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OF CHEMICAL OVICIDES ON THE SURVIVAL OF
THE SOIL-TRANSMITTED INTESTINAL NEMATODES.**

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SOIL-TRANSMITTED INTESTINAL NEMATODES

by

Apollinary Mukasa Mango

A dissertation submitted in partial fulfillment
of the requirements for the degree of
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The University of Michigan
1968

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ABSTRACT

A COMPARATIVE STUDY OF THE EFFECTIVENESS OF CHEMICAL OVICIDES ON THE SURVIVAL OF THE SOIL-TRANSMITTED INTESTINAL NEMATODES

by

Apollinary Mukasa Mango

Co-chairmen: Morton S. Hilbert
Richard J. Porter

The purpose of this investigation was to study the influence of temperature and pH of the medium upon the action of the chemical agents, sodium nitrite, iodine, carbon disulfide and thiabendazole on the eggs and/or larvae of the soil-transmitted nematodes. The following nematode species of domestic animals were used as experimental material: Ancylostoma caninum, Strongyloides fülleborni, Ascaris lumbricoides var. suis and Trichuris vulpis.

Ancylostoma and Strongyloides larvae were cultured from egg-laden stools; Ascaris and Trichuris eggs were isolated from natural media and concentrated after which they were exposed to each of the test chemicals at the various temperature conditions of 15 C, 25 C or 37 C and in media with pH values of 6, 7 or 9 at three different exposure periods. Ancylostoma eggs were exposed in feces to the chemicals in a similar manner. Surviving Strongyloides and Ancylostoma larvae in a given chemical concentration were counted. Ancylostoma, Trichuris and Ascaris eggs were incubated for larval development to motile embryos or larvae; these were recorded as representing surviving eggs. The required lethal doses for the LD₅₀ and LD₉₀ were calculated for the three exposure periods and recorded, but

only the LD₅₀ and LD₉₀ for the third, or final exposure period were reported.

The overall results indicate that it would require 1.6 M NaNO₂, 1.0×10^{-2} M I₂, 0.84 M CS₂ and 3.0×10^{-4} M thiabendazole to kill a mixture of parasitic eggs and larvae (thiabendazole was effective on eggs and 1-2 day-old larvae only) at any temperature range, 13.0 C to 38.0 C in media with pH values 5.7-9.3. It was further noticed that, although the required I₂ concentration decreased with decrease in temperature, in NaNO₂, CS₂ and thiabendazole the required doses decreased with rise in temperature, and increased with decrease in temperature. The pH of the media did not have any influence on the action of thiabendazole, but, in other chemicals, rise in pH resulted in increase in the required lethal concentrations. Feces had little or no effect on the action of CS₂, NaNO₂ and thiabendazole. The action of thiabendazole on developing Ancylostoma larvae revealed that after two days of development, the larvae become insusceptible to thiabendazole doses required for the eggs.

The following conclusions were drawn from the study: parasitic resistance to chemicals from the most resistant was in the following order, Ascaris eggs, Trichuris eggs, hookworm eggs, hookworm larvae and Strongyloides larvae; although feces suppressed the action of I₂, it was nevertheless found to be the most practical ovicide and larvicide, considering its overall required low dosages for the parasites and its low cost.

Rise in temperature resulted in decreased chemical requirement for the parasites, and increase in pH resulted in increase in chemical requirement. Iodine was the only significant

exception, where required dosages increased with rise in temperature, while thiabendazole action was not at all influenced by the pH of the media.

Field applications could be safely carried out at temperatures 13-38 C by adding a chemical solution, for instance, I_2 at a concentration of $6.5 \times 10^{-3} M + 5.0 \times 10^{-4} M$ to parasite-infested feces. Because the pH of natural feces ranges from 6.9 to 8.8, there should be little or no influence of the pH of the medium on the action of the chemical.

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INTRODUCTION

Parasitic worms of man are found in almost all regions of the world, but especially in the tropics and sub-tropics because of climatic and socio-economic factors.

Stoll (1947) emphasized the great parasitic role the lives of worms play in the lives of men when he estimated that there were 2,200 million worm infections among just under 2,200 million people in the world distributed in the following manner in millions: hookworms, 457; *Ascaris*, 644; *Trichuris*, 355; *Strongyloides*, 35; and *Trichostrongylus*, 5.5. On the whole, there were over 2,000 million nematode infections as compared to only 72 million cestode and 148 trematode. The overwhelming number of nematode infections was considered by Stoll (1947) to be the result of the variety and comparative biological efficiency of the nematode life-cycles.

Parasitic nematodes may be grouped in the following categories according to their manner of transmission as suggested by Beaver (1964): contagious or fecal-borne (*Enterobius*); soil-transmitted (*Trichuris*, *Ascaris*, *Strongyloides*, hookworms); arthropod-transmitted (*filaria*); and food-animal-transmitted (*Trichinella*).

The soil-transmitted nematodes lack intermediate hosts as such but they require specific types of soil and favorable soil temperature and moisture for the development of infective stages. The prevalence of worm-infections as a whole can be viewed as providing an index

of a country's progress toward a desirable level of sanitation (Beaver, 1964). Much of the underprivileged world suffers from worm infection, partially as a result of the ever-present sewage-disposal problem. Sewage disposal practices such as application of human feces to the ground surface either as fertilizer or simply as a means of getting rid of it, and in many cases, promiscuous defecation, all increase chances of nematode-worm infection.

In many of the underdeveloped countries, the inhabitants themselves, through their own careless practices, create worm infection problems, and at the same time, the people are not economically or technically capable of eliminating these problems. This is especially true in the tropical and sub-tropical areas of the world where nematode infections are responsible for the highest incidence of diseases, chiefly because of poor or lack of adequate sewage disposal practices (Beaver, 1964).

In so far as their overall prevalence and the severity of the diseases caused by them, as well as their influence on individual health and group productivity throughout the world, the following intestinal nematodes rank highest (Beaver, 1964; Lopez et al., 1965): Ascaris lumbricoides, Linnaeus, 1758; Trichuris trichiura, Linnaeus, 1771; Stiles, 1901; Necator americanus, Stiles, 1902; Stiles, 1903; Ancylostoma duodenale, Dubini, 1843; Creplin, 1845; and Strongyloides stercoralis, Bavay, 1877; Stiles and Hassall, 1902.

Based on social and environmental factors, control of the soil-transmitted nematodes might be accomplished by any of the following methods as suggested by Beaver (1961): destruction of the parasite in the human host; prevention of access of parasite eggs or larvae to the soil; rendering the soil unsuitable for the development and/or survival of the parasite eggs or larvae; and prevention of access of the eggs or larvae from the soil to man.

Considerable emphasis has been placed on the search for a substance that will sterilize the egg-containing fecal matter by killing the nematode eggs or larvae. As early as 1880, the lethal effect of common salt on the larvae of *Ancylostoma* had been observed. More recently Chandler (1929) also noticed the effect of sea water on the survival of hookworm eggs. In addition to sodium chloride, the effects of calcareous lime, gypsum, ammonium sulfate, Chile saltpetre, ashes, kerosene, caustic soda and sulfuric acid were investigated (Ben-Gon, 1960). In Japan, China and Korea where human feces are widely used as fertilizer, a number of investigators have also been engaged in a thorough study of this problem.

The specific purpose of this investigation was to provide information which would assist in evaluating iodine, thiabendazole, sodium nitrite and carbon disulfide as ovicides to be used in the sterilization of night-soil. The study was specifically designed to determine under closely controlled conditions the degree to which

the action on the survival of the eggs and/or larvae of *Ascaris*, *Trichuris*, *Strongyloides* and *Ancylostoma* of the chemical agents was influenced by variations in exposure time, pH and temperature.

The results obtained from the experiments were consequently used in rating the chemical ovicides as to their practical application in the purification or sterilization of infected night-soil and, therefore, rendering it safe when used, for instance, as fertilizer. Some of the factors controlling the selection of such a chemical compound, and which in many cases were not considered by the previous investigators, were also studied.

The conditions under which the chemical agents were evaluated for recommendation were, therefore, that the chemical should be dependably effective in weak concentrations; it should be stable at the desired concentrations; its effects should in no way interfere with the chemistry of the soil; it should not be injurious to plant crops, man and domestic animals; it should be sufficiently inexpensive to permit its wide application; it should be easy to handle and apply under field conditions; and finally, the effects of the chemical should be lasting in any season.

HISTORICAL REVIEW

CHEMICAL AGENTS: GENERAL PROPERTIES AND OVICIDAL ACTIVITY

From the literature concerning in vitro experiments and field trials with a large number of chemical agents (Table I) in order to investigate their ovicidal properties, the following, sodium nitrite, iodine, carbon disulfide and thiabendazole appeared to satisfy many of the requirements as ovicides:

Iodine.

Iodine is one of the oldest antiseptics. It is also effective as a virucide, bactericide, tuberculocide, fungicide, sporicide, amebicide, and as a counter-irritant (Gleason, Gosselin and Hodge, 1963; Gershenfeld et al., 1948, 1950, 1952, 1954, 1955). It has also been used for emergency and small scale water disinfection with satisfactory results at a recommended concentration of 8 ppm (Hitchens, 1922; Pond and Willard, 1937; Renshaw, 1943; Fair et al., 1944).

Internally, iodine is useful against hypothyroidism and colloid goiter, and as an antidote for alkaloid poisons. In veterinary medicine it is used for the removal of roundworms and tapeworms of poultry (Gleason et al., 1963). Recently, Dozanska and Iwanczuk (1959) and Thitasut (1961) have reported iodine's ovicidal

TABLE I

A PARTIAL LIST OF POTENTIAL CHEMICAL OVICIDES

Chemical Agent	Parasite/Stage	Reference
Sodium chloride	Hookworm eggs and larvae	Chandler (1929)
Sodium borate	Dog hookworm larvae	Hoerlein (1950)
Sodium chlorophenate Dinitro-o-cyclohexylphenol Dicyclohexalamine salt Sodium-2, 4, 5-trichlorophenate	Ascaris, liver fluke eggs Liver fluke eggs	Batte and Swanson (1952) Batte and Swanson (1952)
Carbon disulfide	Ascaris eggs Hookworm eggs	Matsumura <u>et al.</u> (1953) Kutsumi and Ote (1955) Komiya <u>et al.</u> (1957) Tomomatsu (1961)
Acetic acid	Dog hookworm larvae	Dozanska and Iwanczuk (1959) Ackert <u>et al.</u> (1922)
Solid quicklime Tincture of iodine Lysol, phenol, Chloroform	Ascaris eggs	Dozanska and Iwanczuk (1959)
Chlorthion Isochlorthion Pentachlorophenol Dichlorodiphenyltrichloroethane	Ascaris eggs	Fujita (1959)
Tolyl mustard oil	Ascaris eggs	Kunii (1959); Komiya <u>et al.</u> (1957)
Ammonium nitrate	<u>N. americanus</u> egg and larvae	Ben Gon (1960)

Table I (continued)

Chemical Agent	Parasite/Stage	Reference
Sodium N-methyl-dithiocarbamate dihydrate	Ascaris eggs <u>Rhabditis</u> <u>hominis</u> and Ciliata	Yokouchi (1960)
Iodine	Hookworm and Strongyloides larvae Ascaris, Toxocara, Trichuris eggs	Thitasut (1961)
Sodium nitrite	Ascaris and Hookworm eggs	Kozai (1960, 1962)
Thiabendazole	Ascaris eggs Trichuris, Hookworm eggs	Kutsumi (1964, 1965) Hitch (1964): Chatterjee, Bandyopadhyay, and Chowdhury (1965, 1966) Stone, Mullins and Willis (1965)

activity against the eggs of *Ascaris*, *Toxocara* and *Trichuris* and the larvae of *Strongyloides* at concentrations of 70 ppm to 250 ppm at 15 C to 30 C, and as a disinfectant for leafy vegetables at concentrations of 500 ppm.

Iodine is very fast-acting as compared to chlorine and chloramines; it kills faster than chloramines, quaternary ammonium and phenol compounds. Gershenfeld (1957) and also Beckwith and Moser (1933) reported that bactericidal tests with dilute halogens (1:5000) revealed that free iodine solutions displayed more effective antibacterial activity than did chlorine or bromine at 37 C or 24 C either in the presence or absence of organic matter.

Chang (1958) considered that the effectiveness of iodine against a wide variety of organisms was probably attributed to its high penetrating ability, which in turn, was very much dependent on the pH of the solutions. Iodine's highest biocidal activity is witnessed in the diatomic form, I_2 , which is the prevalent stage at low pH values. As the pH of the solution is raised, iodine progressively changes into hypoiodous acid (HIO), and then hypoiodous acid ion (IO^-), and finally, iodate ion (IO_3^-) with corresponding decrease in activity.

Iodine acts directly on cells by precipitating proteins (Grollman, 1965). The effect of organic matter is negligible when iodine is used in high concentrations,

but the presence of many kinds of organic matter decreases iodine's activity at low concentrations (Wilson, Mizuno, and Bloomberg, 1960).

If ingested in large quantities, iodine is toxic internally, and it may cause poisoning due largely to its corrosive action on the gastro-intestinal tract; average doses may cause skin lesions, and the solid element is irritating to eyes and mucous membranes as well (Finklestein and Jacobi, 1937).

Advantages and disadvantages of iodine as a disinfecting agent are summarized in Table II as of 1950 (Wilson et al., 1960):

Although Table II shows that iodine has almost as many advantages as disadvantages, when thoroughly examined it can be seen that its advantages outweigh the disadvantages.

Sodium Nitrite.

Sodium nitrite, glyceryl trinitrate, amyl nitrate, and other related compounds are used in medicine to dilate coronary vessels and to reduce blood pressure (Dreisbach, 1963). These compounds act directly on smooth muscle of the vessel wall, or indirectly through the innervation of the blood vessels and so alter their tone (Rubin et al., 1963).

The ovicidal activity of sodium nitrite was first reported by Nagano (1952). This author noticed that NaNO_2 was able to destroy the eggs of *Ascaris* in acid

TABLE II

ADVANTAGES AND DISADVANTAGES OF IODINE*
AS A DISINFECTANT

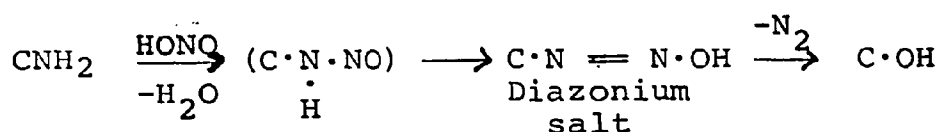
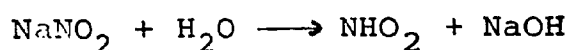
Advantages	Disadvantages
Long history of use	Instability
High activity	Low solubility
Low concentrations	Precipitated upon dilution
Fast killing	High vapor pressure
Penetrating	Skin staining
Non-specific	Vapors irritating
Low temperature coefficient	Objectionable odor
Fairly low toxicity	Primary irritant
Moderately irritating only	Too reactive
Wide pH range	Corrosive
Own color indicator	Easily reduced by organic matter
Tests for, are available	
Not affected by water hardness	
Not affected by detergents	
Inexpensive	
Abundant supply	

*Adapted from Wilson et al. (1960).

medium. Kozai (1960, 1962) got better results with NaNO_2 against *Ascaris* eggs in natural and artificial night-soil by acidifying the medium with calcium superphosphate, $\text{Ca}(\text{H}_2\text{PO}_4)_2$.

Kozai (1960) explained that the increasing activity of NaNO_2 as the pH of the medium was lowered probably resulted from the fact that in acidified medium nitrous acid was released from sodium nitrite to produce diazonium salt, although normally stable at lower temperatures, the acidity of the medium caused it to lose some of its nitrous acid, and therefore becoming more concentrated and unstable.

The instability of diazonium salt in acid medium caused the release of free N_2 and consequently, resulting in an increase in the total reaction and inactivation of the parasitic eggs.



Sodium nitrite has to be swallowed or injected to produce poisoning. An excess of it in drinking water may cause methemoglobinemia in infants (AJPH, 1950; Oppe, 1951). Asbury (1964) reported intoxication and death in livestock which had drunk water to which NaNO_2 had been previously added.

Recently, several authors, including Sinos (1964)

and Kübler (1965) have also observed that cases of methemoglobinemia occurred in young children after being fed on spinach (natural spinach normally contains a certain amount of NaNO_2). Singley (1962) had previously reported cases of methemoglobinemia which had resulted from the ingestion of fish adulterated with NaNO_2 .

Carbon Disulfide.

In medicine, CS_2 is used topically as a counter-irritant and as a local anaesthetic. In veterinary practice, it is used for the removal of roundworms and botfly larvae from horses, and stomach worms from swine (Sollmann, 1957).

Whether as a liquid or vapor, carbon disulfide is hazardous (Gleason et al., 1963); absorption occurs through all portals so that poisoning may occur either by ingestion or by inhalation. The action of CS_2 is chiefly through damage to the central nervous and hemopoietic systems.

McKee et al. (1943) reported in vitro experiments showing that large quantities of CS_2 slightly inhibited the succinic oxidase enzyme system important in intermediate carbohydrate oxidation and normal nerve tissue metabolism.

The ovicidal properties of CS_2 were first recognized by Matsumura et al. (1953). These investigators were able to disinfect night-soil of parasitic eggs

with 0.1 per cent solution of CS_2 at 5 - 20 C in one week. Several years later, Kutsumi and Ote (1955) also reported that 1:250 and 1:500 dilutions of CS_2 completely destroyed the ova of Ascaris and hookworms in one week and four weeks respectively at between 25 C and 30 C.

