxic to in vitro engorging neonymphs at concentrations of 0.001 and 0.0001%, permitting 65.2 - 70.6% survival to adults. When engorging on blood containing 0.01, 0.075, and 0.133% antimony potassium tartrate, 6.70% or fewer of the neonymphs survived to become adults. When engorging in vitro on chicken blood minus the test compounds, 80.73% of the neonymphs survived to become adults.

The only in vitro cadmium chloride treatment to significantly lower fecundity was the 0.01% concentration. The lone surviving female of this treatment deposited only 23.0 ova. Females from the 0.0001% cadmium chloride and cadmium chloride-zinc chloride treatments deposited a mean of 130.00 and 141.86 ova, respectively, which was approximately three times the quantity of ova deposited by the control females. Mean egg production was significantly lowered in the females surviving from neonymphs that had engorged on chicken blood containing 0.001 and 0.0001% antimony potassium tartrate. With one exception, the percentage hatch of ova from in vitro treated ticks equalled or exceeded that of ova from untreated ticks. Likewise, the in vitro treatments did not affect the mean number of days required for the ova to hatch. The exception was 10.46% hatchability of ova from the female surviving the 0.01% antimony potassium tartrate treatment. Examination of histological preparations revealed disruption of gametogenesis in both sexes at

this treatment level, characterized by wrinkled, shrunken spermatids and ruptured ootids. Earlier gametic stages appeared to have escaped damage.

Neonymphs inuncted with lanolin only suffered 69.06% mortality prior to becoming adults. It is thought that the lanolin induced mortality resulted from physical obstruction of the spiracles rather than from chemical toxicity. Neonymphs inuncted with lanolin containing 0.10, 0.25, 0.50, 0.75, and 1.25% cadmium chloride experienced 92.88 - 100% mortality, all of which were significantly greater at the 5% level than mortality in the control group. In most cases mortality occurred to the instar treated. Inuncting neonymphs with cadmium chloride concentrations of 2.50% or less had no inhibitory effects upon fecundity of the surviving females, or fertility of the deposited ova. Histological preparations exhibited no indications of gametic damage in ticks inuncted with cadmium chloride.

Adults inuncted with lanolin only had a mortality rate of 23.10% prior to commencement of oviposition. Adults inuncted with lanolin containing 0.05, 0.10, 0.50, and 1.25% triphenylantimony had a mortality rate of 47.10 - 65.0%. Only those adults inuncted with 0.05% triphenylantimony had a significantly higher percentage mortality than the controls. Deuteronymphs inuncted with the same concentrations as the adults suffered 65.0 - 90.0% mortality.

The inhibitory effects of lanolin and lanolin-triphenylantimony upon the reproduction of inuncted deuteronymphs and adults was pronounced. Inuncting adults with lanolin only decreased the mean number of ova deposited per female by more than 50%, decreased fertility of deposited ova by 19.10%, and increased the mean time required for

hatching by 2.50 days. All groups of triphenylantimony inuncted adults and two of the four deuteronymph groups deposited a smaller mean number of ova than the control females. While some of these differences were statistically significant at the 5% level, there was no pattern and the differences appeared not to be treatment dependent. The ova of all inuncted deuteronymph and adult groups required an average of 1-4 days longer to hatch than ova from the untreated ticks. In general, ova from triphenylantimony inuncted females had a significantly lower percentage hatch than ova from untreated females. Females inuncted with 0.05, 0.10, and 0.50% triphenylantimony, respectively, deposited ova of which 2.40, 15.40, and 29.10% hatched. Of the triphenylantimony inuncted deuteronymphs, only the surviving female of the 1.25% concentration produced ova having a percentage hatch less than that of the control females. The percentage hatch of ova from both triphenylantimony inuncted deuteronymphs and adults was lower than it was for comparable concentrations of cadmium chloride inuncted neonymphs. It is believed that the reproduction inhibition experienced by inuncted deuteronymphs and adults resulted from an interaction of physical effects due to the lanolin and chemical effects due to the triphenylantimony.

Histological preparations showed apparent gametic damage at all concentrations of triphenylantimony used, with inuncted adults being more susceptible than adults surviving from inuncted deuteronymphs. Both sexes of the adults inuncted with 0.50% triphenylantimony displayed gametic damage. The males had about 50% of their spermatids wrinkled and shrunken, while the females contained ootids with cytoplasmic splitting and clumping. Males inuncted with 0.10%

triphenylantimony produced distorted spermatids. Females inuncted with 0.05% triphenylantimony exhibited ootids with cytoplasmic clumping. It appears that gametic damage was limited primarily to the cytoplasm, since only abnormal ootids and spermatids were detected.