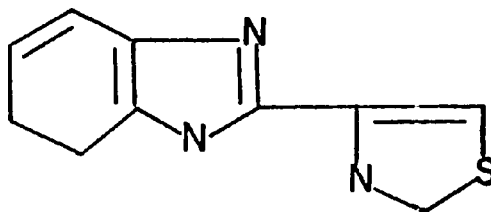
Komiya et al. (1957) demonstrated that a one per cent emulsion of CS_2 at a temperature of 10 C was able to sterilize the eggs of Ascaris and Ancylostoma in the laboratory in seven days. It was, however, demonstrated by Tomomatsu (1961) that CS_2 when applied to night-soil under field conditions, was less effective in the winter season than in summer, and that ovicidal effect was achieved only after increasing the amount of CS_2 during the winter.

However, Komiya et al. (1957) also noticed that full ovicidal activity of CS_2 was possible only in acid medium. The decreased activity of CS_2 under alkaline conditions was attributed to the formation of ammonia from urine and other organic matter. In non-acidified night-soil the NH_3 reacts with CS_2 to form dithiocarbamic acid ammonia, $\text{CS}_2 + 2\text{NH}_3 \longrightarrow \text{H}_2\text{N} - \text{CS}_2\text{NH}_4$. This resulting product ($\text{H}_2\text{N} - \text{CS}_2\text{NH}_4$) is a weaker oviicide than CS_2 .

Except for its poisonous nature and flammability Tomomatsu (1961) regarded CS_2 as a favorable ovicide because it was cheap, it did not reduce the value of the night-soil as fertilizer, and it was also easy to apply.

Thiabendazole.

Thiabendazole, 2-(4'-thiazolyl)-benzimidazole, is a white crystalline compound having a molecular weight of 201.3. The empirical formula for thiabendazole is $C_{10}H_7N_3S$ and the structural formula,



It is stable as a solid and in solution; it is only slightly soluble in dilute acid and alkali with maximum solubility at pH 2.5 (Brown, 1961).

Thiabendazole has been reported to have marked anthelmintic activity on a variety of gastrointestinal parasites of livestock, and to interfere with the migrating larval stages of *Ascaris*, *Ancylostoma* and pinworms in man (Cuckler, 1961; Brown et al., 1961).

When taken internally in large doses, thiabendazole may have side effects like nausea, vomiting, anorexia, dizziness and abdominal cramps (Bailey, 1965).

Leland and Bogue (1964) and Stone, Mullins and Willis (1964, 1965) reported from in vitro tests that thiabendazole was capable of inhibiting the development of *Strongyloides* and hookworm larvae in eggs. Similar experiments were conducted against a variety of saprophytic and pathogenic fungi (Robinson, Phares and Graessle, 1964) and

these also showed that thiabendazole had inhibitory effects on the organisms.

Although thiabendazole had been reported by various workers to be lethal to most developing nematode eggs, Hitch (1964) and Stone et al., (1965) discovered that, in vitro, the drug had no lethal effect on the third larval (filariform) stages of the dog hookworm, Ancylostoma caninum. The drug was, however, effective against the earlier developing larval stages (first and second) as well as those stages in the animal body after infection.

EXPERIMENTAL ORGANISMS:

Life-Cycles and Control Approaches

Before any attempts are made in the control of the soil-transmitted nematodes, it is necessary that their nature and life-cycles be well understood.

The life-cycles of these nematodes in general depend on the presence of infected and potential hosts among the population, and on the favorable soil conditions of temperature and moisture. The adult forms of the worms inhabit the small intestine of man, but a part of their life-cycle is spent in the soil as eggs (*Ascaris*, *Trichuris* and hookworms) and/or larvae (hookworms and *Strongyloides*).

The soil receives feces containing undeveloped or non-infective stages of the parasites; conditions for the development of the eggs or larvae are provided by the soil until the infective stages are reached under favorable conditions of temperature, soil texture and humidity. The

soil also provides protection for the infective stages for a period during which they may be brought into contact with susceptible hosts (Beaver, 1961).

The embryonated infective eggs of *Trichuris* and *Ascaris* are transmitted to the next host by ingestion, whereas in the case of hookworms and *Strongyloides* the third stage infective filariform larvae find their way to the next host by penetrating the latter's skin.

Based on the knowledge of the life-cycles of the worms, their control may be theoretically achieved as suggested in part by Beaver (1961) by: treatment of the infected individuals; complete prohibition of fecal contamination of the soil by sewage treatment, composting or igniting, or by use of privies, septic tanks and oxidation ponds (Katayama, 1955; Hodgson, 1964); rendering the contaminated soil unsuitable for the development or survival of infective stages; wearing of shoes; or barring contact with the infective soil for a period long enough to ensure natural loss of infection from people.

However, many of these control methods are not possible in many parts of the world for economic reasons or because of ignorance. Moreover, some authors, but especially Legler (1950), Bhaskaran et al. (1956), Vosta (1958), and Voute (1961) gave reports indicating that so long as the general population is heavily infected with worms, conventional methods of sewage treatment are not in themselves adequate in the control of worm infection. Treated sludge has been shown to contain viable parasite

eggs, while surfaces of pit privies have also been found to be contaminated with parasite eggs and larvae.

Therefore, a simplified, practical and effective method of control for the exogenous stages of the soil-transmitted nematodes has yet to be found for those parts of the world with high prevalence of worm infection where conventional methods of sewage disposal are lacking.

The search for an economical chemical ovicide which is capable of interrupting the life-cycles of the intestinal nematodes by destroying their eggs or larvae in the soil was the objective of this investigation. A chemical was needed whose ovicidal activity is non-specific and is not influenced by variations in temperature and pH of the medium, as determined by in vitro tests on the parasitic eggs and/or larvae.

The prevalence of the common human soil-transmitted nematodes, and their life-cycles and distinguishing features are shown in Tables III and IV below.

TABLE III

RECENT PREVALENCE RECORDS (%) OF ANKYLOSTOMIASIS, ASCARIASIS
AND TRICHURIASIS IN VARIOUS REGIONS

Region	Ankylostomiasis	Ascariasis	Trichuriasis	Reference
Korea	37-46	81-82	78-81	Brooks et al. (1956)
China (mainland)	30-90	30-97	-	Hou et al. (1959)
Taiwan	79-94	20-72	59-89	Frick et al. (1956)
Thailand (2 areas)	3-28	20-48	1-12	Sadun (1953, 1955)
Philippines (Palo)	60-90	80-82	87-90	Pesigan et al. (1958)
New Guinea (5 areas)	94-98	0-70	4-88	Bearup and Lawrence (1950)
Tahiti	38	13	44	Kessel et al. (1964)
Lebanon (urban)	-	22	16	Salam et al. (1955)
Jordan (2 areas)	-	51-78	44-78	Alicata and Dajani (1955)
N. Egypt (13 areas)	2-7	2-79	0-27	Kuntz et al. (1958)
N. Rhodesia (12 schools)	30-52	0-9	0-1	McCullough and Fris- Hansen (unpublished data, 1955)
Natal, S. Africa (3 areas)	4-6	26-50	39-60	Elsdon-Dew and Horner (1958)

Table III (continued)

Region	Ankylostomiasis	Ascariasis	Trichuriasis	Reference
Mauritius (1952-1955)	25-55	28-36	33-49	Scott (1961)
Seychelles (ages 5-60)	23-35	43-78	82-95	Spitz (1960)
Puerto Rico (6 areas)	19-41	30-50	72-89	Malsonado and Oliver-Gonzalez (1960)
Surinam	8	42	9	Blumberg et al. (1953)
Costa Rica (students)	1-6	6-7	31-33	Jimenez-Quiros et al. (1958)
Peru (Iquitos)	91	75	81	Gonzalez-Mugaburu (1955)
Bolivia (2 areas)	50-61	3-16	1-10	Hilburg (1955)

*Adapted from Beaver (1961).

TABLE IV
CHARACTERISTICS AND LIFE-CYCLES OF PARASITES

Name	<u>Ascaris lumbricoides</u>	<u>Ancylostoma duodenale</u>	<u>Strongyloides stercoralis</u>	<u>Trichuris trichiura</u>
Egg size (u)	60x45	60x38	54x32	52x23
Stage of progeny leaving human host	Unembryonated egg	Egg in early cleavage	Rhabditoid larva	Unembryonated egg
Time of incubation after leaving host	10-15 days	Less than 24 hrs	None	20-40 days
Rhabditiform larvae at hatching (u)	250x14 Posterior bulbar swelling of cylindrical esophagus	275x16 Posterior bulbar swelling of esophagus, long, narrow pre-esophageal chamber	225x16 Posterior bulbar swelling of esophagus, short, pre-esophageal chamber	125x8 Slight posterior bulbar swelling of cylindrical esophagus
Free-living larvae (u)	None	850x35 Sharply, pointed tail	700x28 Minute fork at tail	None
Invasion of host, infective stage	Unhatched ovum (rhabditiform larva) - Swallowed	Filariform larva - penetrates skin	Filariform larva penetrates skin	Unhatched ovum (rhabditiform larva) swallowed

Table IV (continued)*

Name	<u>Ascaris lumbricoides</u>	<u>Ancylostoma duodenale</u>	<u>Strongyloides stercoralis</u>	<u>Trichuris trichiura</u>
Development	Indirect, to intestine via intestine	Lymph, blood, lungs and glottis	Indirect, to intestine via lymph, blood, lungs and glottis.	Direct
Minimal length of life cycle.** (weeks)	11	6	4	15
Host - definitive	Man and hog	Man	Man; experimentally dog, cat and ape.	Man, monkey, lemur and hog.

*Adapted from Beaver (1961) and Belding (1965).

**From infection to adult.

Classification, Description and Differentiation

The experimental nematode worms, namely, *Ascaris*, *Trichuris*, *Strongyloides* and hookworms, belong to the phylum *Nematoidea*, or roundworms.

Man and some domesticated animals are infected by similar or closely related nematode worms. Since it was more convenient to obtain and maintain under laboratory conditions representative worms from domestic animals, the following nematode worms were used in the experiments:

Ascaris.

Ascaris lumbricoides Linnaeus, 1758; syn. *A. suis*, Goeze, 1782, the roundworm of the domestic pig. According to Baylis (1926) as described by Lapage (1962), the worms found in man and pig are morphologically and serologically indistinguishable, but are apparently 'physiological' or 'host' varieties.

Sprent (1952) differentiated the existing human and domestic pig worm species on the basis of variations in shape and size of the denticulous ridges of the *Ascarids*. He pointed out that the denticles of the pig *Ascaris* form a conspicuous row of more or less equilateral triangles while those of the human *Ascaris* are relatively much smaller and their ridges are concave.

Many transmission tests from man to pig and vice-versa have failed, but pigs kept on diets deficient in Vitamin A have been successfully infected with human *Ascaris* (Sprent, 1952). Normally, the pig *Ascaris* does not develop beyond the larval stage in man (Takata, 1951).

The adult worms inhabit the small intestine of the domestic pig. The adult worm is cylindrical, whitish to reddish yellow in color; the male measures 150 to 310 by 3 mm and the female 220 to 350 by 5 mm. The anterior end is conical; there are three papillae at the mouth. The posterior end is blunt with a ventral curvature in the male and bluntly pointed in the female (Beaver, 1952).

The fertile eggs are broadly oval and they measure from 45 to 70 by 35 to 50 u and average about 60 by 45 u. The egg shell has three layers, an inner, highly impermeable lipoid layer which is secreted just after fertilization; a middle, thick transparent chitinous layer or true shell synthesized from glycogen and ovarian nitrogen; and an outer, coarse, albuminous covering of yellowish or golden hue, which serves as an auxiliary barrier to permeable substances (Jaskoski, 1952; Pollak et al., 1955). The infertile eggs measure 88 to 94 by 39 to 44 u; they are larger and narrower and have a thinner egg shell covering.

Nishimura (1956) reported that adult A. lumbricoides had survived in five per cent glucose-saline and human bile for 13 to 15 days. Ascaris eggs were viable and capable of developing after maintenance for eight months at 6 - 35 C in 2.4 per cent formaldehyde, but failed to develop in a 7.4 per cent solution (Sandars, 1951).

Trichuris.

Trichuris vulpis Fröhlich, 1789; the dog whipworm, served as the experimental material. The adult worm inhabits the cecum and other parts of the intestine of the dog and

fox.

The adult dog whipworm is larger than the human species, and measures 45 to 75 mm long as compared to T. trichiura (25 to 50 mm). About three quarters of the body length of the dog whipworm is made up by the anterior portion; in T. trichiura the anterior portion constitutes about two-thirds. The spicule in T. vulpis is 9 to 11 mm and in T. trichiura it measures only 2.0 to 3.35 mm long (Lapage, 1962).

The most important differentiation of T. vulpis from T. trichiura is based on the size of the eggs, and on the vulvar and vaginal characteristics (Chitwood, 1930; Hall and Sonnenberg, 1956). The eggs are oval in shape and yellowish-brown in color. They measure 70 to 89 by 37 to 40 u in T. vulpis as compared to those of T. trichiura which measure 50 to 60 by 21 to 25 u.

Trichuris eggs are less resistant to heat, cold, desiccation and chemical agents than those of *Ascaris* (Brown, 1927). The eggs are killed instantly at high temperatures of about 52 C to 54 C and at low temperatures of about -9 C to 12 C.

Strongyloides.

Strongyloides fülleborni von Linstow (1905) is a parasite of monkeys, and it was first described by Linstow (1905). The adult inhabits the small intestine of the monkey. Chandler (1923) pointed out that the eggs of S. fülleborni usually hatch outside the host intestine unlike those of the human species S. stercoralis which hatch into

larvae before leaving the body of the host.

According to Sandground (1925), S. fülleborni can be differentiated from say, S. stercoralis, by the fact that in the former species, the eggs usually do not hatch until after they have left the body of the host. Other differentiating features are that in S. fülleborni, the female vulva in the bisexual generation has conspicuous lips; the most reliable character is that the anterior of the body is greater than it is posterior to the vulva, and this fact alone may also be used to differentiate S. fülleborni from other species in primates. Additional features of differentiation are the shape of the ovaries and stoma (Sandground, 1925; Goodey, 1926; Premvati, 1958; Little, 1966).

The larvae of S. fülleborni are killed at 43 C, and above 37 C only a few complete their life-cycle; below 8 C the rhabditiform larvae are killed in 10 hours, and the filariform larvae in seven days. The larvae develop best in water-saturated soils with abundant organic material (Coutinho et al., 1952).

Ancylostoma.

Ancylostoma caninum Ercolani, 1859; Hall, 1913; syn. Uncinaria canina Ercolani, 1859; Railliet, 1900, inhabits the small intestine of the dog, cat, fox and, rarely, also man.

The adult male dog hookworm measures 10 to 20 mm and the female 14 to 16 mm. The eggs measure 56 to 65 by 37 to 43 u, and they contain embryos of about eight

cells when laid.

The larvae of A. caninum are larger, but the shape is similar to those of Necator americanus. The third stage, infective filariform larvae average 660 by 22 u. The cuticle has faint circular annulations. The oral buccal capsule measures 15 u deep and 3 u in diameter, and there are heavy cuticular walls. In optical plane, the capsule walls appear as two spears 12 u long with anterior ends directed toward the opposite member; there are no concavities like those in Necator (Nichols, 1956).

The dog hookworm eggs fail to develop at temperatures below 13 C. Salt water inhibits the development and hatching of ova; the ova fail to hatch in the absence of oxygen, or in the presence of urine or night-soil after a period of time (Chandler, 1929; Matsusaki, 1963). The larvae require relatively little moisture, but they cannot withstand drying.

MATERIALS AND METHODS

1. PARASITES

a. Source of Organisms

The nematode parasites in this study were obtained from the following sources:

Strongyloides.

The larvae of S. fulleborni were obtained by culturing egg-laden stools from a naturally infected African monkey, Macaca mulatta, supplied by Dr. K. W. Cochran, Department of Epidemiology, the University of Michigan, Ann Arbor;

Ancylostoma.

The dog hookworm, A. caninum eggs and larvae were obtained from a naturally infected dog.* The dog was maintained by the Animal Care unit, The University of Michigan;

Trichuris.

The eggs of the dog whipworm, T. vulpis, were obtained from a continuous supply of egg-laden stools from the same dog that was used for the source of Ancylostoma eggs and larvae.

Ascaris.

The eggs of the pig Ascaris, A. lumbricoides

*Purchased from E.S. Rogers, Meadowbrook Farms, Richmond, Michigan.

var. suis, were obtained from the uteri of gravid worms in recently killed domestic pigs in a slaughter house* through the courtesy of Dr. Val van Giesen, Veterinarian, Wayne County Department of Health, Eloise, Michigan.

b. Preparation of Test Organisms

The larvae of Strongyloides fulleborni and Ancylostoma caninum were obtained by the animal bone-charcoal technique supplemented by the filter-paper-strip method as described by Harada and Mori (1955).

The Bone-Charcoal Technique

A sample of feces of about two gm was mixed on a filter paper in a petri dish with an equal amount of granular animal bone-charcoal.** The mixture was moistened with a little water and incubated in covered petri dishes at 28 C for four to six days, until larvae appeared in the culture.

For better yields of larvae, a sand-charcoal-fecal culture mixture was prepared by mixing equal parts of animal bone-charcoal, sand and feces. The larvae were then recovered by the Baermann apparatus method. The Baermann apparatus was set up as described by Faust et al. (1964). The charcoal culture containing hookworm or Strongyloides larvae was placed on two layers of cheesecloth over a wire screen. Lukewarm

*Arnoldy & Sons Slaughterhouse, Dearborn, Michigan.

** Lots # C7 and E7, purchased from Sargent & Company.

water, 10 degrees above room temperature was added into the funnel until the level of the water in the funnel rose just above the lower surface of the culture. The mixture was left to stand for about 30 minutes at room temperature (25 C) after which 10-20 ml of the larval suspension were drawn off and examined for larvae. The procedure was repeated until no more larvae could be recovered. The larvae were washed and kept at 20 C until needed.

The Filter-Paper-Strip Cultivation Method

This is a simplified method, and one by which it was possible to recover cleaner larvae. The procedure was carried out as described by Harada and Mori (1955). Filter paper strips, one inch by seven inches, were prepared. A small sample of egg-laden feces (0.5 - 1.0 gm) from freshly collected infected stools was smeared about one inch from one end of the strip.

Each treated filter paper strip was fitted into a wide-mouthed jar filled with about 30 ml of distilled water so that the smeared end of the strip was about one-half inch in the water, leaving about another half inch distance between the surface of the water and the lower border of the smeared area. The upper end of the strip was secured in place with masking tape. The jars were closed and then incubated at 28 C for four to six days until larvae could be detected in the clear water below.

Exposure of *Ancylostoma* eggs to chemical agents was done in feces, but *Ascaris* and *Trichuris* eggs were first recovered from their natural media and then exposed to the chemical agents in aqueous suspension.

Trichuris eggs were recovered by sedimentation, as described by Chatterjee et al. (1965). Fresh feces of the dog infected with *Trichuris* were thoroughly comminuted in a small quantity of water; more water was added, and the suspension was strained through a wire screen to remove coarse material. The fine suspension was left to settle in a jar for one hour before pipetting off the supernatant and then washing the sediment several times with clean water. The clean egg suspension was filtered through three or four layers of cheesecloth to remove the coarse matter. The filtrate was left to settle, and the procedure repeated until the egg suspension was clear. The eggs in the last suspension were concentrated by further settling and drawing off as much of the supernatant as possible. Recovered eggs were stored at 8 - 10 C.

Ascaris eggs were obtained from gravid uteri in the intestines of recently slaughtered pigs. The uteri of the female worms containing eggs were dissected and the eggs removed by squeezing them out into distilled water in a beaker. After washing them several times, the eggs were concentrated by settling.

for two hours, at the end of which time the supernatant was pipetted off leaving the egg-laden sediment. The eggs were stored in a cool room at 3 - 10 C.

Counting Technique for Hookworm and Strongyloides Larvae

In order to estimate the number of larvae in a given worm suspension, Whipple's Chamber or counting cell,* as employed by Whipple et al (1927) in the examination of water, was used. In order to count, one ml of the larval suspension in a counting chamber was placed under a dissecting microscope. The cover of the cell was replaced and the larvae were counted at a magnification of 14 (2x by 10x by 0.7).

Only the living larvae, as judged by their motility in the suspension, were counted. No attempt was made to count the dead worms since it was not possible to distinguish them from moulted sheaths. The larval suspension was discarded after each count and the procedure was repeated with a fresh sample. The mean of the counts was calculated and recorded.

Microscope Standardization for Measuring Hookworm and Strongyloides Larvae

In order to be able to estimate the growth of the larvae, a compound microscope was calibrated as outlined by Tanner (1950) for viewing at low power (100x). The larval suspension was examined for larvae whose motility had been considerably reduced by addition of approximately

*Purchased from Arthur H. Thomas & Son, Philadelphia, Pennsylvania.

1 - 2 drops of 70% I_2 solution in 2 ml suspension. The length and width of the larvae were measured and expressed in microns.

Counting Technique for Ascaris and Trichuris Eggs

A 1-ml pipette was calibrated and found to deliver 0.05 ml of the egg suspension for each drop.

The egg suspension was thoroughly mixed and one drop (0.05 ml) was transferred to a glass slide and examined microscopically for eggs under low power (100x). The whole surface of the slide containing the egg suspension was examined and the eggs counted.

c. Viability Studies

Environmental conditions, such as, temperature, light, humidity, alkalinity, and acidity have been reported to have lethal effects on the eggs and larvae of the soil-transmitted nematodes (Brown, 1927; Matsusaki, 1963). Therefore, before any experiments concerning the ovicidal effects of chemical agents were carried out, tests on the effects of the temperature range of 8 C - 37 C and the pH range of 6 and 9 upon the survival of the parasite eggs and larvae were conducted. The temperature and pH ranges are those that had been selected for ovicidal testing of the chemical agents against the various parasites.

The aim of the tests was to show whether the environmental conditions within the selected temperature and pH parameters had any lethal effects on the

parasitic eggs or larvae.

The hookworm eggs and Strongyloides larvae are more susceptible to physical and chemical agents than the Ascaris and Trichuris eggs (Brown, 1927) and for this reason, hookworm eggs and Strongyloides larvae were chosen for preliminary studies on their susceptibility to temperature, (15 C, 25 C and 37 C), pH, (6, 7 and 9) and storage or holding time. It was assumed that selected temperature and pH parameters would therefore, have less effect on Ascaris and Trichuris eggs.

TEMPERATURE EFFECTS: The Effects of Temperature on the Survival and Development of Strongyloides Larvae

The aim of this test was to learn whether the temperature range of 8 C - 37 C had any effect upon the survival, growth and development of newly hatched larvae of Strongyloides fülleborni.

Strongyloides larvae were cultivated, recovered, washed and concentrated. A measured number of larvae was placed in four pairs of vials. Each of the vials in a pair contained the same number of larvae, after which the vials were incubated at specified controlled temperatures (8 C, 20 C, 25 C and 37 C). Surviving larvae at each temperature were counted daily and recorded.

The Effects of Temperature on the Survival, Hatchability and Development of the Hookworm Eggs

Hookworm eggs, freshly isolated and mostly in 2

to 8-cell developmental embryo stages, were exposed to three different temperature conditions, 8 C, 25 C and 37 C. The objective of this test was to observe the survival rate of the eggs and their ability to hatch and develop at various temperatures.

The Effects of Temperature upon the Survival and Development of Hookworm Larvae at 8 C, 25 C and 37 C

Hookworm larvae were cultivated, recovered and on the second day, they were divided into three paired groups of vials. The three groups of vials were incubated at 8 C, 25 C and 37 C respectively in order to observe the larval survival and growth.

pH EFFECTS: The eggs and larvae of hookworm and the larvae of *Strongyloides* were each exposed to acid, neutral and alkaline media covering a pH range of 6 and 9. The objective of the tests was to show whether the pH of the holding medium for the test organisms had any influence on their survival and development.

Influence of pH on the Survival and Development of Hookworm Eggs

Three pairs of egg-suspensions in test tubes were buffered, two of them with 0.07 M Na_2HPO_4 and 0.07 M KH_2PO_4 to approximate pH values of 6 and 7; the third pair was buffered with $\text{NaB}_4\text{O}_7 \cdot 10 \text{ H}_2\text{O}$ (Borax) to pH 9. The final pH of the egg-suspensions was checked by the Beckman pH meter, and the samples were then incubated

at 28 C.

The survival and development of the eggs were determined by daily examination in order to observe the growth changes in the embryos. The larvae that hatched were maintained in the same media, and their size was measured.

The Effect of pH 6 - 9 on the Survival of Hookworm Larvae

Three sets of hookworm larvae were placed in media buffered to approximate pH values of 6, 7 and 9; the control medium was not buffered. The samples were incubated at room temperature (25 C).

Survival of the larvae was estimated by counting the larvae every day and making a record of the survivors.

The Effect of pH 6-9 on the Survival of Strongyloides Larvae

Three pairs of Strongyloides larval suspensions were buffered to approximate pH values of 6, 7 and 9. The samples were then incubated at room temperature for six days. The death rate in each medium was estimated by daily counts of the survivors.

2. CHEMICAL AGENTS

a. Source of Chemicals

The chemical ovicides used in this study were obtained from the following sources: sodium nitrite, NaNO_2 , reagent A.C.S. Code 2265, was bought from the Allied

Chemical Company, General Chemical Division, Morristown, New Jersey; carbon disulfide, CS_2 , reagent A.C.S. Code 1552, was purchased from the same company from which NaNO_2 was bought; thiabendazole (2-(4'thiazolyl)-benzimidazole), $\text{C}_{10}\text{H}_7\text{N}_3\text{S}$, Lot 67 RTS 781, was donated by the Merck Sharp and Dohme Research Laboratories, Division of Merck & Co., Inc., Rahway, New Jersey; iodine, I_2 , Lot No. 32132, was purchased from the J. T. Baker Chemical Company, Phillipsburg, New Jersey.

b. Preparation of the Stock Solutions of the Test Chemicals and Their Maintenance

The stock solutions of the experimental chemical agents, namely, sodium nitrite, carbon disulfide, iodine and thiabendazole were prepared at approximate molar concentrations previously tested and believed to be effective.

Preliminary tests were done with each chemical against each parasite in order to establish the actual dilution range of effectiveness. The ranges of test concentrations were either increased or lowered, depending on the results of the preliminary tests.

Sodium Nitrite, 1 M Solution

A 1M stock solution of sodium nitrite, NaNO_2 , molecular weight 69.00, was prepared by dissolving 69 gm of NaNO_2 crystals in a small amount of distilled water and then diluting it to one liter. The solution was stored at 20 C.

Carbon Disulfide, 0.1 M Solution

A 0.1 M solution of CS_2 , a liquid with molecular weight of 76.14 and specific gravity of 1.26 was prepared by dissolving 7.6 gm of the liquid in about 100 ml of ethyl alcohol (95 per cent). The volume was made up to one liter with distilled water.

The solution was stored in a well-stoppered dark bottle at 8 C - 10 C to eliminate loss by evaporation, since the boiling point of CS_2 is only 46.3 C.

Thiabendazole, 0.001 M Solution

A 0.001 M stock solution of thiabendazole, $\text{C}_{10}\text{H}_7\text{N}_3\text{S}$, molecular weight 203.1, was prepared by dissolving 0.2 gm of the powder in about 10 ml of dimethylsulfoxide.* The pH of the mixture was adjusted to between 2 and 2.5 with two or three drops of concentrated HCl in order to achieve maximum solubility (Brown, 1961). After all the thiabendazole powder had completely dissolved, the solution was diluted with distilled water to one liter and then stored at 20 C.

Iodine, 0.1 N Solution

A 0.1 N aqueous solution of I_2 was prepared as recommended in the Standard Methods for the Examination of Water and Waste Water (APHA, 1960) in the following manner: about 12.6 gm of iodine crystals, atomic weight 126.91, were dissolved in potassium iodide solution and then diluted with distilled water to one

*D-1483, purchased from Crown-Zellerbach.

liter.

The solution was transferred to a dark bottle wrapped in aluminum foil, and then stored in a dark room at 8 C - 10 C. No solution was kept for more than one week.

The actual concentration of the stock iodine solution was determined by titration with sodium thiosulfate (Pierce et al., 1960; APHA, 1960).

Whereas the practical concentrations of the solutions of NaNO_2 and CS_2 were found to be toxic or irritating to the skin on contact, those of I_2 and thiabendazole were mild and easy to work with.

c. Choice of Serial Chemical Concentrations for Ovicidal Testing

A series of concentration values was employed in making up test samples from stock solutions of known strength based on progressive bisection of intervals on a logarithmic scale as recommended by Duodoroff (1951). In each case, aliquot portions of the stock solution were used according to the scale, 10.0, 8.7, 7.5, 6.5, 5.6, 4.9, 4.2, 3.7, 3.2, 2.8, 2.4, 2.1, 1.8, 1.55, 1.35, 1.15 and 1.0, either as milliliters or fractions of a milliliter.

The advantage of using this scale is that when plotted, the values lie evenly spaced in a straight line (Duodoroff, 1951).

d. Selection of the Test pH and Temperature Conditions

The choice of the experimental temperature range of 15 C - 37 C was an attempt to simulate the typical climatic conditions that prevail in the parasite-ridden regions of the world, and at the same time to satisfy the optimum conditions under which the parasite stages are able to survive and develop uninhibited.

Three test temperatures, 15 C, 25 C and 37 C representing low, medium and high were selected. The 15 C condition was made possible by use of running cold water with addition of ice. For tests requiring longer intervals for instance, more than 24 hours, a refrigerator set at 15 C was provided. Two incubator spaces were used to provide the 25 C and 37 C conditions for long test periods, and a water-bath for shorter periods.

Acid-base conditions were selected on the principle that in nature, the pH of night-soil varies between 6.9 and 8.8 (Kozai, 1960). Subsequently, approximate pH values of 6, 7 and 9 were selected to cover the range of normal night-soil medium in which the eggs and larvae of the soil-transmitted nematodes are usually found.

Final test suspensions were buffered to respective pH values by means of Sorensen's 0.07 M sodium phosphate dibasic, Na_2HPO_4 and 0.07 M potassium phosphate monobasic, KH_2PO_4 mixtures in the ratio of 10:90 for pH 5.91 and 60:40 for pH 6.98 (Pearse, 1960). For the pH value of 9, a solution of sodium borate decahydrate (borax), $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{ H}_2\text{O}$ was employed (APHA, 1960). Actual pH

values of the buffered test suspensions were confirmed by the Beckman pH meter at the beginning and end of each exposure period.

3. OVICIDAL TESTING PROCEDURES

a. Larvicidal Tests for Strongyloides and Hookworm Larvae

The larvae of hookworms and Strongyloides were exposed to chemical agents in five pairs of 50-ml plastic vials; there were also five control samples to which no chemical was added. To each of the larval-suspensions in the vials, a predetermined concentration of the chemical was added. Each of the test samples received a different chemical concentration in descending order and according to logarithmic scale so that the five test concentrations covered the dilution range of the chemical previously obtained from exploratory tests.

The suspensions were prepared in the order, chemical agent, buffer solution, distilled water and larval suspension adding up to a final volume of 20 ml. In the control samples distilled water replaced the chemical. The procedure was carried out at a given test temperature and maintained at the same temperature throughout the testing period. Separate tests were done at each of the temperatures, 15 C, 25 C and 37 C, and pH values, 6, 7 and 9. Therefore, in all, there were nine sets of experiments for each chemical agent tested against

each species of parasite.

The killing effect of the chemical agent was evaluated at the end of one-, two- and three-hour periods of exposure by examination of the test suspension and counting the surviving larvae. Normally, two counts were taken for each sample and their mean was recorded. Survivors in the control samples were also counted. The mean pH of the samples throughout the testing period was also calculated.

As a demonstration of the procedure discussed above, the exposure of hookworm larvae to sodium nitrite solution at room temperature (25 C) and pH 7 follows:

i Estimation of Larvae in Suspension

Larval count per ml in suspension	52
Volume of larval suspension to be used	7 ml
∴ Estimated number of larvae to be added (7 x 52)	364

Since the final volume of the test-suspension was 20 ml, each ml of the mixture contained 364/20	18 larvae
--	-----------

ii Buffer Solution

Three ml of the mixture of 0.07 M Na_2HPO_4 and 0.07 M KH_2PO_4 were added in the ratio of 60:40 in order to buffer the final test mixture to a pH of about 7.

iii Serial Dilutions of 1 M NaNO_2

The final selection of effective dilution range (10.0, 6.5, 4.2, 2.4 and 1.6 ml) within 1 M NaNO_2 was

based on the results of exploratory tests which had shown that addition of these quantities to the larval suspension resulted in 100 per cent kill or no kill at all depending on the quantity added.

Each of the quantities was placed in a corresponding labelled vial.

iv Final Mixture

The contents of each vial as shown in Table V, were thoroughly mixed, after which the final pH was checked and recorded. The samples were incubated at room temperature (25 C) for one hour. At the end of the hour, one ml of the larval suspension was examined for surviving larvae. The procedure was repeated for both test and control samples and the results were recorded for one, two and three-hours of exposure.

The same testing procedure was repeated for all temperatures, 15 C, 25 C, and 37 C and pH 6, 7 and 9 with each chemical agent against the larvae of hookworms and Strongyloides.

b. Ovicidal Tests for Hookworm, Trichuris and Ascaris Eggs.

Hookworm Eggs

The hookworm eggs were exposed to chemical agents in a fecal suspension medium. Exposure of hookworm eggs to NaNO_2 solution at 15 C and pH 7 is a typical example of the experiments in this category.

TABLE V

CONSTITUENT FRACTIONS IN A LARVICIDAL

TEST SAMPLE:* NaNO_2 AGAINST

HOOKWORM LARVAE AT 25 C AND pH 7

Sample Number	Constituents in Test Sample in ml	
	1 M NaNO_2	H_2O
1	10.0	0
2	6.5	3.5
3	4.2	5.8
4	2.4	7.6
5	1.6	8.4

*The following 7 ml, of larval suspension, 1.8 ml of 0.07 M Na_2HPO_4 and 1.2 ml of 0.07 M KH_2PO_4 were added to each sample adding up to a final volume of 20 ml.

Test suspensions were prepared in the order, chemical agent, buffer solution, fecal sample and distilled water adding up to a final volume of 20 ml.