Submerging A. persicus ova in physiological saline containing cadmium chloride concentrations of 0.01 - 10.0% for up to 30 minutes increased the percentage hatch over that for ova dipped in saline only. Ova dipped in cadmium chloride solutions required a mean of 10.7 - 12.7 days to hatch, compared with 13.0 days for those dipped in saline alone. Dipping ova in saline containing 0.10 - 0.40% antimony potassium tartrate had the same general effects upon hatching as the cadmium chloride solutions. Ova dipped in the antimony potassium tartrate solutions had a higher percentage hatch than ova dipped in saline, with the percentage hatch increasing as submergence time increased.

Incidental to the main line of investigation, two peripheral studies were conducted. Cadmium chloride administered to one week chicks via drinking water at concentrations of 0.05% or greater produced 100% mortality. Nearly 67% of the chicks survived when consuming drinking water containing 0.025% cadmium chloride. A 50:50 ratio of cadmium chloride-zinc chloride in the water at concentrations of 0.025 and 0.05%, respectively, was lethal to 16.67 and 100% of the chicks. Injection of saline containing 1.0 and 5.0% cadmium chloride into gravid female lone star ticks, Amblyomma americanum, caused 100% mortality. Injecting the gravid females with saline containing 0.01, 0.001, and 0.0001% cadmium chloride reduced the mean number of ova deposited per female and significantly reduced (5% level) the

percentage hatch of ova. There was a direct dosage-dependence effect upon mortality, mean ova deposited per female, and percentage hatch of ova.

LITERATURE CITED

Abdel-Razig, Mohamed El Tahir. 1966. Evaluation of compounds of cadmium and related metals as reproductive inhibitors in insects. Unpublished Ph. D. dissertation, Oklahoma State University, Stillwater, Oklahoma.

Abdussalam, M., and M. M. Sarwar. 1953. Trees as habitats of the fowl tick Argas persicus (Oken). Bull. Entomol. Res. 44 (3):419-420.

Arthur, Don R. 1962. Ticks and Disease, XVI + 445 pp. Pergamon Press, Inc., New York.

Baader, E. W. 1952. Chronic cadmium poisoning. Ind. Med. Surg. 21 (9):427-430.

Balashov, Y. S., and Y. L. Goroshchenko. 1962. The development and functioning of the male sexual system in argasid ticks. Parazitol. Sb. 19:16-25, 1960. [Biol. Abstr. 37(1):12008].

Barger, E. H., and L. E. Card. 1949. Diseases and Parasites of Poultry, 4th ed., p. 32. Lea and Febiger, Philadelphia.

Bedi, U. 1962. Morphological and histological observations on the male germ cells of fowl tick, Argas persicus, and cattle tick, Hyalomma aegyptium. Res. Bull. Panjab Univ. Sci. 13 (1/2):31-46.

Belyaeva, A. P. 1967. Effect of antimony on the reproductive function. Gig. Tr. Prof. Zabol. 11 (1):32-37. [Biol. Abstr. 50 (12):64628].

Beton, D. C., G. S. Andrews, H. J. Davies, L. Howells, and G. F. Smith. 1966. Acute cadmium fume poisoning. Five cases with one death from renal necrosis. Brit. J. Ind. Med. 23 (4):292-301. [Biol. Abstr. 48(2):23315].

Blejer, H. P. 1966. Cadmium oxide fumes deadly occupational hazard to man. Calif. Dept. of Health. 23(10/11):165-166.

Bouissou, H., and M. T. Fabre. 1966. Sulfate de cadmium et acetate de zinc: leur action sur la spermatogenese. Laval Med. 37(5):576-578.

Bologov, V. P., and E. V. Kalinin. 1968. Histological and histochemical changes in different sections of the brain in animals with chronic cadmium poisoning treated by vitamin B_{12}. Nauch Tr. Ruazon Med. Inst. 27:135-143. [Biol. Abstr. 50 (12):644527].

Bologov, V. P., and G. A. Uzbekov. 1966. Quantitative change in the thiol groups of protein and low-molecular compounds of the brain under the action of cabalamine and nicotinic acid, under natural conditions and in cadmium poisoning. Saratov. 2:258-159. [Biol. Abstr. 49(3:29316].

Bosworth, A. B. 1969. Some effects of cadmium and related compounds on reproductive organs and growth of Culex quinque-fasciatus Say (Culicidae). Unpublished master's thesis. Oklahoma State University, Stillwater, Oklahoma.

Brooks, R. R., and M. G. Rumsby. 1967. Studies on the uptake of cadmium by the oyster, Ostrea sinuata (Lamarack). Austr. J. Mar. Freshwater Res. 18(1):53-61.

Bunde, C. A., H. E. Blair, G. R. Burch, and J. E. Lee. 1954. Ascarid action of cadmium. Proc. Soc. Experimentl. Biol. and Med. 87:549-550.

Burch, G. E., and H. E. Blair. 1955. A new ascaricide for swine. J. Amer. Vet. Assoc. 126:304-308.

Byerrum, R. U., R. A. Anwar, and C. A. Hoppert. 1960. Toxicity of small amounts of cadmium and chromium in water. Tech. Eau (Brussels) 165:24. [Chem. Abstr. 54:25320].

Cameron, E. 1965. The effects on intratesticular injection of cadmium chloride into the rabbit. J. Anat. 99 (4):907-912. [Biol. Abstr. 47 (1):6709].

Carroll, Robert E. 1966. The relationship of cadmium in the air to cardiovascular disease death rates. J. Amer. Med. Assoc. 198 (3): 267-269.

Chatterjee, S. N. 1966. Sterilization of scrub cows by intra-ovarian injection of cadmium chloride--a preliminary study. Ind. Vet. J. 43 (4):331-338.

Chatterjee, S. N., and A. B. Kar. 1965. Sterilization of female goats by cadmium chloride. Vet. Rec. 77:1108-1111.

Chiquoine, A. Duncan. 1965. The effects of cadmium on the female reproductive system. Anat. Rec. 151 (3):335.

Chiquoine, A. Duncan, and V. Suntzeef. 1965. Sensitivity of mammals to cadmium necrosis of the testis. J. Reprod. Fert. 10 (3):455-457.

Cotten, M. V., and M. E. Logan. 1966. Effects of antimony on the cardiovascular system and intestinal muscles. J. Pharmacol. Experimentl. Therap. 151 (1):7-22.

Cotzias, G. C., D. C. Borg, and B. Selleck. 1961a. Specificity of zinc pathway in the rabbit: zinc-cadmium exchange. Amer. J. Physiol. 102:63-66.

Cotzias, G. C., D. C. Borg, and B. Selleck. 1961b. Virtual absence of turnover in cadmium metabolism: Cd^{109} studies in the mouse. Amer. J. Physiol. 102:927-930.

Davis, J. T., and J. G. Congilio. 1967. The effects of cryptorchidism, cadmium and anti-spermatogenic drugs on fatty acids composition of rat testis. J. Reprod. Fert. 14(3):407-413.

Deschiens, R., and M. Tahiri. 1961. Selective molluscicidal action of cadmium sulfate. Bull. Soc. Pathol. Exot. 54(5):944-946.

DeWitt, W. B. 1965. Effect of frequency of administration on antimony activity against Schistosoma mansoni in mice. Amer. J. Trop. Med. Hyg. 14(6):1003-1006.

Dimov, G., and D. Knorre. 1967. Enzyme histochemical and enzyme electrophoretic studies on rat testes during the first 48 hours of experimental cadmium poisoning. Virchows Arch. Pathol. Anat. Physiol. Klin Med. 342(3):252-257. [Biol. Abstr. 49(5):2154].

Drummond, R. O. 1960. Ticks, habits, biology, contol. Pest Control 28(12):9-14.

El-Toraei, I. 1968. Personal experience in the treatment of [human] filarial lymphedema. Angiologica (Barcelona) 20(6):289-300. [Biol. Abstr. 50(3):71716].

Ercoli, N. 1968. Chemotherapeutic and topological properties of antimonyl-tartrate-dimethyl-cysteine chelates. Proc. Soc. Exp. Biol. Med. 129(1):284-290. [Biol. Abstr. 50(10):54919].

Fairhall, L. T. 1957. Industrial Toxicology, 2nd ed., pp. 30-33. The Williams and Wilkins Co., Baltimore, Md.

Favino, A., and G. Aazari. 1967. Renal lesions induced by a single subcutaneous $CdCl_2$ injection in rat. Lavoro. Umano. 19(8):367-372. [Biol. Abstr. 40(13):67997].

Feldman-Muhsam, B. 1964. Physiology, behavior and genetics of Acari: Some contributions to the understanding of the reproduction of ticks. Acarologia 6 (H.S):294-298.

Feldman-Muhsam, B. 1967. Spermatophore formation and sperm transfer in Ornithodorus ticks. Science 156(3779):1252-1253.

Feldmesser, J., and R. V. Rebois. 1966. Nematocidal effects of several cadmium compounds. Nema. 12(1):9.

Ferm, Vergil H., and Stanley J. Carpenter. 1968. The relationship of cadmium and zinc in experimental mammalian teratogenesis. Lab. Invest. 18(4):429-432.

Forny, R. B., C. A. Bunde, and G. R. Burch. 1955. Tissue storage in cadmium treated pigs. Proc. Soc. Exp. Biol. (New York) 90:13-14.