Aliquot portions of 0.01 M NaNO_2 solution in quantities of 4.2, 3.7, 3.2, 2.8 and 2.4 ml were placed in 5 pairs of labelled corresponding centrifuge tubes; to each of the tubes 2 ml of a mixture of 0.07 M Na_2HPO_4 and KH_2PO_4 in the ratio of 60:40 were added. Two grams of the egg-laden dog stools were added to each pair of the test samples and five control tubes. Distilled water was added to each sample to bring the final volume to 20 ml as shown in Table VI. After mixing the contents of the centrifuge tubes by shaking, the actual pH of each sample was checked and recorded. The samples were incubated at 15 C for three hours. At the end of the exposure period, the chemical was removed from the test samples by diluting with water, centrifuging and discarding the supernatant. This process of washing was repeated three times. The control samples were also subjected to centrifugation in order to remove excess water.

The sediment in each tube was separately cultured with bone charcoal as already described in the preceding pages. The cultures were incubated at 28 C for four to six days for larval development. The cultures were examined and the number of larvae were counted in both test and control samples. The larvae were assumed to represent the number of eggs that had not been affected

TABLE VI

CONSTITUENT FRACTIONS IN AN OVICIDAL

TEST SAMPLE: NaNO_2 AGAINST

HOOKWORM ECGS AT 15 C AND pH 7

Sample Number	Constituents in Test Sample in ml	
	0.01 M NaNO_2	H_2O
1	4.2	11.8
2	3.7	12.3
3	3.2	12.8
4	2.8	13.2
5	2.4	13.6

*The following, 2 gm of feces, 1.2 ml of Na_2HPO_4 and 0.8 ml of KH_2PO_4 were added to each sample making up a final volume of 20 ml.

by the chemical in the test samples. Separate tests were done at 3-, 6- and 12-hour exposure periods, and for all the temperatures and all pH values.

Ascaris and Trichuris Eggs

The eggs of *Ascaris* and *Trichuris* were exposed to the chemical solutions in aqueous suspension. After the eggs had been collected and concentrated, testing proceeded as follows, as demonstrated by the activity of iodine solution upon *Ascaris* eggs at room temperature (25 C) and pH 7.

Test suspensions were prepared in the order, chemical agent, buffer solution, egg suspension and distilled water adding up to a final volume of 10 ml. Aliquot portions of 0.001 M I_2 in amounts of 6.5, 4.9, 3.7, 2.8 and 2.1 ml were placed in five pairs of corresponding centrifuge tubes; to each of the test samples and five control tubes, 2 ml of 0.07 M Na_2HPO_4 and KH_2PO_4 in the ratio of 60:40 were added. Into each of the tubes 0.3 ml of egg suspension were added, and the final mixture was made up to 10 ml with water as shown in Table VII.

After mixing the samples and recording their pH, they were incubated at room temperature (25 C) for three hours. At the end of the exposure period iodine was removed by diluting, centrifuging and discarding the supernatant two or three times. The remaining sample at the bottom of each tube was diluted with 2 - 3 ml

TABLE VII

CONSTITUENT FRACTIONS IN AN OVICIDAL

TEST SAMPLE:* 0.001 M I_2 AGAINST

ASCARIS EGGS AT 25 C AND pH 7

Sample Number	Constituents in Test Sample in ml	
	0.001 M I_2	H_2O
1	6.5	1.2
2	4.9	2.8
3	3.7	4.0
4	2.8	4.9
5	2.1	5.6

*The following, 0.3 ml of egg suspension, 2 ml of 0.07 M Na_2HPO_4 and KH_2PO_4 in the ratio of 60:40 were added to each sample making up a total final volume of 10 ml.

of distilled water, after which the samples were incubated at 30 C for 5 - 10 days.

The eggs were examined daily to observe the development of the embryos. Surviving eggs usually developed to motile embryos between 5 and 10 days in *Ascaris*, and 10 and 14 days in *Trichuris*. Eggs with motile embryos were counted and recorded. Control samples were also examined for motile embryos.

c. Calculation of the Chemical Concentration in the Final Test Mixture

The actual concentrations of the chemical agent in the final test mixtures were determined in the following manner using the effect of 1 M NaNO_2 on the larvae of hookworms as an example.

An aqueous stock solution (1 M), was prepared by dissolving 69 gm of NaNO_2 in distilled water and then diluting it to 1 liter. Dilution series in ml of 10.0, 6.5, 4.2, 2.4 and 1.6 were drawn from 1 M NaNO_2 and mixed with larval suspension, buffer solution and water adding up to a final volume of 20 ml in each sample. The final concentration of NaNO_2 in the final mixture was therefore, as shown in Table VIII.

d. Calculation of the 50 and 90 Per Cent Endpoints

An endpoint in this study was taken to mean the dilution within the test dilution series at which a certain desired proportion of the test parasite larvae or eggs were killed.

TABLE VIII

SODIUM NITRITE CONCENTRATION IN THE FINAL
TEST MIXTURE IN MOLES PER LITER (M) *

Sample Number	1 M NaNO ₂ (ml)	Final NaNO ₂ Concentration in moles per liter
1	10.0	$\frac{10 \text{ ml}}{20 \text{ ml}} \times 1 \text{ M NaNO}_2 = 0.500$
2	6.5	$\frac{6.5 \text{ ml}}{20 \text{ ml}} \times 1 \text{ M NaNO}_2 = 0.325$
3	4.2	= 0.210
4	2.4	= 0.120
5	1.6	= 0.080

*Based on the final volume of 20 ml.

The 50 per cent endpoint (LD_{50}) is therefore, the dose at which half of the parasite population was killed by the chemical agent. The 50 per cent endpoint is usually preferred in bioassay experiments because at this dilution the chances of variation are minimum (Gaddum, 1933).

In this study both the 50 and 90 per cent endpoints were estimated. The 90 per cent endpoint was included in order to give a rough idea of how concentrated the chemical would have to be in order to achieve, or nearly achieve, a total kill.

The method for estimating the endpoints was that of Reed and Muench (1938), and in certain cases where it was also necessary to include the confidence limits or standard errors of the doses, a supplementary method by Pizzi (1950) was applied. The latter method is basically the Reed-Muench method, but introduces the following formula by which it is possible to calculate the standard error of the LD_{50} .

$$S E_{LD_{50}} = \sqrt{\frac{0.79 \text{ hR}}{n}}$$

where 0.79 = Constant
 h = interval between doses
 n = number of animals
 R = interquartile range

An illustration of the procedure for estimating LD_{50} and LD_{90} endpoints is given in Table IX, below

TABLE IX

ESTIMATION OF THE LD₅₀ AND LD₉₀ ENDPOINTS BY
THE REED-MUENCH METHOD*

Dosage Moles/liter	Parasite/ Dose	Living (s)	Dead (d)	Cumulated Per Cent	
				Living	Dead
0.500	15	2	13	2	48
0.325	15	4	11	6	35
0.210	15	5	10	11	24
0.120	15	7	8	18	14
0.080	15	9	6	27	6
					48/50=96.00
					35/41=85.36
					24/35=68.57
					14/34=43.75

*Based on the results of the treatment of hookworm larvae with NaNO₂
at 37 C and pH 9 for 3 hours.

based on the results of exposure of hookworm larvae to aliquot portions of 1 M NaNO_2 at 37 C and pH 9.0.

Fifty and Ninety Per Cent Interpolation

LD_{50} :	<u>Per cent mortality</u>	<u>Dosage: moles/liter</u>
	68.57	0.210 M
	50.00	
	43.75	0.120 M
∴ 50%	$\frac{6.25\% \times 0.09 \text{ M}}{24.82\%}$	+ 0.120 M
	$\text{LD}_{50} = 0.143 \text{ M}$	
LD_{90} :	96.00	0.500 M
	90.00	
	85.36	0.325 M
∴ 90%	$\frac{4.64\% \times 0.175 \text{ M}}{10.64\%}$	+ 0.325 M
	$\text{LD}_{90} = 0.401 \text{ M}$	

Estimation of the Standard Errors for the LD_{50} and LD_{90} by the Pizzi Method

The standard errors for the fifty and ninety per cent endpoints obtained above were estimated by means of the formula by Pizzi (1950) as shown above.

Standard Error for LD_{50}

$$\text{LD}_{50} = 0.143 \text{ M}$$

$$h = 0.210 \text{ M} - 0.120 \text{ M} = 0.09 \text{ M}$$

$$n = 15$$

$$\begin{aligned} R &= \text{LD}_{75} - \text{LD}_{25} = 0.265 \text{ M} - 0.091 \text{ M} \\ &= 0.174 \text{ M} \end{aligned}$$

$$\begin{aligned}
 S. E_{LD_{50}} &= \sqrt{\frac{0.79 \times 0.09 \text{ M} \times 0.174 \text{ M}}{15}} \\
 &= \sqrt{8.0 \times 10^{-4}} \\
 S. E_{LD_{50}} &= 2.8 \times 10^{-2} = 0.028 \text{ M}
 \end{aligned}$$

The results show that one standard error (68%) for the LD_{50} of 0.143 M is 0.028 M, and therefore, two standard errors (95%) is $2 \times 0.028 \text{ M} = 0.050 \text{ M}$. The confidence limits for the LD_{50} are therefore $0.143 \text{ M} \pm 0.05 = 0.093 \text{ M}$ and 0.193 M .

Standard Error for LD_{90}

Estimation of the standard error for the LD_{50} showed that the confidence limits for 0.401 were $0.401 \text{ M} \pm 0.08 = 0.481 \text{ M}$ and 0.321 M .

e. Exposure of Developing Hookworm (*Ancylostoma caninum*) Larvae to Thiabendazole

An experiment was carried out to study the susceptibility of the growing larvae of the dog hookworm, A. caninum to thiabendazole, determined by daily survivors.

Experimental Design and Test Procedure

Freshly collected stools of a dog infected with hookworms were cultured in charcoal in petri dishes at 28 C. The hatched larvae in 2-3 petri dishes were separated daily by the Baermann apparatus method.

The larval suspension was placed in a petri dish under a dissecting microscope and then the larvae were

picked out individually by means of an eye dropper and placed in a graduated centrifuge tube until a desired number had been collected.

Two ml of 10^{-4} M solution of thiabendazole were added to the larval suspension. The mixture was then buffered to a pH of about 7 with 2 ml of Na_2PO_4 and KH_2PO_4 at a ratio of 60:40. Sufficient distilled water was added to make up a final volume of 20 ml. The procedure was in the order of, larval suspension, chemical agent, buffer solution and distilled water.

The test sample was incubated at 28 C for 12 hours. At the end of the exposure period the larval suspension was placed in a petri dish under a microscope and the surviving larvae were counted again. Per cent mortality of the larvae was calculated and the results were compared with the control.

Another set of developing larvae was exposed to a higher concentration, 2×10^{-2} M of thiabendazole in the same manner and the results were recorded as above.

EXPERIMENTAL RESULTS

A. Preliminary Tests

The effects of temperature, pH and storage or holding time on the survival and development of parasitic eggs and/or larvae in the absence of test chemicals were first studied. The aim of these experiments was to make sure that selected test conditions alone were not harmful to the parasites.

Parasitic eggs and/or larvae were exposed to various temperature and pH conditions. The survival and development or growth of the parasitic forms were determined by counting and/or measuring.

The data on the effect of temperature on the survival and growth of *Strongyloides* larvae are given in Table X and Figure 1 respectively. It was evident from Table X and Figure 1 that temperatures, 8 C, 20 C, 25 C and 37 C did not greatly increase the death rate of the *Strongyloides* larvae in five to six days. The decrease in the numbers of the larvae at any of the temperatures was gradual and nearly identical.

These data further show that larval growth in the temperatures, 20 C, 25 C, and 37 C was not suppressed. However, they show that there was an unusually rapid growth in the larvae kept at 8 C. Ordinarily, low temperatures have a retarding effect on the development of organisms (Matsusaki, 1963; Brown, 1927). There was no

TABLE X
TEMPERATURE EFFECTS ON THE SURVIVAL AND DEVELOPMENT
OF STRONGYLOIDES LARVAE

Day of Incubation	Larvae Surviving at				Larval Measurements (u)*			
	20 C				20 C			
	8 C	25 C	37 C		8 C	25 C	37 C	
1	68	22	27	28	240x16	296x15	256x15	272x16
2	68	20	26	26	264x15	296x16	288x16	326x15
3	67	21	24	26	526x16	348x16	448x16	396x16
4	63	20	25	25	656x16	540x16	560x17	580x16
5	64	16	24	22	656x16	572x16	620x16	628x16
6		16	22			656x16	664x16	

*Based on the daily measurements of two larvae at each temperature.

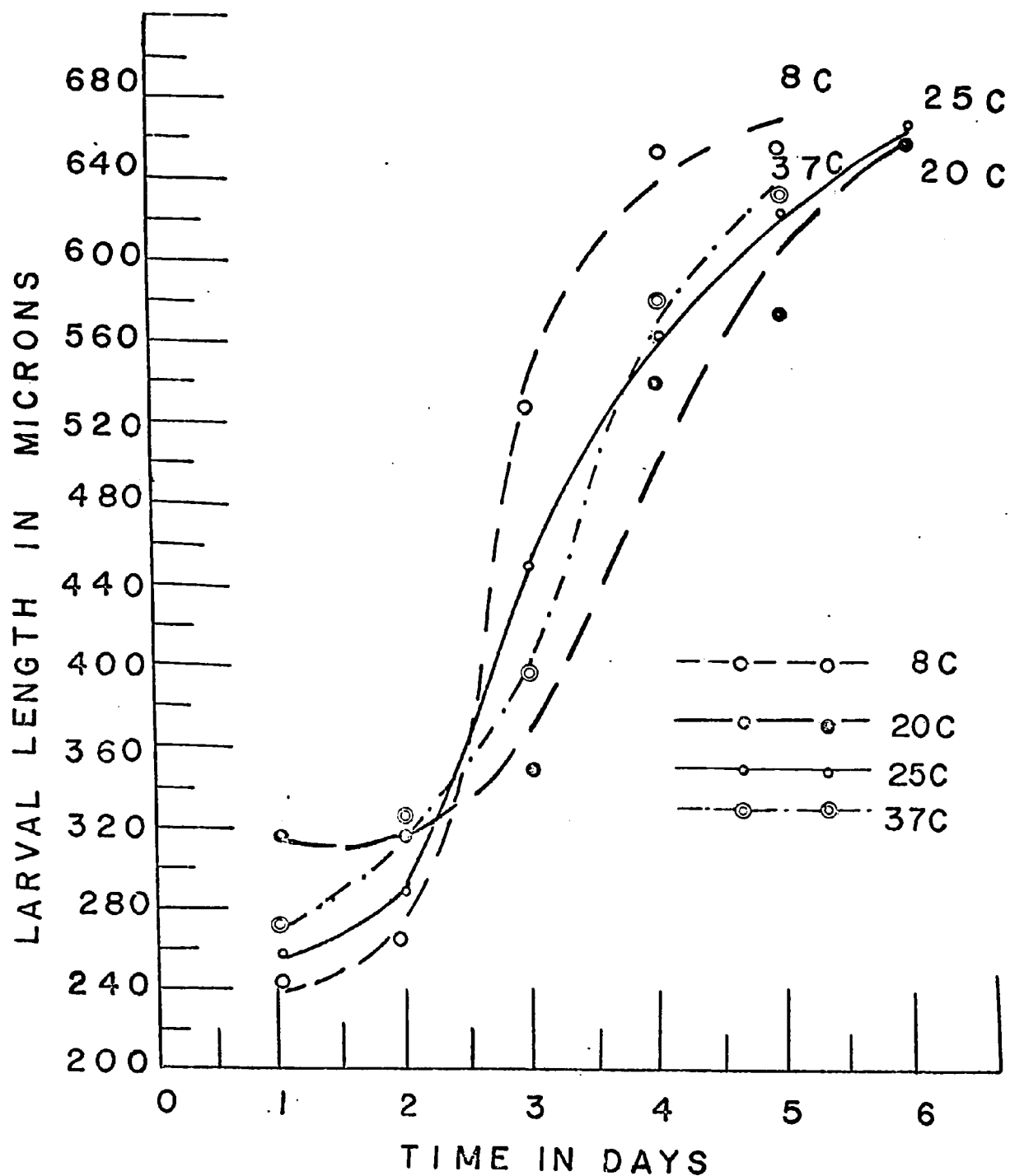


Figure 1.--Growth of *Strongyloides* Larvae: Length increase at Selected Temperatures.*

*Regression Lines in Figures 1-7 were fitted by Observation.

satisfactory explanation for this unique phenomenon from the experimental results.

Table XI below is a summary of the temperature effects upon the survival, development and hatchability of the hookworm eggs at temperatures, 8 C, 25 C and 37 C.

The results in the table indicate that these temperatures, 25 C and 37 C had no retarding effect on the development and hatchability of the hookworm eggs, and that at 8 C, the eggs were not able to develop beyond the 11-cell stage. None of the eggs hatched at the latter temperature, but those eggs that were transferred and incubated at 25 C were able to develop and hatch into larvae as expected.

The results of the effect of temperature on the survival and development of hookworm larvae at 8 C, 25 C, and 37 C appear in Table XII and Figure 2. It appears from these data that the influence of temperature range 8 C to 37 C had insignificant effect on the survival and growth of the hookworm larvae. Most of the larvae survived and grew to maximum size at the various incubation temperatures of 8 C, 25 C and 37 C.

The results on the suspension of hookworm eggs in media of pH values of 6, 7 and 9 at room temperature appear in Table XIII. These results indicate that there was no significant difference in the development and hatching of the hookworm eggs and growth of the larvae in the media ranging in pH from 5.9 and 9.0.