Freund, John E. 1960. Modern Elementary Statistics, 2nd ed., pp. 247-281. Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Friberg, L. 1948. Proteinuria and kidney injury among workmen exposed to cadmium and nickel dusts. J. Ind. Toxicol. 30(1):32-36.

_____. 1952. Further investigations on chronic cadmium poisoning. A. M. A. Arch. Ind. Hyg. 5:30-36.

Gabbiani, G. 1966. Action of cadmium chloride on sensory ganglia. Experientia 22(4):261-262. [Biol. Abstr. 48(2):28370].

Gabbiani, G., D. Baic, and C. Diziel. 1967a. Studies on tolerance and ionic antagonism for cadmium or mercury. Can. J. Physiol. Pharmacol. 45(3):443-450.

Gabbiani, G., D. Baic, and C. Diziel. 1967b. Toxicity of cadmium for the central nervous system. Exp. Neurol. 18(2):154-160.

Galun, R., and S. H. Kindler. 1965. Glutathione as an inducer of feeding in ticks. Science 147(3654):166-167.

Gaujolle, F., P. H. Chanh, P. Kan, and F. Moulas. 1965. Toxicity comparee du zinc et du cadmium a l'egard des oeufs d'Artemia saline. Ann. Pharm. Franc. 23(2):81-84. [Biol. Abstr. 47(5): 69809].

Ginsberg, J. M. 1934. Toxicity of cadmium to chewing insects. Science 80(2073):269.

Glukhov, V. E. 1966. Life cycle of Argas persicus in the Northern Caucasus. Ref. Zh. Biol. No. 10 K 191. [Biol. Abstr. 48(13): 67359].

Gregson, J. D. 1942. Notes on the laboratory rearing of some Canadian ticks (Acarina). Proc. Entomol. Soc. Brit. Columbia. 39:32-35.

Gunn, S. A., T. C. Gould, and W. A. D. Anderson. 1961a. Zinc protection against cadmium injury to rat testis. Arch. Pathol. 71:274-281.

_____, _____, and _____. 1961b. Competition of cadmium for zinc in rat testes and dorsolateral prostate. Acta. Endocrin. Copenhagen 37:24-30.

_____, _____, and _____. 1963. Cadmium induced interstitial tumors in rats and mice and their prevention by zinc. J. Natl. Cancer Inst. 31(4):745-759.

_____, _____, and _____. 1964. Effect of zinc on cancerogenesis by cadmium. Soc. Exp. Biol. Med. 115(3):653-657.

_____, _____, and _____. 1966a. Loss of selective injurious vascular response to cadmium in regenerated blood vessels of testis. Amer. J. Pathol. 48(6):959-969. [Biol. Abstr. 47(1): 2899].

_____, _____, and _____. 1966b. Protective effect of thiol compounds against cadmium induced vascular damage to testis. Proc. Soc. Exp. Biol. Med. 47(4):1036-1039. [Biol. Abstr. 47(4): 48352].

_____, _____, and _____. 1967. Specific response of mesenchymal tissues to cancerogenesis by cadmium. Arch. Pathol. 83(6):493-499.

_____, _____, and _____. 1968a. Specificity in protection against lethality and testicular toxicity from cadmium. Proc. Soc. Exp. Biol. Med. 128(2):591-593. [Biol. Abstr. 59(1):59085].

_____, _____, and _____. 1968b. Mechanics of zinc, cysteine and selenium protection against cadmium induced vascular injury to mouse testis. J. Reprod. Fert. 15(1):65-70.

Gupta, R. K., G. U. Barnes, and F. R. Skelton. 1967. Lightmicroscopic and immunopathological observations on cadmium-induced injury in mature rat testis. Amer. J. Pathol. 5(1):191-199.

Guthrie, J. E. 1954. Further observations on the efficiency of cadmium anthranilate as an ascaricide in swine. Vet. Med. 49:500.

Hey, D. H. 1966. Kingzetl's Chemical Encyclopedia, 9th ed., pp. 73-74. Bailliere, Tindall and Cassell. London.

Hill, C. H., G. Matrone, W. L. Payne, and C. W. Barber. 1963. In vivo interactions of cadmium with copper, zinc, and iron. J. Nutr. 80:227-235.

Hindle, E., and G. Merriman. 1912. The sensory perceptions of Argas persicus (Oken). Parasitol. 5:203-216.

Hodgen, G. D., W. R. Butler, and W. R. Gomes. 1969. In vivo and in vitro effects of cadmium chloride on enzyme carbonic anhydrase activity. J. Reprod. Fert. 18(1):156-157.

Hoogstraal, H. 1957. Bat ticks of genus Argas (Ixodoidea, Argasidae). 2. Secretargas new subgenus and A. transgariepinus White, 1846, its adult and immature stages; with a defination of the subgenus Argas. Ann. Entomol. Soc. Amer. 50(6):544-549.

Hoogstraal, H., and G. M. Kohls. 1960. Observations on the subgenus Argas (Ixodoidea, Argasidae, Argas). 1. Study of A. reflexus reflexus (Fabricius 1794), the European bird argasid. Ann. Entomol. Soc. Amer. 53(5):611-618.

_____, and _____. 1967. Bat ticks of the genus Argas (Ixodoidea, Argasidae). 7. A. (Chiroptargas) cordiformis, new species, a heart-shaped parasite from South-West Africa. Ann. Entomol. Soc. Amer. 60(1):63-67.

Hoogstraal, H., M. N. Kaiser, and G. M. Kohls. 1968a. The subgenus Persicargas (Ixodoidea, Argasidae, Argas). 4. Argas (P.) robertsi, a new species, a parasite of Australian fowl, and Keys to Australian argasid species. Ann. Entomol. Soc. Amer. 61(2): 535-539.

_____, _____, and _____. 1968b. The subgenus Persicargas (Ixodoidea, Argasidae, Argas). 5. A. (P.) zumpti, new species, a parasite of the South African Cape Vulture. Ann. Entomol. Soc. Amer. 61(3):744-551.

Hoogstraal, H., and V. McCarthy. 1965. The subgenus Persicargas (Ixodoidea, Argasidae, Argas). 2. A. (P.) abdussalami, new species, associated with wild birds on trees and buildings near Lahore, Pakistan. Ann. Entomol. Soc. Amer. 58(5):756-762.

Hooker, W. A., F. C. Bishopp, and H. P. Wood. 1912. The life history and bionomics of some North American ticks. USDA. Bur. of Entomol. Bull. 106:45-61.

Humason, Gretche L. 1962. Animal Tissue Techniques, 2nd ed., p. 18. W. H. Freeman and Co., San Francisco, Calif.

Johnson, A. D., and N. L. VanDenmark. 1969. Early effects of cadmium and zinc on testis and body temperatures. J. Reprod. Fert. 18(1):157-158.

Kaiser, M. N. 1966a. Viruses in ticks. 1. Natural infections of Argas (Persicargas) arboreus by Quaranfil and Nyamanini viruses and absence of infections in A. (P.) persicus in Egypt. Amer. J. Trop. Med. Hyg. 15:964-975.

_____. 1966b. The subgenus Persicargas (Ixodoidea, Argasidae, Argas). 3. The life cycle of A. (P.) arboreus and a standardized rearing method for argasid ticks. Ann. Entomol. Soc. Amer. 59(3):496-502.

_____. 1966c. Viruses in ticks. 2. Experimental transmission of Quaranfil viruses by Argas (Persicargas) arboreus and A. (P.) persicus. Amer. J. Trop. Med. Hyg. 15:976-985.

Kaiser, M. N., H. Hoogstraal, and G. M. Kohls. 1964. The subgenus Persicargas, new subgenus (Ixodoidea, Argasidae, Argas). 1. A. (P.) arboreus, new species, and Egyptian persicus-like parasite of wild birds, with a redefinition of the subgenus Argas. Ann. Entomol. Soc. Amer. 57(1):60-69.

Kar, A. B. 1961. Chemical sterilization of male Rhesus monkey. Endocrinol. 69(6):1116-1119.

_____. 1962. Chemical sterilization of male goats. India Vet. Sci. Animal Husb. 32(1):70-73. [Biol. Abstr. 43(2):20305].

Kar, A. B., and R. P. Das. 1962. Sterilization of males by intertesticular administration of cadmium chloride. Acta. Endocrinol. 40:321-331.

_____ and _____. 1963. The nature of protective action of selenium on administration of cadmium induced degeneration of rat testis. Proc. Nat. Sci. India. Part B. Biol. Sci. 29(3):297-307.

Kar, A. B., R. P. Das, and J. N. Karkun. 1959. Ovarian changes in prepuberal rats after treatment with cadmium chloride. Acta. Biol. et Med. Germ. [Biol. Abstr. 36(6):75714].

Kar, A. B., P. R. Dasgupta, and R. P. Das. 1962. Effect of low dose of cadmium chloride on the genital organs and fertility of male rats. J. Sci. Indust. Res. 20(1):322-326. [Biol. Abstr. 39(1): 1952].

Kar, A. B., and V. P. Kandoj. 1963. Sterilization of males by scrotal inunction of cadmium chloride. 7th International Congress on Planned Parenthood, 1963. J. Reprod. Fert. 5(3):461.