The larvae of hookworm were suspended in media of

TABLE XI

SURVIVAL, DEVELOPMENT AND HATCHABILITY OF HOOKWORM EGGS
AT SELECTED TEMPERATURES

Day of Incubation	Egg Condition and/or Developmental Stage at		
	8 C	25 C	37 C
1-4	Most eggs at 2- and 11-cell stages; a small number in the tadpole stage		
5	Most eggs in 4- to 11-cell stages; a few tadpoles.	Half of the eggs embryonated; rest hatched to larvae measuring 456 (240-600) u by 14.7 (12-16) u	Two-thirds of the eggs hatched to larvae with similar measurements as those at 25 C; rest of the eggs still in motile embryo stage.
6	No change.	All eggs hatched to larvae; measuring 270 - 680 u by 16-20 u	As in 25 C; size 640 by 16 u
7	Nearly all eggs in 11-cell stage.	Only about 25% of the larvae living; showing feeble movements.	Most larvae grown to average size of 696 by 16 u
8	No change in the condition of the eggs. One-half transferred to 25 C	No living larvae seen.	Only one live larva seen; size 560 by 16 u
9	Left at 8 C No change	Transferred to 25 C Damaged eggs, 2; hatched (larvae 2; size, 296 by 16 u)	All larvae dead

Table XI (continued)

Day of Incubation	8 C		25 C	37 C
	Left at 8 C	Transferred to 25 C		
10	No change	All normal eggs hatched (larvae), size: 224-400 by 16u	.	
11	Egg condition unchanged	Larvae all dead		

TABLE XII
SURVIVAL AND DEVELOPMENT OF HOOKWORM LARVAE AT SELECTED TEMPERATURES

Day of Incubation	Larvae Surviving at			Larval Measurements* (u) at		
	8 C	25 C	37 C	8 C	25 C	37 C
1	74	97	67	272x16	240x16	240x16
2	70	63	61	272x20	280x16	264x16
3	73	90	64	286x16	396x17	296x17
4	79	80	62	400x16	500x16	428x16
5	76	80	59	536x17	636x17	596x17
6	76	86	61	744x16	688x16	640x16
7	72	81	61	659x16	728x18	720x16
8	76	80	64	720x24	720x16	720x20
9	72	76	58	700x18	728x16	744x16
10				648x16	720x16	680x16
11				765x18	720x17	720x16
12				700x16	648x16	716x17
13				656x18	764x20	720x17
14				680x18	720x18	728x16
15				680x16	712x17	720x16
16				728x16	720x17	716x16
17				680x18	728x17	
18				728x18		
				272-765 by 16-24	240-764 by 16-20	240-744 by 16-20

*Based on daily larval measurements of two larvae at each temperature.

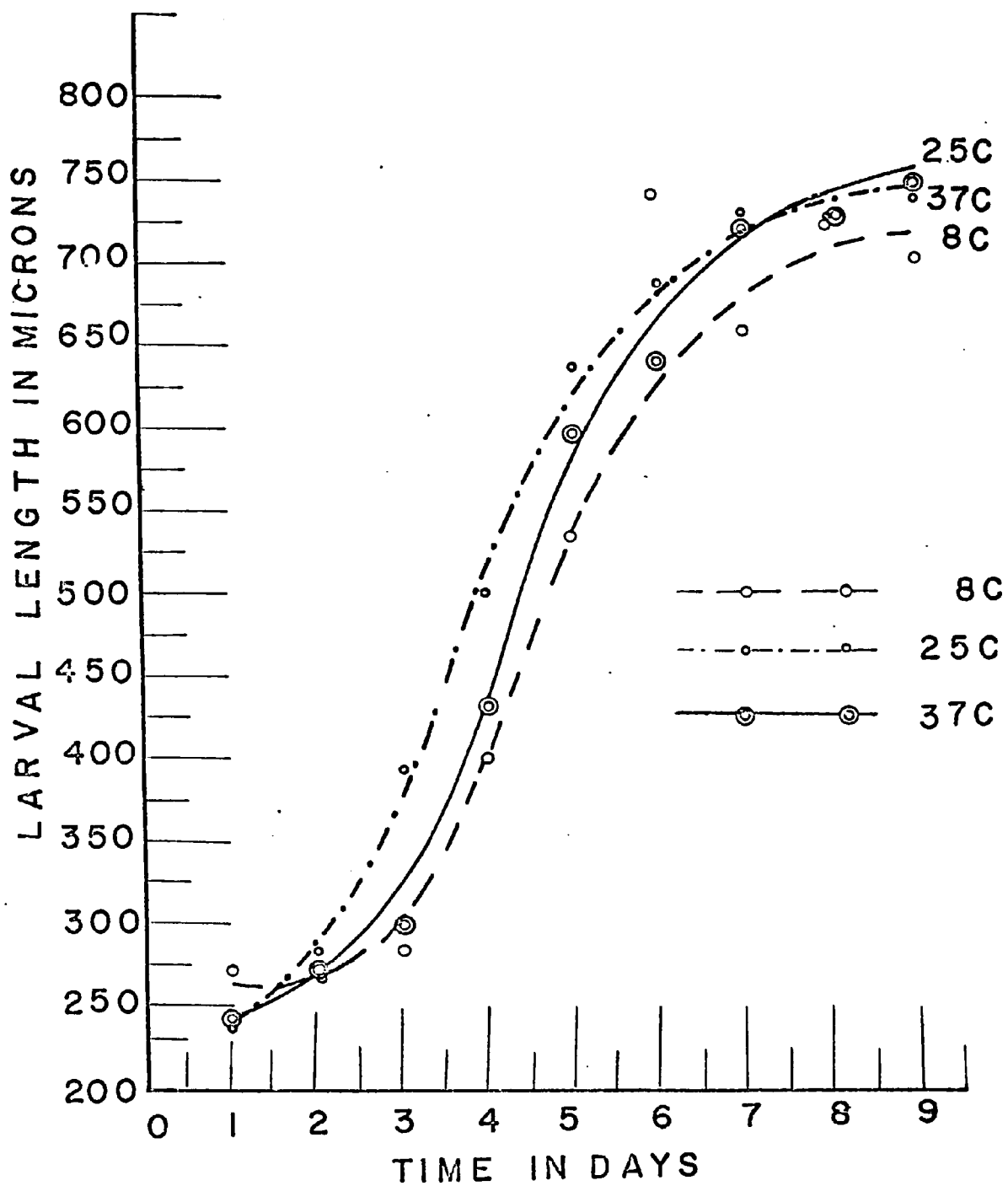


Figure 2.--Growth of Hookworm Larvae: Length increase at Selected Temperatures.

TABLE XIII

SURVIVAL, DEVELOPMENT AND HATCHABILITY OF
 HOOKWORM EGGS AND LARVAE AT THE pH VALUES OF
 6, 7 AND 9 AND ROOM TEMPERATURE (25 C)

A. Egg Development and Hatchability

Day of Incubation	Egg Stage at pH		
	5.9 - 6.1	7.2 - 7.3	8.8 - 9.0
1	2-4 cell	2-4 cell	2-4 cell
2	2-11 cell	2-11 cell	2-11 cell
3	Multiple cell	Multiple cell	Multiple cell
4	Multiple cell	Multiple cell	Multiple cell
5	Motile embryo	Motile embryo	Motile embryo
6	Hatching	Hatching	Hatching
7	Larvae	Larvae	Larvae
8	Larvae	Larvae	Larvae

Table XIII (continued)

B. Larval Development

Day of Incubation	Larval Measurements* (u) at pH		
	5.9-6.1	7.2-7.3	8.8-9.0
1	256 x 16	240 x 16	264 x 16
2	280 x 16	-	320 x 17
3	360 x 16	-	-
4	375 x 16	472 x 16	-
5	680 x 16	696 x 20	481 x 16
6	596 x 18	630 x 16	580 x 16
7	656 x 18	580 x 17	616 x 16
8	690 x 16	646 x 16	690 x 18
9	696 x 18	648 x 16	616 x 16
10	680 x 16	696 x 16	636 x 16
11	516 x 16	616 x 16	696 x 17
12	696 x 16	-	-
13	680 x 16	-	-
Range	256 - 696 by 16 - 18	240 - 696 by 16 - 20	264 - 696 by 16 - 18

*Based on daily measurements of two larvae at each pH range.

pH values of 6, 7 and 9 at room temperature (25 C) for seven days. A record of surviving larvae was made daily as shown in Table XIV. It can be seen from these results that the rate of decrease in the number of hookworm larvae was nearly the same in all the media, buffered and unbuffered and covering pH values of 6.0-6.2, 7.3, 7.5-8.0 and 8.7-8.9.

The larvae of *Strongyloides* were suspended in media of approximate pH values of 6, 7 and 9 at room temperature (25 C). The number of survivors was recorded daily as shown in Table XV. It can be seen from the table that the larvae of *Strongyloides* were not significantly affected by pH between 5.8 and 9.0. The rate of decrease in the buffered media, acid, alkaline and neutral was comparable to that in the normal or unbuffered medium.

B. Larvicidal and Ovicidal tests

The Effects of Chemical Agents on the Survival of Hookworm and Strongyloides Larvae

The first series of experiments was concerned with the effects of sodium nitrite, carbon disulfide, iodine and thiabendazole on the survival of *Ancylostoma* and *Strongyloides* larvae under controlled conditions of temperature and pH.

1. The Effect of Sodium Nitrite on the Larvae of Ancylostoma and Strongyloides

Nine experiments were performed on each kind of worm at the following temperature-- pH combinations: 15 C -

TABLE XIV

THE EFFECT OF MEDIA OF DIFFERENT pH VALUES
ON THE SURVIVAL OF HOOKWORM LARVAE

Day of Incubation	Surviving Larvae at pH			
	7.5-8.0 (unbuffered)	6.0-6.2	7.3	8.7-8.9
1	17	16	16	13
2	13	11	13	16
3	15	15	16	14
4	14	13	13	12
5	15	13	12	12
6	12	11	11	13
7	13	12	10	11

TABLE XV

THE EFFECT OF MEDIA OF DIFFERENT pH VALUES
ON THE SURVIVAL OF STRONGYLOIDES LARVAE

Day of Incubation	Surviving Larvae at pH			
	7.5-8.1*	5.8-6.0	7.2-7.4	8.8-9.0
1	14	19	16	18
2	16	17	14	16
3	16	19	15	18
4	15	19	15	19
5	12	13	13	17
6	12	14	13	15

*Unbuffered

pH 6, 7, and 9; 25 C - pH 6, 7, and 9; and 37 C - pH 6, 7, and 9. After cultivating and recovering the larvae their numbers in the suspension were estimated. A known number of larvae in suspension was added to each of the five pairs of 50-ml vials containing NaNO_2 and covering a concentration range of 0.08 M to 0.50 M for *Ancylostoma* and 0.025 M to 0.16 M for *Strongyloides*. The concentration ranges were selected on the basis of exploratory tests using 1 M NaNO_2 stock solution against the larvae. The samples were buffered to respective pH values and then incubated at the desired temperature (15 C, 25 C or 37 C) for one, two and three hours. At the end of each exposure period, surviving larvae in each vial were counted in a small portion of the sample. The remaining larval suspension was reincubated for another hour. The procedure was repeated for the second and third hour exposure periods.

The results were calculated for fifty and ninety per cent endpoints and then recorded as moles per liter. Tables XVI and XVII show detailed data on the treatment of hookworm larvae with NaNO_2 in various combinations of temperature, pH and exposure periods. The endpoints (LD_{50} and LD_{90}) appearing in Table XVII represent the means of those in Table XVI at the three-hour exposure period at given temperature and pH ranges.

The data in Tables XVI (1-9) and XVII show that the dose needed to achieve the LD_{50} or LD_{90} decreased with increase in exposure period and that the pH of the medium

TABLE XVI (1-9)

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF SODIUM NITRITE ON HOOKWORM LARVAE

(1) Effect of Sodium Nitrite, NaNO_2 , Against Hookworm Larvae at 14.0-15.0 C and pH 6.0-6.2*

Exper. No.	Time Inter- val (hour)	Mean Temp. °C	Mean pH	Control Sur- vivors	Survivors at Concentration (M)						Endpoints**			
					0.500	0.325	0.210	0.120	0.080	0.050	LD50	LD90		
i	1	15.0	6.0	16	4	7	9	10	14			0.24	0.50	
ii	1	15.0	6.0	16	6	6	9	12	12			0.25	0.53	
iii	1	14.0	6.1	23	7	9	12	18	24			0.26	0.52	
iv	1	14.0	6.1	23	7	8	8	16	26			0.21	0.51	
					Mean						0.24		0.52	
i	2	15.0	6.0	15	4	4	6	11	15			0.24	0.50	
ii	2	15.0	6.0	15	3	3	7	10	11			0.18	0.45	
iii	2	14.0	6.1	21	2	4	10	14	19			0.19	0.39	
iv	2	14.0	6.2	21	2	3	8	15	21			0.18	0.36	
					Mean						0.20		0.43	
i	3	15.0	6.1	14	3	3	4	7	14			0.18	0.45	
ii	3	15.0	6.1	14	2	2	4	8	10			0.15	0.35	
iii	3	14.0	6.1	21	1	2	6	12	19			0.16	0.30	
iv	3	14.0	6.2	21	1	3	5	12	18			0.15	0.31	
					Mean						0.16		0.35	

*Unless otherwise stated, the pH of the controls was within the range of the test-

samples.

** (M)

Table XVI (1-9) (continued)

(2) Effect of Sodium Nitrite, NaNO_2 Against Hookworm Larvae at 14.5-15.0 C and pH 6.9-7.2

Exper. No.	Time Interval (hour)	Mean Temp. °C	Mean pH	Control Survivors	Survivors at Concentration (M)						Endpoints*	
					0.500	0.325	0.210	0.120	0.080	0.050	LD ₅₀	LD ₉₀
i	1	14.5	7.1	17	6	9	11	12	16		0.29	0.54
ii	1	14.5	7.0	17	4	8	10	10	13		0.23	0.49
iii	1	15.0	7.0	22	9	9	18	21	21		0.32	0.58
iv	1	15.0	7.0	22	8	9	17	23	24		0.32	0.56
					Mean						0.29	0.54
i	2	14.5	7.1	16	2	3	5	11	15		0.18	0.40
ii	2	14.5	7.1	16	1	2	6	10	16		0.18	0.32
iii	2	15.0	7.0	23	4	7	13	16	21		0.23	0.46
iv	2	15.0	7.0	23	3	5	7	10	18		0.15	0.39
					Mean						0.18	0.39
i	3	14.5	7.1	17	1	2	3	6	13		0.11	0.28
ii	3	14.5	7.1	17	0	1	3	6	12		0.11	0.29
iii	3	15.0	7.0	22	1	1	4	11	14		0.12	0.25
iv	3	15.0	7.0	22	0	1	6	10	15		0.12	0.26
					Mean						0.12	0.22

* (M)

Table XVI (1-9) (continued)

(3) Effect of Sodium Nitrite NaNO_2 Against Hookworm Larvae at 14.5-14.8 C and pH 8.8-9.0

Exper. No.	Time Inter- val (hour)	Mean Temp. °C	Mean pH	Control Sur- vivors	Survivors at Concentration (M)						Endpoints*		
					0.500	0.325	0.210	0.120	0.080	0.050	LD ₅₀	LD ₉₀	
i	1	14.8	8.8	16	7	9	10	15	15			0.33	0.58
ii	1	14.8	8.8	16	6	8	9	13	16			0.30	0.55
iii	1	14.5	8.8	20	7	10	12	16	19			0.29	0.54
iv	1	14.5	8.8	20	8	8	11	15	17			0.26	0.53
					Mean						0.30	0.55	
i	2	14.8	8.8	16	5	6	8	11	14			0.24	0.51
ii	2	14.8	8.8	16	5	5	8	10	15			0.23	0.52
iii	2	14.5	8.8	20	7	9	10	14	18			0.26	0.53
iv	2	14.5	8.8	20	6	9	10	14	17			0.24	0.51
					Mean						0.24	0.52	
i	3	14.8	8.8	14	3	4	6	8	10			0.18	0.46
ii	3	14.8	8.8	14	3	3	6	8	12			0.20	0.33
iii	3	14.5	8.8	20	3	5	7	11	15			0.16	0.42
iv	3	14.5	8.8	20	3	6	7	11	16			0.17	0.43
					Mean						0.18	0.41	

* (M)

Table XVI (1-9) (continued)

(4) Effect of Sodium Nitrite, NaNO_2 , Against Hookworm Larvae at 24.4-25 C and pH 6.0-6.6

Exper. No.	Time Inter-val (hour)	Mean Temp. °C	Mean pH	Control Survivors	Survivors at Concentration (M)						Endpoints*	
											LD ₅₀	LD ₉₀
					0.500	0.325	0.210	0.120	0.080	0.050		
i	1	24.4	6.2	25	0	4	13	19	26	22	0.21	0.32
ii	1	24.4	6.2	25	1	4	7	21	27	28	0.18	0.33
iii	1	25.0	6.2	20	0	5	11	20	18	18	0.24	0.40
iv	1	25.0	6.1	20	1	7	12	18	19	20	0.25	0.44
					Mean						0.22	0.37
i	2	24.4	6.3	26	0	3	5	18	22	26	0.15	0.28
ii	2	24.4	6.3	26	0	1	7	17	21	25	0.15	0.26
iii	2	25.0	6.3	19	0	2	7	17	19	20	0.19	0.31
iv	2	25.0	6.2	19	0	5	8	16	22	19	0.21	0.38
					Mean						0.18	0.31
i	3	24.4	6.3	25	0	1	2	8	19	24	0.08	0.20
ii	3	24.4	6.4	25	0	1	3	15	22	21	0.13	0.21
iii	3	25.0	6.3	19	0	3	4	12	19	21	0.16	0.31
iv	3	25.0	6.3	19	0	1	6	12	19	20	0.16	0.28
					Mean						0.13	0.25