Kazantzis, G., and W. J. Hanburg. 1966. The induction of sarcoma in the rat by cadmium sulphide and by cadmium oxide. Brit. J. Cancer 20(1):190-199. [Biol. Abstr. 48(5):78044].

Keathley, J. P. 1966. Growth inhibition and mortality to larvae of the bollworm, Heliothis zea (Boddie), on rearing media treated with cadmium salts and two chlorophenoxy-thiazine compounds. Unpublished Master's Thesis, Oklahoma State University, Stillwater, Oklahoma.

Kench, J. E., A. C. Gain, and E. M. Sutherland. 1965. A biochemical study of the minialbumin to be found in the urine of men and animals poisoned by cadmium. South African Med. J. 39(46):1191-1198.

Kench, J. E., and E. M. Sutherland. 1967. Tryptophan content of the serum albumen of normal and cadmium poisoned monkeys. Brit. J. Ind. Med. 24(4):326-329.

Kennedy, A. 1966. Hypocalcemia in experimental cadmium poisoning. Brit. J. Ind. Med. 23(4):313-317. [Biol. Abstr. 48(2):23247].

Knorre, D. 1968. Enzymatic studies of rat testis during 30 days after cadmium induced necrosis. Virchows Archiv. ABt A Pathol. Anat. 345(3):255-263. [Biol. Abstr. 50(10):52272].

Kubota, Joe, V. A. Logan, and Fred Losee. 1968. Copper, Zinc, Cadmium, and lead in human blood from 19 locations in the United States. Arch. Environ. Health 16(6):788-793.

Kunz, S. E. 1967. The effects of cadmium compounds on reproduction and growth of the stable fly, Stomoxys calcitrans (Linnaeus). Unpublished Ph. D. Dissertation, Oklahoma State University, Stillwater, Oklahoma.

Lapanje, S. 1964. Polarographic studies in ion binding in solutions of pepsin. 1. Binding in cadmium ions. Biopolymers 2(6):585-592.

Lease, J. G. 1968. Effect of graded levels of cadmium on tissue uptake of ^{65}Zn by chicks over time. J. Nutr. 96(3):294-302.

Lees, A. D. 1946. Chloride excretion and function of coxal glands in ticks. Parasitol. 37:172-184.

Levine, N. D., and I. Ives. 1954. Nematocidal screening tests of cadmium compounds against horse strongyle larvae. J. Parasitol. (Suppl) 40:15.

Lofts, B., and R. K. Murton. 1967. The effects of cadmium on the avian testis. J. Reprod. Fert. 13(1):155-164.

Loomis, E. C. 1961. Life histories of ticks under laboratory conditions (Acarina, Ixodoidea and Argasidae). J. Parasitol. 47(1): 91-99.

Maekawa, K., Y. Tsunenari, K. I. Mamura, and J. Kono. 1968. Injurious effect of orally administered cadmium on the testis of rats. Zoo. Mag. 77(2):52-58. [Biol. Abstr. 50(9):48134].

Maleki, M. A. 1967. An epidemiological study, clinical forms, and treatment of leishmaniasis of the skin in Iran. Dermatol. Int. 6(3):152-153. [Biol. Abstr. 50(13):71508].

Mansour, M. M. 1967. Anti-bilharzial antimony drugs. Nature (London) 214(5090):819-820.

Maruyama, K. 1957. Sea anemone Apyrase. III. Effect of metals on the enzyme action. Sci. Papers Coll. Gen. Educ. Univ. Tokyo Biol. 7(1):11-20.

Mason, K. E., J. O. Young, and J. E. Brown. 1964. Effectiveness of selenium and zinc in protecting against cadmium induced injury of the rat testis. 77th Session of the Amer. Anat., Denver, 1964. Anat. Rec. 148(2):309.

Medley, J. G., and E. H. Ahrens. 1968. Fluorescent dyes for marking and recovering fowl ticks in poultry houses treated with insecticides. J. Econ. Entomol. 61(1):81-84.

Meek, E. S. 1959. Cellular changes induced by cadmium in mouse testis and liver. Brit. J. Exptl. Pathol. 40:503-506.

Micks, Don W. 1951. The laboratory rearing of the common fowl tick, Argas persicus. J. Parasitol. 37(1):102-105.

Miller, W. J., D. M. Blackmon, and Y. G. Martin. 1968. Cadmium absorption, excretion, and tissue distribution following single tracer oral and intravenous doses in young goats. J. Dairy Sci. 51(1):1836-1839.

Miller, W. J., B. Lampp, G. W. Powell, C. A. Salotti, and D. M. Blackmon. 1967. Influence of a high level of dietary cadmium and cadmium in milk, excretion, and cow performance. J. Dairy Sci. 50(9):1404-1408.

Murthy, G. K. 1968. Cadmium and silver content of market milk. J. Dairy Sci. 51(4):610-613.

Osintseva, V. P., T. I. Bonashevskaya, and E. V. Arzamastsen. 1966. Effect of tri-valent antimony on the organism of experimental animals. Arkh. Pathol. 28(7):60-64. [Biol. Abstr. 48(5):75357].

Paufler, S. K., and R. H. Foote. 1967. Effect of triethylene-melamine and cadmium chloride on spermatogenesis in rabbits. J. Reprod. Fert. 14(2):348-349.

Parizek, J. 1957. The destructive effect of cadmium ion on testicular tissue and its prevention by zinc. J. Endocrinol. 15:56-63.

_____. 1960. Sterilization of the male by cadmium salts. J. Reprod. Fert. 1:294-309.

_____. 1964. Vascular changes at sites of oestrogen biosynthesis produced by parenteral injection of cadmium salts: The destruction of placenta by cadmium salts. J. Reprod. Fert. 7:263-265.

_____. 1965. The peculiar toxicity of cadmium during pregnancy. An experimental toxemia of pregnancy induced by cadmium salts. J. Reprod. Fert. 9:11-112.

Parizek, J., I. Ostadalova, I. Benes, and A. Babicky. 1968. Pregnancy and trace elements: The protective effect of compounds of an essential trace element, selenium, against the peculiar toxic effects of cadmium during pregnancy. J. Reprod. Fert. 16(3):507-509.

Perry, H. Mitchell, Jr., Margaret Erlanger, Anice Yunice, and Elizabeth F. Perry. 1967. Mechanics of the acute hypertensive effect of intra-arterial cadmium and mercury in anesthetized rats. J. Lab. Clin. Med. 70(6):963-972.

Perry, H. M., Jr., and A. Yunice. 1965. Acute pressor effects of intra-arterial cadmium and mercury ions in anesthetized rats. Proc. Soc. Exp. Biol. Med. 120:805.

Petering, H. G., M. Johnson, and K. Stemmes. 1969. Effect of cadmium on dose response relationships of zinc in rats. Fed. Proc. 28(2): 691.

Powell, G. W., W. J. Miller, and C. M. Clifton. 1964a. Effect of cadmium on the palatability of calf starters. J. Dairy Sci. 47(9): 1017-1018.

Powell, G. W., W. J. Miller, J. D. Morton, and C. M. Clifton. 1964b. Influence of dietary cadmium level and supplementary zinc on cadmium toxicity in bovine. J. Nutr. 84(4):205-214.

Powell, G. W., W. J. Miller, and D. M. Blackmon. 1967. Effects of dietary EDTA and cadmium absorption, excretion and retention of orally administered ^{65}Zn in various zinc-deficient and normal goats and calves. J. Nutr. 93(2 pt. 1):203-212.

Prescott, R. T. M., and H. F. Clinton. 1945. External parasites of poultry. J. Dept. Agri. Victoria. 43(1):473-475.

Princi, F. 1947. A study of industrial exposure to cadmium. J. Ind. Toxicol. 29(5):315-320.

Prodan, L. 1932a. Cadmium poisoning. I. The history of cadmium poisoning and uses of cadmium. J. Ind. Hyg. Toxicol. 14:132-155.

Prodan, L. 1932b. Cadmium poisoning. II. Experimental cadmium poisoning. J. Ind. Hyg. Toxicol. 14:174-196.

Proverbs, M. D., 1969. Induced sterilization and control of insects. Ann. Review Entomol. 14:81-102.

Robinson, L. E., and J. Davidson. 1913a. The anatomy of Argas persicus (Oken 1818). I. Parasitol. 6(1):20-48.

_____ and _____. 1913b. The anatomy of Argas persicus (Oken 1818). II. Parasitol. 6(2):217-256.

_____ and _____. 1914. The anatomy of Argas persicus (Oken 1818). III. Parasitol. 6(3):382-424.

Roveda, R. J. 1940. Primera contribucion al estudis de la bionomia del Argas persicus. Univ. Buenos Aires Fac. Agron. y Vet. Inst. Parasitol. y Enferm. Parasitarias 1 (6):3-32. [Biol. Abstr. 17(1): 3518].

Salvant, W., and H. Connet. 1920. The influence of heavy metals on the isolated frog heart. J. Pharmacol. Experimentl. Therap. 15: 217-232.

Schroeder, Henry A. 1966. Cadmium, chromium, and cardiovascular disease. Circulation 34(2):39-40. Suppl. III.