*(M)

Table XVI (1-9) (continued)

(5) Effect of Sodium Nitrite, NaNO_2 , Against Hookworm Larvae at 24.0-25.6 C and pH 7.0-7.2

Exper. No.	Time Inter-val (hour)	Mean Temp. °C	Mean pH	Control Sur-vivors	Survivors at Concentration (M)						Endpoints*	
											LD50	LD90
					0.500	0.325	0.210	0.120	0.080	0.050		
i	1	24.0	7.0	19	1	2	5	12	14	19	0.15	0.30
ii	1	24.0	7.0	19	1	2	5	11	15	16	0.14	0.30
iii	1	25.6	7.1	16	2	3	6	10	13	17	0.17	0.39
iv	1	25.6	7.1	16	1	2	4	13	15	16	0.18	0.32
					Mean						0.16	0.33
i	2	24.0	7.0	18	0	1	3	9	12	18	0.12	0.23
ii	2	24.0	7.0	18	0	1	4	9	13	18	0.12	0.26
iii	2	25.6	7.1	17	0	2	5	8	12	16	0.12	0.28
iv	2	25.6	7.1	17	1	2	4	6	14	17	0.08	0.30
					Mean						0.11	0.26
i	3	24.0	7.1	18	0	1	2	7	10	19	0.10	0.20
ii	3	24.0	7.1	18	0	0	3	9	12	16	0.11	0.21
iii	3	25.6	7.1	18	0	1	3	10	11	16	0.11	0.22
iv	3	25.6	7.1	18	0	1	3	7	14	16	0.11	0.23
					Mean						0.11	0.22

* (M)

Table XVI (1-9) (continued)

(6) Effect of Sodium Nitrite NaNO_2 Against Hookworm Larvae at 24.5-25.0 °C and pH 8.7-8.9

Exper. No.	Time Interval (hour)	Mean Temp. °C	Mean pH	Control Survivors	Survivors at Concentration (M)					Endpoints*	
					0.500	0.325	0.210	0.120	0.080	0.050	LD ₅₀ LD ₉₀
i	1	24.5	8.8	10	1	2	5	7	8	9	0.18 0.38
ii	1	24.5	8.8	10	1	1	3	6	7	10	0.15 0.31
iii	1	25.0	8.8	14	0	2	8	9	11	14	0.11 0.32
iv	1	25.0	8.8	14	1	2	6	7	11	15	0.10 0.32
Mean										0.14	0.33
i	2	24.5	8.8	10	1	1	2	5	8	9	0.13 0.30
ii	2	24.5	8.8	10	0	1	3	4	9	10	0.13 0.28
iii	2	25.0	8.8	12	0	1	6	9	10	14	0.18 0.31
iv	2	25.0	8.8	12	0	1	3	5	8	14	0.11 0.22
Mean										0.14	0.28
i	3	24.5	8.8	10	0	0	1	2	7	11	0.10 0.18
ii	3	24.5	8.8	10	0	1	2	4	6	8	0.10 0.25
iii	3	25.0	8.8	12	0	1	4	7	10	11	0.16 0.28
iv	3	25.0	8.8	12	0	1	3	6	8	15	0.12 0.27
Mean										0.12	0.25

* (M)

Table XVI (1-9) (continued)

(7) Effect of Sodium Nitrite, NaNO_2 , Against Hookworm Larvae at 36.0-36.5 C and pH 6.1-6.4

Exper. No.	Time Interval (hour)	Mean Temp. °C	Mean pH	Control Survivors	Survivors at Concentration (M)						Endpoints*	
					0.500	0.325	0.210	0.120	0.080	0.050	LD ₅₀	LD ₉₀
i	1	36.0	6.2	10	1	4	7	8	9		0.26	0.46
ii	1	36.0	6.2	10	1	2	6	8	9		0.23	0.42
iii	1	36.5	6.2	13	1	3	7	10	12		0.22	0.41
iv	1	36.5	6.3	13	1	2	6	9	12		0.19	0.36
					Mean						0.23	0.41
i	2	36.0	6.2	9	1	3	6	8	9		0.26	0.46
ii	2	36.0	6.2	9	1	2	4	7	9		0.21	0.43
iii	2	36.5	6.2	12	1	3	6	8	10		0.20	0.41
iv	2	36.5	6.3	12	1	2	6	8	13		0.20	0.38
					Mean						0.22	0.42
i	3	36.0	6.3	8	0	2	4	5	7		0.18	0.36
ii	3	36.0	6.2	8	0	2	3	4	8		0.17	0.35
iii	3	36.5	6.3	11	0	1	6	8	11		0.20	0.31
iv	3	36.5	6.3	11	0	1	6	6	11		0.18	0.31
					Mean						0.18	0.33

*(M)

Table XVI(1-9) (continued)
 (8) Effect of Sodium Nitrite, NaNO_2 , Against Hookworm Larvae at 36.0-36.5°C and pH 6.8-7.2

Exper. No.	Time Inter-val (hour)	Mean Temp. °C	Mean pH	Control Survivors	Survivors at Concentration (M)						Endpoints*	
					0.500	0.325	0.210	0.120	0.080	0.050	LD ₅₀	LD ₉₀
					Mean						0.26 0.46	
i	1	36.5	7.0	11	0	6	7	8	10		0.26	0.44
ii	1	36.5	7.0	11	1	7	7	8	10		0.28	0.47
iii	1	36.0	7.0	13	3	4	7	9	15		0.24	0.49
iv	1	36.0	7.0	13	1	4	7	9	15		0.24	0.45
					Mean						0.26 0.46	
i	2	36.5	7.0	10	0	3	6	7	8		0.21	0.38
ii	2	36.5	6.8	11	1	3	6	8	8		0.23	0.44
iii	2	36.0	7.0	13	1	2	7	8	11		0.18	0.35
iv	2	36.0	7.0	13	1	2	7	8	11		0.18	0.35
					Mean						0.20 0.38	
i	3	36.5	7.0	9	0	3	5	7	8		0.22	0.41
ii	3	36.5	6.8	9	0	3	5	7	8		0.22	0.41
iii	3	36.0	7.0	13	0	2	4	5	11		0.28	0.30
iv	3	36.0	7.0	13	0	2	4	5	11		0.28	0.30
					Mean						0.25 0.36	

* (M)

Table XVI (1-9) (continued)
 (9) Effect of Sodium Nitrite, NaNO_2 , Against Hookworm Larvae at 36.0-36.5 C and pH 8.7-8.9

Exper. No.	Time Inter-val (hours)	Mean Temp. °C	Mean pH	Control Survivors	Survivors at Concentration (M)						Endpoints*	
					0.500	0.325	0.210	0.120	0.080	0.050	LD ₅₀	LD ₉₀
i	1	36.0	8.8	13	4	7	8	9	10		0.26	0.52
ii	1	36.0	8.9	13	1	6	7	8	11		0.22	0.45
iii	1	36.5	8.8	13	5	6	8	7	9		0.24	0.53
iv	1	36.5	8.8	13	3	6	7	7	10		0.22	0.40
					Mean						0.24	0.48
i	2	36.0	8.8	13	1	6	7	8	8		0.23	0.45
ii	2	36.0	9.0	13	1	4	6	8	9		0.18	0.41
iii	2	36.5	8.8	14	3	6	6	8	9		0.18	0.47
iv	2	36.5	8.8	14	3	6	6	6	8		0.16	0.46
					Mean						0.18	0.45
i	3	36.0	8.8	14	0	4	5	5	8		0.12	0.33
ii	3	36.0	8.8	14	0	3	5	6	7		0.12	0.31
iii	3	36.5	8.8	15	2	4	5	7	9		0.14	0.40
iv	3	36.5	8.8	15	2	5	5	6	9		0.14	0.42
					Mean						0.13	0.36

*(M)

TABLE XVII

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF NaNO_2^* ON HOOKWORM LARVAE

Temperature/ pH Ranges	Endpoints (M)	
	LD ₅₀	LD ₉₀
<u>14.0 - 15.0 C</u>		
6.0 - 6.2	0.16	0.35
6.9 - 7.2	0.12	0.22
8.8 - 9.0	0.18	0.41
<u>24.0 - 25.6 C</u>		
6.0 - 6.6	0.13	0.25
7.0 - 7.2	0.11	0.22
8.7 - 8.9	0.12	0.25
<u>36.0 - 36.5 C</u>		
6.1 - 6.4	0.18	0.33
6.8 - 7.2	0.25	0.36
8.7 - 8.9	0.13	0.36

*Based on three-hour exposure period.

did not seem to have any influence on the killing effect of NaNO_2 . However, they show that at high exposure temperatures (36.0 - 36.5 C) more of the chemical was needed to give the same effect as at 14.0 - 15.0 C and 24.0 - 25.0 C ranges. It was observed in vitro that the larvae which had survived after three hours of exposure at any concentration did not show any weakening and that they were just as active as those in the controls.

The results of the effect of NaNO_2 on Strongyloides larvae at various temperatures and pH values are summarized in Table XVIII. These data show that temperature had no influence on the effect of NaNO_2 . But at pH of 8.8 to 9.1 the amount of the chemical required to achieve the LD_{50} and LD_{90} was nearly double that required at acid and neutral pH values. The data further show that the concentration of NaNO_2 required to kill hookworm larvae (Table XVI (1-9)) was almost two to three times that needed to give fifty and ninety per cent endpoints for Strongyloides larvae (Table XVIII). The surviving Strongyloides larvae were also seen to be as active as those of Ancylostoma mentioned above.

2. The Effect of Iodine on the Survival of Ancylostoma and Strongyloides Larvae

The larvae of Ancylostoma and Strongyloides were exposed to iodine solution at 15 C, 25 C and 37 C and pH values of 6.0, 7.0 and 9.0 for one, two and three hours exposure. An estimated number of larvae of each kind in suspension were put in five pairs of vials containing

TABLE XVIII

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF NaNO_2^* ON STRONGYLOIDES LARVAE

Temperature/ pH Ranges	Endpoints (M)	
	LD ₅₀	LD ₉₀
<u>13.5 - 15.5 C</u>		
6.0 - 6.2	0.04	0.06
7.0 - 7.2	0.04	0.06
8.8 - 9.1	0.08	0.12
<u>23.0 - 24.5 C</u>		
6.0 - 6.1	0.04	0.05
7.0 - 7.1	0.04	0.06
8.8 - 9.1	0.08	0.12
<u>35.0 - 37.0 C</u>		
5.9 - 6.1	0.04	0.05
6.9 - 7.2	0.05	0.06
8.8 - 9.1	0.08	0.13

*Based on three-hour exposure period.

iodine solution.

The concentration of iodine in the vials covered a range of 8.0×10^{-5} M to 3.3×10^{-4} M for Ancylostoma larvae and 9.0×10^{-5} M to 3.8×10^{-4} M for Strongyloides larvae based on the results of preliminary tests. Potassium iodide solution replaced the iodine in the control samples. The rest of the procedure was like that described above for sodium nitrite against hookworm and Strongyloides larvae. The results of the experiments appear in Tables XIX and XX for Ancylostoma and Strongyloides respectively.

Data in both Tables XIX and XX show that pH had no influence on the activity of iodine on hookworm and Strongyloides larvae. On the other hand they show that the temperature of 35.5 to 37.5 C had some influence, in that relatively stronger solutions were required to give the same effect as at lower temperature ranges, 13.5 C and 26.0 C. The data further indicate that nearly similar concentrations of iodine were necessary for both hookworm and Strongyloides larvae in order to achieve the LD₅₀ and LD₉₀.

It was observed that the action of iodine on either of the larvae was very fast, killing most of the larvae in higher concentrations in less than two hours of exposure.

3. The Effect of Carbon Disulfide on the Larvae of Ancylostoma and Strongyloides

The test samples were prepared as already described

TABLE XIX

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF IODINE* ON ANCYLOSTOMA LARVAE

Temperature/ pH Ranges	Endpoints x 10 ⁻⁴ M	
	LD ₅₀	LD ₉₀
<u>13.5 - 15.5 C</u>		
5.9 - 6.1	1.4	1.8
7.0 - 7.2	1.5	2.3
8.8 - 9.1	1.3	2.1
<u>23.5 - 25.0 C</u>		
6.1 - 6.3	1.6	2.4
7.0 - 7.2	1.4	2.3
8.7 - 9.1	1.6	2.5
<u>35.5 - 37.5 C</u>		
5.9 - 6.2	1.9	3.1
6.8 - 7.1	1.9	2.7
8.8 - 9.1	1.6	2.5

*Based on three-hour exposure period

TABLE XX

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF IODINE* ON STRONGYLOIDES LARVAE

Temperature/ pH Ranges	Endpoints x 10 ⁻⁴ M	
	LD ₅₀	LD ₉₀
<u>13.5 - 15.2 C</u>		
6.0 - 6.1	1.3	1.8
6.8 - 7.1	1.4	2.4
8.8 - 9.1	1.6	2.1
<u>23.5 - 26.0 C</u>		
5.9 - 6.1	1.3	2.1
6.9 - 7.1	1.5	2.4
8.8 - 9.0	1.4	2.3
<u>35.5 - 37.0 C</u>		
5.9 - 6.1	2.1	3.0
6.8 - 7.2	1.8	2.6
8.8 - 9.0	2.0	3.2

*Based on three-hour exposure period

in the procedure for NaNO_2 and I_2 . The dilution ranges of CS_2 used here were 1.6×10^{-3} M to 5.0×10^{-3} M for hookworm larvae and 9.0×10^{-4} M to 5.0×10^{-3} M for *Strongyloides* larvae. The control samples contained similar amounts of ethyl alcohol (95%) without the CS_2 . The samples were incubated at the desired temperatures for one, two and three hours. The results of the tests are shown in Tables XXI for hookworm and XXII for *Strongyloides* larvae.

The data in Table XXI show that at low temperature (14.0 - 16.0 C) and high pH values 8.8 - 9.2, slightly more CS_2 was required to kill the hookworm larvae than at higher temperatures 24.0 - 37.8 C and lower pH values 5.9 - 7.2. On the other hand, the data in Table XXII concerning the effect of CS_2 on *Strongyloides* larvae show that temperature had little or no effect, but they show a slight influence of pH between 8.7 and 9.3. At this pH range higher concentrations of CS_2 were required to reach the LD_{50} and LD_{90} in most conditions except for the LD_{50} at 14.0 C - 16.0 C.

4. The Effect of Thiabendazole on the Larvae of Ancylostoma and Strongyloides

Preliminary tests were conducted with thiabendazole solution ranging in strength from 0.001 M to one molar on the filariform larvae of hookworms and *Strongyloides*. The drug was found not to be at all lethal to the larvae. The resistance of the filariform larvae to thiabendazole led to an attempt to find out how the developing larval

TABLE XXI

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF CS₂* ON HOOKWORM LARVAE

Temperature/ pH Ranges	Endpoints x 10 ⁻³ M	
	LD ₅₀	LD ₉₀
<u>14.0 - 16.0 C</u>		
5.9 - 6.2	3.4	4.1
6.9 - 7.2	3.7	4.6
8.8 - 9.2	3.8	4.7
<u>24.0 - 25.5 C</u>		
6.0 - 6.2	2.0	3.1
6.9 - 7.2	2.1	3.5
8.8 - 9.2	2.8	4.5
<u>36.0 - 37.8 C</u>		
5.9 - 6.2	2.3	3.6
6.9 - 7.2	2.8	4.2
8.8 - 9.1	3.0	4.6

*Based on three-hour exposure period

TABLE XXII

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF CS₂* ON STRONGYLOIDES LARVAE

Temperature/ pH Ranges	Endpoints x 10 ⁻³ M	
	LD ₅₀	LD ₉₀
<u>14.0 - 16.0 C</u>		
5.8 - 6.1	2.0	3.1
6.8 - 7.1	2.1	3.6
8.7 - 9.3	2.0	4.0
<u>23.0 - 25.0 C</u>		
5.8 - 6.1	2.2	3.3
6.9 - 7.1	2.2	3.3
9.0 - 9.1	2.5	3.6
<u>36.0 - 37.5 C</u>		
5.7 - 6.1	1.8	2.6
6.7 - 7.1	2.1	3.2
8.7 - 9.1	2.3	3.3

*Based on three-hour exposure period

stages responded at various periods of growth. Previous experiments on eggs had indicated that they were susceptible to thiabendazole at a concentration of 5.0×10^{-6} M. Therefore, freshly passed dog stools were cultured in charcoal and incubated at 28 C. A sample of the culture was subjected to the Baermann method in order to recover the developing larvae. The larvae were exposed to thiabendazole at concentrations of 5.0×10^{-3} M to 5.0×10^{-5} M. This concentration range was arbitrarily selected on the basis of preliminary observation that it generally took a smaller amount of each of the chemicals to kill larvae than it did the eggs.

The tests were carried out at room temperature (25 C) and neutral pH (7.0), and the procedure was as described above in the section on materials and methods. The results of the experiment are shown in Table XXIII. The data show that hookworm larvae less than three days old were readily susceptible to the action of thiabendazole, and that after the third day larval response decreased, so that by the third and fourth day, thiabendazole effect was not recognizable.