_____. 1967. Cadmium, chromium, and cardiovascular disease. Circulation 35(3):570-582.

Schroeder, H. A., M. Mathur, J. J. Balassa, M. Kanisawa, and A. P. Nason. 1968. Zirconium, niobium, antimony and fluorine in mice: Effects on growth, survival and tissue levels. J. Nutr. 95(1): 95-101.

Schroeder, H. A., A. P. Nason, I. H. Tipton, and J. J. Balassa. 1967. Essential trace metals in man: Zinc. Relation to environmental cadmium. J. Chronic Dis. 20:179-210.

Schroeder, H. A., W. H. Vinton, and J. J. Balassa. 1963. Effect of chromium, cadmium and other trace metals on the growth and survival of mice. J. Nutr. 80(1):39-47.

Shabilina, L. P. 1967a. The action of dust of cadmium compounds on the respiratory organs. Ref. Zh. Otd. Vyp. Farmakol Khimioter. Sredstva Toksikol 6(54):883. [Biol. Abstr. 49(6):29520].

_____. 1967b. Information on the comparative toxicity of some cadmium compounds. Ref. Zh. Otd. Vyp. Farmakol. Khimioter Sredstva Toksikol 6(54):885. [Biol. Abstr. 49(6):2636].

_____. 1968. Problems of industrial hygiene in production and use of cadmium stearate. G. G. Sanite 33(8):29-34. [Biol. Abstr. 50 (7):36627].

Singh, K., and R. S. Mathur. 1968. Action of cadmium on some testicular enzymes of the desert gerbil Meriones hurrianae Jerdon. J. Reprod. Fert. 17(3):509-513.

Smith, J. P., J. C. Smith, and A. J. McCall. 1960. Chronic poisoning from cadmium fume. J. Pathol. Bacteriol. 80:287-296.

Snedecor, George W. 1956. Statistical Methods, pp. 18-32. The Iowa State Univ. Press, Ames, Iowa.

Spolyar, L. W., J. F. Keppler, and H. G. Porter. 1944. Cadmium poisoning in industry: Report of five cases, including one death. J. Ind. Hyg. Toxicol. 26(7):232-240.

Steinhaus, Edward A. 1967. Insect Microbiology, pp. 196-213. Hafner Pub. Co., New York.

Supple, W. C. 1961. Production of zinc deficiency in turkey poults by dietary cadmium. Poultry Sci. 40(3):827-828.

Takkar, G. L., S. R. Chowdhury, A. B. Kar, and V. P. Kamboj. 1968. Calcification of the seminiferous tubules after cadmium administration; prevention by zinc and selenium. Acta. Biol. Med. Ger. 20(1):97-102. [Biol. Abstr. 50(12):63441].

Tatchell, R. J. 1964. Digestion in the tick, Argas persicus (Oken) Parasitol. 54:423-440.

_____. 1967. Salivary secretion in the cattle tick as a means of water elimination. Nature 213(5079):940-941.

Taylor, R. M., H. S. Hurlbut, T. H. Work, J. R. Kingston, and H. Hoogstraal. 1966. Arboviruses isolated from Argas ticks in Egypt: Quaranfil, Chenuda, and Nyamanini. Amer. J. Trop. Med. Hyg. 15:76-86.

Timm, F., and G. Schulz. 1966. The testis and heavy metals. Histochemie 7(1):15-21. [Biol. Abstr. 48(1):1709].

Timme, A. H. 1965. A report on the histology of the cadmium poisoned monkey F. South African Med. J. 39(46):1198.

Turpaev, T. M., and L. I. Mamedova. 1956. The mechanism of action of cadmium ions on the contractibility of heart muscles. Biokhemiya 21:478-481. [Chem. Abstr. 51(1):2173f.].

Tsuchiya, Kenzaburo. 1967. Proteinuria of workers exposed to cadmium fumes. Arch. Environ. Health. 14(6):875-880.

Waites, G. M. H., and B. P. Setchell. 1966. Changes in blood flow and vascular permeability of the testis, epididymis and accessory reproductive organs of the rat after administering cadmium chloride. J. Endocrinol. 34(3):329-342. [Biol. Abstr. 47(5):71758].

Watkins, D. B. 1964. Cadmium sulfide in the management of tinea versicolor. Dermatol. Tropica Ecol. Geographica 3(2):111-114.

Weast, R. C. 1969. CRC Handbook of Chemistry and Physics, 50th ed., p. B-11. The Chemical Rubber Co., Cleveland, Ohio.

White, I. G. 1955. The toxicity of heavy metals to mammalian spermatozoa. Anat. J. Exp. Biol. Med. Sci. 33:359-366.

Wilson, R. H., E. DeEds, and L. J. Cox, Jr. 1941. The effect of continued cadmium feeding. J. Pharmacol. Therap. 71:22-235.

VITA

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