The Effects of Chemical Agents on the Survival of Parasitic Eggs

The following experiments were concerned with activity of chemical agents on the survival and development of the eggs of *Ascaris* and *Trichuris* in aqueous media and of *Ancylostoma* in feces.

TABLE XXIII

SUSCEPTIBILITY OF DEVELOPING HOOKWORM LARVAE TO THIABENDAZOLE*

Day of Incubation	Exper. No.	Experimental Dose (M)						Control Larvae				
		5.0 x 10 ⁻⁵			5.0x10 ⁻³			Unexposed	Dead	% Mor- tality	% Effecti- veness**	
		Larvae Tested	Dead	% Mor- tality	Larvae Tested	Dead	% Mor- tality					
1		Larvae still in eggshell										
2	i	31	26	83.8	10	8	80.0	31	1	3.2	80.6	
	ii	14	13	92.8	10	10	100.0	14	1	7.2	85.6	
	iii	10	8	80.0	--			10	0	0	80.0	
3	i	35	4	11.4	16	5	31.2	35	1	2.8	8.6	
	ii	17	3	17.6	18	4	22.2	15	1	6.6	11.0	
	iii	16	2	12.5	--	--	--	--	--	--	--	
4	i	34	1	2.9	12	0	0	34	1	2.9	0	
	ii	18	1	5.6	18	3	16.6	18	1	5.6	0	
	iii	18	1	5.6	12	1	4.5	--	--	--	--	
5	i	12	1	8.3	12	2	16.6	12	1	8.3	0	
	ii	11	1	9.0	11	0	0	11	0	0	9.0	

*For 12-hour exposure periods at room temperature (25 C) and neutral pH (7.0).

**Controls were based on test at concentration of 5.0x10⁻⁵ M.

1. The Effect of Sodium Nitrite on the Survival and Development of Ancylostoma, Trichuris and Ascaris Eggs at Various Temperatures and pH Values

Test samples containing NaNO_2 at a concentration range of 1.2×10^{-3} M to 2.1×10^{-3} M for hookworm eggs and 0.50 M to 1.7 M for both Ascaris and Trichuris eggs were buffered to desired pH values of, (6, 7 or 9) and then incubated at selected temperatures (15 C, 25 C or 37 C). The hookworm and Trichuris eggs samples were treated for 3, 6 or 12 hours and those of Ascaris for 36, 48 or 60 hours. Distilled water replaced NaNO_2 solution in the control samples. At the end of the exposure periods the samples were prepared for incubation as described in the section on materials and methods.

The larvae in each hookworm culture were counted and recorded assuming that they represented the eggs that had resisted the action of NaNO_2 . Motile embryos in Ascaris and Trichuris eggs were counted and recorded as surviving eggs. Calculated LD_{50} and LD_{90} given in Tables XXIV for hookworm, XXV for Ascaris, and XXVI for Trichuris eggs show that concentrations of NaNO_2 lethal to the eggs of Ascaris and Trichuris were nearly three times higher than those necessary for hookworm eggs. At high pH values a slightly higher concentration of NaNO_2 was required against hookworm as compared to that in feces at lower pH values; this behavior was not evident in aqueous suspensions of Ascaris and Trichuris eggs. It was observed that at lower temperatures (15 C) larger amounts of NaNO_2 were required to kill the eggs

TABLE XXIV

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF NaNO_2^* ON HOOKWORM EGGS

Temperature/ pH Ranges	Endpoints x 10^{-3} M	
	LD ₅₀	LD ₉₀
<u>13.0 - 15.5 C</u>		
5.9 - 6.2	1.6	1.9
7.0 - 7.2	1.5	2.0
8.7 - 9.1	1.6	2.0
<u>24.5 - 26.0 C</u>		
5.8 - 6.0	1.2	1.8
6.8 - 7.0	1.2	1.8
8.8 - 9.0	1.6	2.0
<u>36.2 - 37.5 C</u>		
5.9 - 6.1	1.0	1.6
6.8 - 7.1	1.1	1.6
8.9 - 9.2	1.7	2.0

*Based on 12-hour exposure period.

TABLE XXV

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF NaNO_2^* ON ASCARIS EGGS

Temperature/ pH Ranges	Endpoints (M)	
	LD ₅₀	LD ₉₀
<u>14.8 - 16.0 C</u>		
5.8 - 6.0	1.3	1.6
7.0 - 7.4	1.3	1.6
8.8 - 9.1	1.4	1.6
<u>24.5 - 25.0 C</u>		
5.8 - 6.4	1.2	1.6
6.9 - 7.2	1.3	1.6
8.6 - 9.0	1.3	1.6
<u>35.8 - 37.2 C</u>		
5.8 - 6.2	0.8	1.3
6.8 - 7.1	0.8	1.3
8.8 - 9.2	0.9	1.5

*Based on 60-hour exposure periods.

TABLE XXVI
THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF NaNO_2^* ON TRICHURIS EGGS

Temperature/ pH Ranges	Endpoints, (M)	
	LD ₅₀	LD ₉₀
<u>14.8 - 16.0 C</u>		
5.8 - 6.1	1.2	1.6
6.8 - 7.1	1.3	1.6
8.6 - 9.0	1.3	1.6
<u>24.5 - 25.0 C</u>		
6.0 - 6.3	0.8	1.3
6.9 - 7.2	1.1	1.6
8.7 - 9.0	0.8	1.3
<u>35.8 - 36.8 C</u>		
5.9 - 6.2	0.9	1.4
6.8 - 7.2	0.8	1.3
8.9 - 9.2	0.8	1.3

*Based on 12-hour exposure period.

than at elevated temperatures (25 - 37 C).

The concentration of NaNO_2 prepared for use in these experiments was found to be very irritating to the skin on contact. *Ascaris* and *Trichuris* eggs were susceptible to nearly similar NaNO_2 concentrations; however, it required 36-60 hours of exposure for *Ascaris* eggs as compared to 3-12 hours for *Trichuris* eggs.

2. The Effect of Iodine on the Survival and Development of Ancylostoma, Ascaris and Trichuris Eggs at Various Temperatures and pH Values

Samples of eggs of hookworm in feces and those of *Ascaris* and *Trichuris* in aqueous media were prepared in centrifuge tubes and exposed to iodine solution in the same way described above for sodium nitrite.

The concentration ranges of iodine used were 1.8×10^{-4} M to 7.5×10^{-4} M for *Ascaris*, 2.1×10^{-4} M to 8.7×10^{-4} M for *Trichuris*, and 5.0×10^{-3} M to 1.1×10^{-2} M for hookworm eggs. The exposure periods were one, two and three hours for hookworm and *Trichuris* eggs and for 6, 12 and 24 hours for *Ascaris* eggs. Potassium iodide solution was used in control samples. The results expressed in LD_{50} and LD_{90} at respective temperature and pH values are shown in Tables XXVII for hookworm, XXVIII for *Ascaris* and XXIX for *Trichuris*.

The data in Tables XXVII-XXIX show first, that the concentration of iodine required to kill hookworm eggs in feces was about ten times stronger than that needed against *Ascaris* and *Trichuris* eggs; the data also show that the concentration of iodine needed increased with

TABLE XXVII

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF I_2^* ON HOOKWORM EGGS

Temperature/ pH Ranges	Endpoints x 10^{-3} M	
	LD ₅₀	LD ₉₀
<u>14.5 - 16.0 C</u>		
5.7 - 6.0	5.6	7.4
7.0 - 7.3	6.1	7.7
8.8 - 9.2	6.4	8.1
<u>24.0 - 26.4 C</u>		
6.0 - 6.4	5.9	7.6
6.7 - 7.1	6.5	8.1
8.6 - 8.9	7.0	8.6
<u>35.5 - 38.0 C</u>		
5.8 - 6.1	7.6	9.3
6.9 - 7.2	7.8	9.7
8.8 - 9.0	8.0	10.0

*Based on three-hour exposure period.

TABLE XXVIII

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF I_2^* ON ASCARIS EGGS

Temperature/ pH Ranges	Endpoints x 10^{-4} M	
	LD ₅₀	LD ₉₀
<u>15.0 - 16.0 C</u>		
6.0 - 6.3	3.2	5.2
6.8 - 7.0	3.2	5.3
8.8 - 9.1	3.1	5.4
<u>25.0 - 26.2 C</u>		
5.7 - 6.0	3.0	5.3
6.9 - 7.1	3.8	6.2
8.9 - 9.2	3.8	6.5
<u>36.5 - 37.5 C</u>		
5.7 - 6.0	4.0	6.6
6.9 - 7.2	4.3	6.8
8.8 - 9.1	4.6	6.8

*Based on 24-hour exposure period.

TABLE XXIX

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF I_2^* ON TRICHURIS EGGS

Temperature/ pH Ranges	Endpoints x 10^{-4} M	
	LD ₅₀	LD ₉₀
<u>14.0 - 15.5 C</u>		
5.8 - 6.0	3.2	5.4
6.8 - 7.3	3.2	5.0
8.7 - 9.0	3.7	5.4
<u>24.5 - 26.0 C</u>		
6.0 - 6.4	3.0	5.2
6.6 - 6.9	3.2	4.6
8.9 - 9.2	3.2	4.8
<u>35.8 - 37.5 C</u>		
5.8 - 6.0	4.6	6.8
6.7 - 7.0	5.1	7.6
8.8 - 9.0	5.6	7.9

*Based on 3-hour exposure period.

increasing pH and temperature in nearly all the experiments.

3. The Effect of Thiabendazole on the Survival and Development of Ancylostoma, Ascaris and Trichuris Eggs at Various Temperatures and pH Values

The same procedure as described above was followed in the experiments below. Ancylostoma eggs in feces were exposed to thiabendazole at concentrations ranging from 1.5×10^{-6} M to 7.0×10^{-6} M; Ascaris and Trichuris eggs were exposed in aqueous media to thiabendazole at concentrations of 1.0×10^{-4} M to 3.2×10^{-4} M for Ascaris and 5.0×10^{-6} M to 1.6×10^{-4} M for Trichuris. The exposure periods were three, six and twelve hours for hookworm eggs, and 12, 24 and 36 hours for Ascaris and Trichuris eggs. After the removal of the chemical, hookworm samples were incubated at 28 C and those of Ascaris and Trichuris at 30 C. The results are shown in Tables XXX for hookworm eggs, XXXI for Ascaris eggs, and XXXII for Trichuris eggs.

The results of the experiments concerning the effect of thiabendazole on Ancylostoma, Ascaris and Trichuris eggs (Tables XXX, XXXI and XXXII) show that the lethal doses of the chemical decreased with rise in temperature, and that the pH of the media did not have a marked effect on the activity of the chemical. The data also show that it took about 100 times less chemical and shorter exposure period to kill hookworm eggs in feces than it did Ascaris and Trichuris eggs in aqueous suspension.

TABLE XXX

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF THIABENDAZOLE* ON HOOKWORM EGGS

Temperature/ pH Ranges	Endpoints x 10 ⁻⁶ M	
	LD ₅₀	LD ₉₀
<u>14.8 - 16.0 C</u>		
5.7 - 6.3	5.1	6.7
6.8 - 7.0	5.0	6.6
8.9 - 9.1	4.8	6.1
<u>24.5 - 26.0 C</u>		
5.9 - 6.2	3.5	5.4
6.9 - 7.2	3.9	5.5
8.7 - 9.0	3.6	5.4
<u>36.0 - 37.0 C</u>		
6.0 - 6.3	2.7	4.5
6.9 - 7.2	2.7	4.5
8.9 - 9.2	2.6	4.4

*Based on 12-hour exposure period.

TABLE XXXI

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF THIABENDAZOLE* ON ASCARIS EGGS

Temperature/ pH Ranges	Endpoints x 10 ⁻⁴ M	
	LD ₅₀	LD ₉₀
<u>14.4 - 15.2 C</u>		
5.8 - 6.1	2.2	3.0
7.0 - 7.3	2.5	3.0
8.6 - 9.1	2.5	3.0
<u>24.8 - 26.0 C</u>		
5.8 - 6.3	1.8	2.8
7.0 - 7.4	2.0	2.9
8.8 - 9.2	2.1	2.7
<u>35.8 - 37.5 C</u>		
6.0 - 6.2	1.9	2.6
6.8 - 7.0	1.7	2.5
8.6 - 9.0	1.8	2.7

*Based on 36-hour exposure period.

TABLE XXXII

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF THIABENDAZOLE* ON TRICHURIS EGGS

Temperature/ pH Ranges	Endpoints x 10 ⁻⁴ M	
	LD ₅₀	LD ₉₀
<u>15.2 - 16.0 C</u>		
5.9 - 6.2	1.2	1.5
6.8 - 7.2	1.2	1.5
8.8 - 9.2	1.2	1.5
<u>25.0 - 26.0 C</u>		
5.9 - 6.3	1.1	1.4
7.0 - 7.4	1.2	1.5
8.7 - 9.0	1.2	1.5
<u>36.2 - 37.2 C</u>		
6.0 - 6.2	0.9	1.4
6.8 - 7.1	0.8	1.3
8.8 - 9.2	1.0	1.5

*Based on 36-hour exposure period.

4. The Effect of Carbon Disulfide on the Survival and Development of Ancylostoma, Ascaris and Trichuris Eggs at Various Temperatures and pH Values

The testing procedure with CS₂ was similar to those already described above for other chemicals. Carbon disulfide was used at concentration ranges of 0.14 M to 0.50 M for hookworm eggs in feces, 0.28 M to 0.87 M for Trichuris eggs, and 0.49 M to 0.87 M for Ascaris eggs in aqueous media. Ethyl alcohol replaced CS₂ solution in control samples because it had been used as a solvent for the latter.

Hookworm samples were exposed for 3, 6 and 12 hours and Ascaris and Trichuris samples for 60-, 72- and 84-hour exposure periods. The results of the experiments are shown in Tables XXXIII for hookworm eggs, XXXIV for Ascaris eggs, and XXXV for Trichuris eggs.

The data in Tables XXXIII - XXXV show that both Ascaris and Trichuris eggs required nearly similar concentrations of CS₂ to give LD₅₀ and LD₉₀ at exposure periods of 84 hours, but more than hookworm required at a shorter exposure period. The data also show that the pH did not have any significant influence, and that it took higher concentrations of CS₂ at lower temperature ranges to reach the endpoints.

It was further observed that it was very difficult to work with CS₂ at 1 M stock solution, because of its toxic nature and loss through evaporation. Working with stock solutions of more than 1 M showed that the effect of CS₂ for which ethyl alcohol had been used as

TABLE XXXIII

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF CS₂* ON HOOKWORM EGGS

Temperature/ pH Ranges	Endpoints (M)	
	LD ₅₀	LD ₉₀
<u>14.8 - 16.0 C</u>		
6.1 - 6.3	0.36	0.46
7.0 - 7.3	0.38	0.48
8.9 - 9.2	0.38	0.48
<u>24.5 - 26.0 C</u>		
6.0 - 6.4	0.24	0.32
6.9 - 7.3	0.23	0.34
9.0 - 9.2	0.35	0.41
<u>35.8 - 37.8 C</u>		
5.9 - 6.2	0.23	0.36
6.8 - 7.1	0.24	0.39
8.9 - 9.3	0.29	0.45

*Based on 12-hour exposure period.

TABLE XXXIV

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF CS₂* ON ASCARIS EGGS

Temperature/ pH Ranges	Endpoints (M)	
	LD ₅₀	LD ₉₀
<u>14.5 - 15.2 C</u>		
6.0 - 6.3	0.66	0.83
6.8 - 7.2	0.65	0.81
8.7 - 9.1	0.68	0.83
<u>24.8 - 25.0 C</u>		
6.0 - 6.2	0.68	0.82
7.0 - 7.2	0.66	0.81
9.0 - 9.2	0.64	0.82
<u>36.5 - 37.0 C</u>		
5.8 - 6.2	0.60	0.80
6.7 - 7.0	0.62	0.78
8.7 - 9.1	0.64	0.82

*Based on 84-hour exposure period.

TABLE XXXV

THE INFLUENCE OF TEMPERATURE AND pH ON THE
EFFECT OF CS₂* ON TRICHURIS EGGS

Temperature/ pH Ranges	Endpoints (M)	
	LD ₅₀	LD ₉₀
<u>14.8 - 15.8 C</u>		
6.0 - 6.2	0.63	0.81
6.8 - 7.2	0.66	0.82
8.7 - 9.1	0.69	0.84
<u>24.5 - 25.0 C</u>		
5.9 - 6.0	0.44	0.73
6.9 - 7.2	0.46	0.75
8.6 - 9.0	0.49	0.78
<u>35.6 - 37.0 C</u>		
5.8 - 6.1	0.44	0.75
7.0 - 7.3	0.50	0.75
8.8 - 9.0	0.49	0.76

*Based on 84-hour exposure period.

a solvent was the same as that of ethyl alcohol alone as judged from control samples.

The overall activity of the chemical agents on the various parasites are given below in Tables XXXVI - XXXIX which show the required concentrations for the 50% endpoints (the ratio of 50% to 90% endpoints was 5:9 in all experiments). Examination of the data in the tables shows that on the whole, greater amounts of NaNO_2 than any other chemical were required for the parasites. The next chemical which was required in large amounts was CS_2 . On the extreme end of the scale was thiabendazole. However, the latter's failure to kill the third stage filariform larvae of hookworms and *Strongyloides*, leaves I_2 as the best of the chemicals with value as an ovicide and larvicide.

In all the experiments concerning the effects of chemicals on parasitic eggs and/or larvae, it was noticed, as might be expected, that in most cases the increase in exposure period resulted in a decrease in the chemical dose necessary to give the 50% and 90% endpoints.

This trend is shown in Figures 3 - 6 based on the effect of NaNO_2 , I_2 , CS_2 , and thiabendazole on hookworm eggs at room temperature (24.5 - 26.0 C) and neutral pH (6.7 - 7.3) at corresponding exposure periods.

It can be seen in Figure 3 that the decrease in the required NaNO_2 concentration was directly proportional to the increase in exposure time. On the other hand, the decrease in the required I_2 , CS_2 and thiabendazole

TABLE XXXVI

COMPARATIVE SUSCEPTIBILITY OF PARASITES TO THE ACTION
OF SODIUM NITRITE: FIFTY PER CENT ENDPOINTS

Temperature/ pH Ranges	Parasite at Concentration (M)				
	Hookworm Eggs** m/lx10 ⁻³	Strongyloides Larvae*	Hookworm Larvae*	Trichuris Eggs**	Ascaris Eggs***
13.0 - 16.0 C					
5.8-6.2	1.6	0.04	0.16	1.2	1.3
6.8-7.4	1.5	0.04	0.12	1.3	1.3
8.6-9.1	1.6	0.08	0.18	1.3	1.4
23.0 - 26.0 C					
5.8-6.6	1.2	0.04	0.13	0.8	1.2
6.8-7.2	1.2	0.04	0.11	1.1	1.3
8.6-9.1	1.6	0.08	0.12	0.8	1.3
35.0 - 37.5 C					
5.8-6.4	1.0	0.04	0.18	0.9	0.8
6.8-7.2	1.1	0.05	0.25	0.8	0.8
8.7-9.2	1.7	0.08	0.13	0.8	0.9

*3-hour exposure.

**12-hour exposure.

***60-hour exposure.

TABLE XXXVII
COMPARATIVE SUSCEPTIBILITY OF PARASITES TO THE ACTION
OF IODINE: FIFTY PER CENT ENDPOINTS

Temperature/ pH Ranges	Parasite at Concentration				
	Ancylostoma Larvae* m/1x10 ⁻⁴	Strongyloides Larvae* m/1x10 ⁻⁴	Ascaris Eggs** m/1x10 ⁻⁴	Trichuris Eggs* m/1x10 ⁻⁴	Ancylostoma Eggs* m/1x10 ⁻³
13.5-16.0 C					
5.7-6.3	1.4	1.3	3.2	3.2	5.6
6.8-7.3	1.5	1.4	3.2	3.2	6.1
8.7-9.2	1.3	1.6	3.1	3.7	6.4
23.4-26.4 C					
5.7-6.4	1.6	1.3	3.0	3.0	5.9
6.6-7.2	1.4	1.5	3.8	3.2	6.5
8.6-9.2	1.6	1.4	3.8	3.2	7.0
35.5-38.0 C					
5.7-6.2	1.9	2.1	4.0	4.6	7.6
6.6-7.2	1.9	1.8	4.3	5.1	7.8
8.8-9.1	1.6	2.0	4.6	5.6	8.0

*3-hour exposure.

**24-hour exposure.

TABLE XXXVIII

COMPARATIVE SUSCEPTIBILITY OF PARASITES TO THE ACTION
OF CARBON DISULFIDE: FIFTY PER CENT ENDPOINTS

Temperature/ pH Ranges	Parasite at Concentration				
	Strongyloides Larvae* m/lx10 ⁻³	Hookworm Larvae* m/lx10 ⁻³	Hookworm Eggs** m/l	Trichuris Eggs*** m/l	Ascaris Eggs*** m/l
<u>14.0-16.0 C</u>					
5.8-6.3	2.0	3.4	0.36	0.63	0.66
6.8-7.3	2.1	3.7	0.38	0.66	0.65
8.7-9.3	2.0	3.8	0.38	0.69	0.68
<u>23.0-26.0 C</u>					
5.8-6.4	2.2	2.0	0.24	0.44	0.68
6.9-7.3	2.2	2.1	0.23	0.46	0.66
8.6-9.2	2.5	2.8	0.35	0.49	0.64
<u>35.6-37.8 C</u>					
5.7-6.2	1.8	2.3	0.23	0.44	0.60
6.7-7.3	2.1	2.8	0.24	0.50	0.62
8.7-9.3	2.3	3.0	0.29	0.49	0.64

*3-hour exposure

**12-hour exposure

***84-hour exposure

TABLE XXXIX
COMPARATIVE SUSCEPTIBILITY OF PARASITES TO THE ACTION
OF THIABENDAZOLE: FIFTY PER CENT ENDPOINTS

Temperature/ pH Ranges	Parasite Eggs at Concentration		
	Hookworm Eggs* m/l x 10 ⁻⁶	Trichuris Eggs** m/l x 10 ⁻⁴	Ascaris Eggs** m/l x 10 ⁻⁴
<u>14.4 - 16.0 C</u>			
5.7 - 6.3	5.1	1.2	2.2
6.8 - 7.3	5.0	1.2	2.5
8.6 - 9.2	4.8	1.2	2.5
<u>24.5 - 26.0 C</u>			
5.8 - 6.3	3.5	1.1	1.8
6.9 - 7.4	3.9	1.2	2.0
8.7 - 9.2	3.6	1.2	2.1
<u>35.8 - 37.5 C</u>			
6.0 - 6.3	2.7	0.91	1.9
6.8 - 7.2	2.7	0.86	1.7
8.6 - 9.2	2.6	1.0	1.8

*12-hour exposure

**36-hour exposure

concentrations was rapid at first and then there was a slight decline in the rate of decrease. In the case of I_2 , an attempt to increase the treatment periods to beyond 36 hours did not markedly improve nor change the I_2 concentrations required.

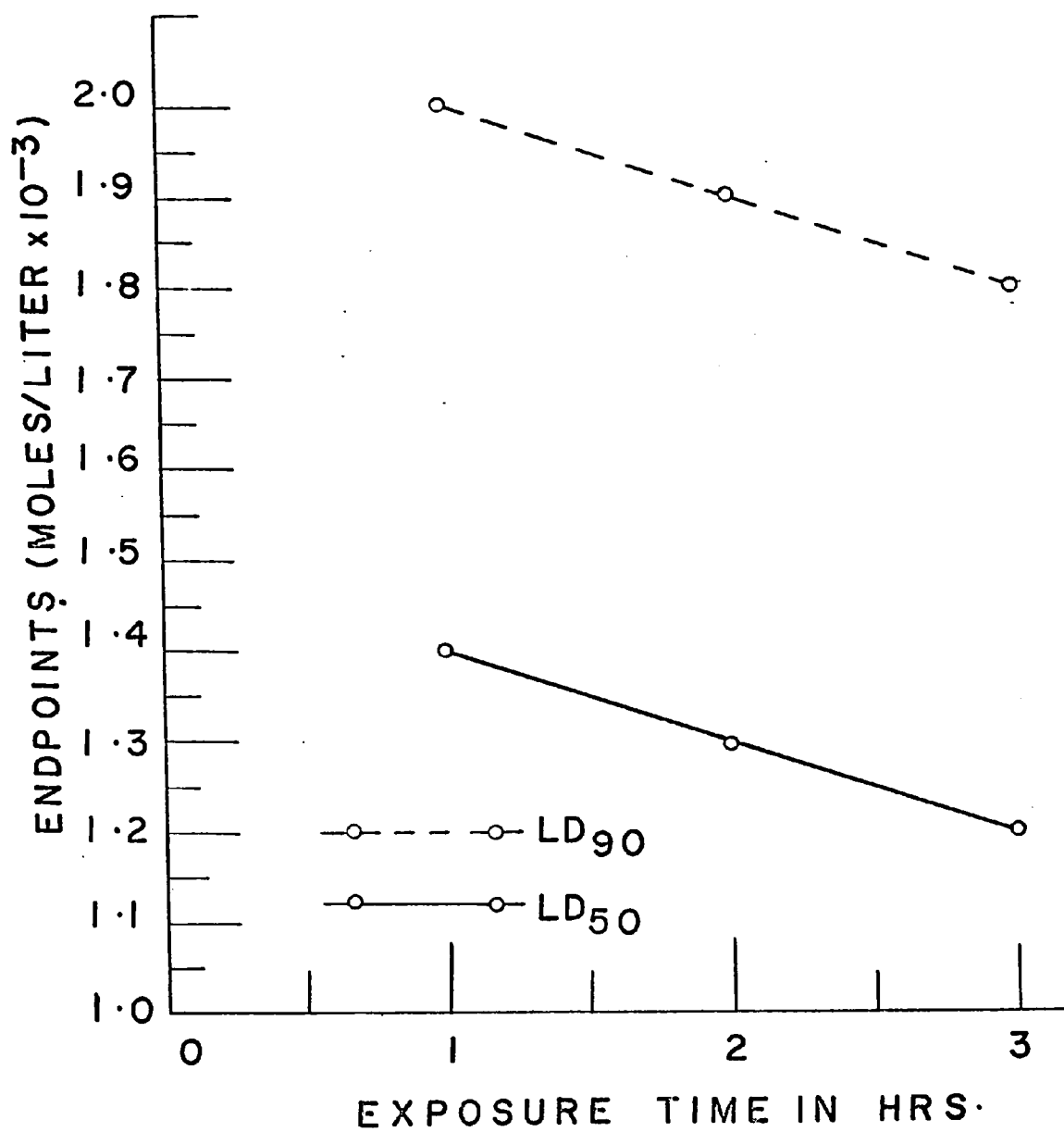


Figure 3.--Time-Dose Concentration Relationship for NaNO_2 in the Treatment of Hookworm Eggs at Room Temperature (24.5 C) and Neutral pH (6.8-7.0).

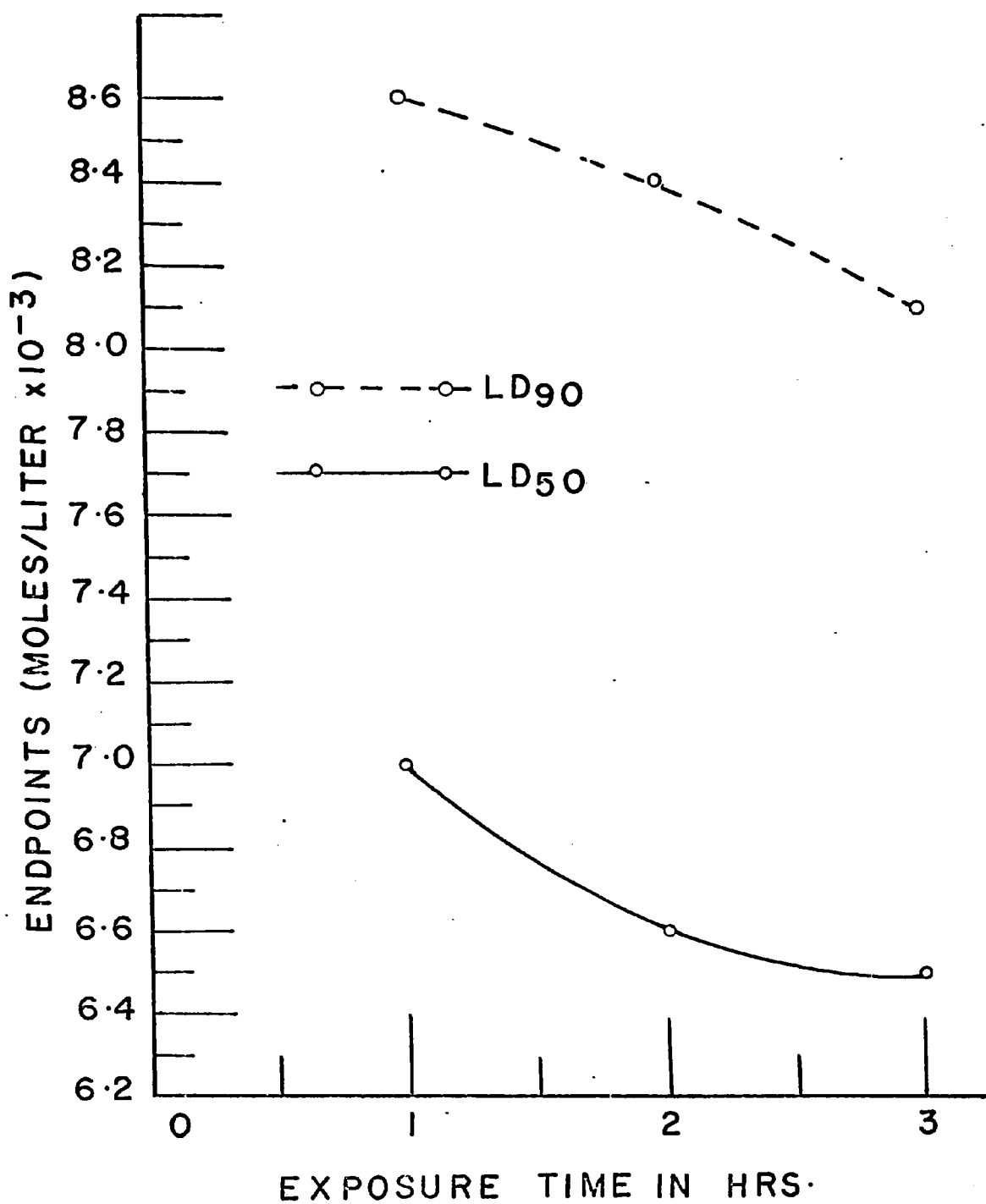


Figure 4.--Time-Dose Concentration Relationship for I_2 in the Treatment of Hookworm Eggs at Room Temperature (25.4 C) and Neutral pH (6.7-7.0).

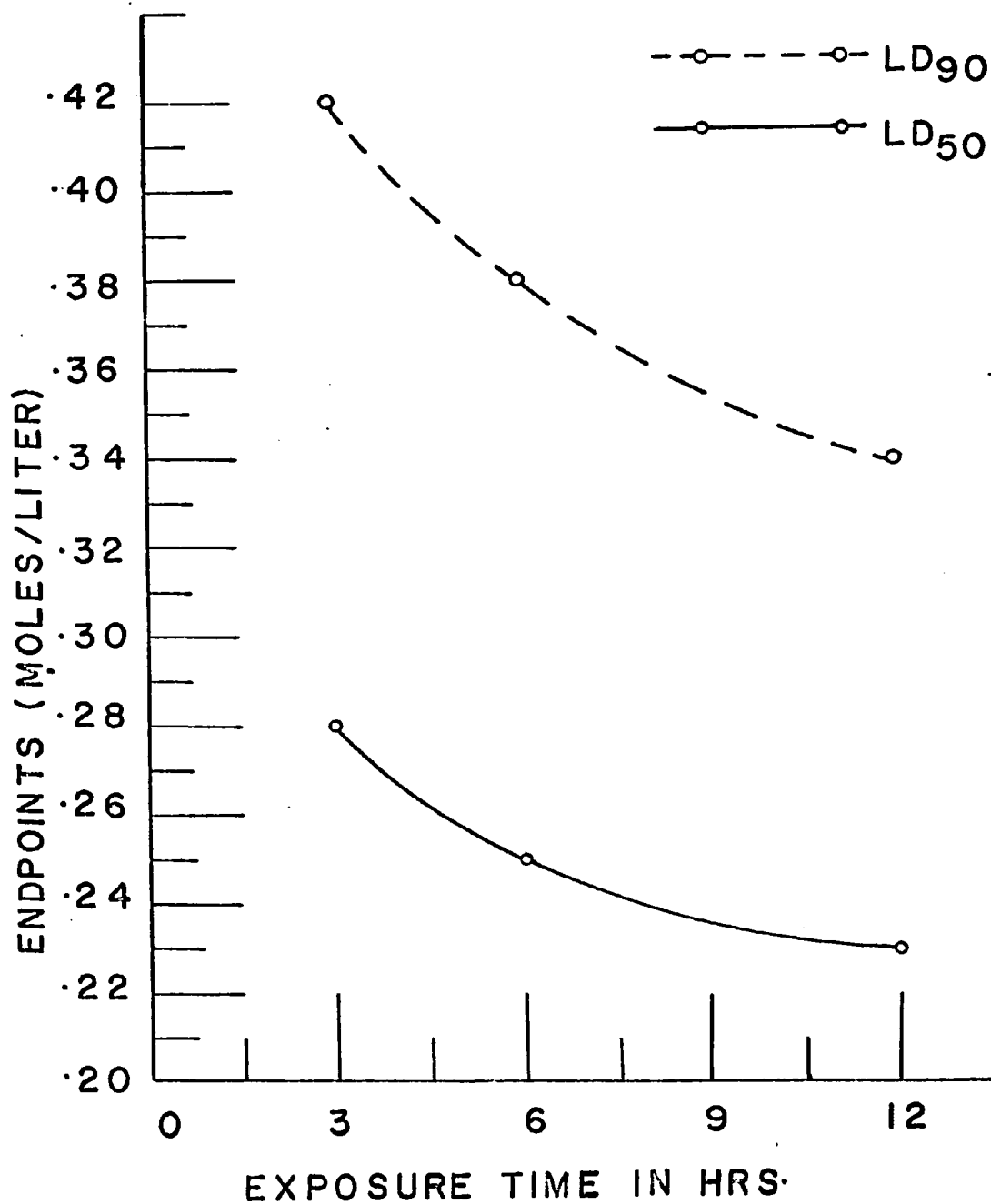


Figure 5.--Time-Dose Concentration Relationship for CS₂ in the Treatment of Hookworm Eggs at Room Temperature (25 C) and Neutral pH (6.9-7.3).

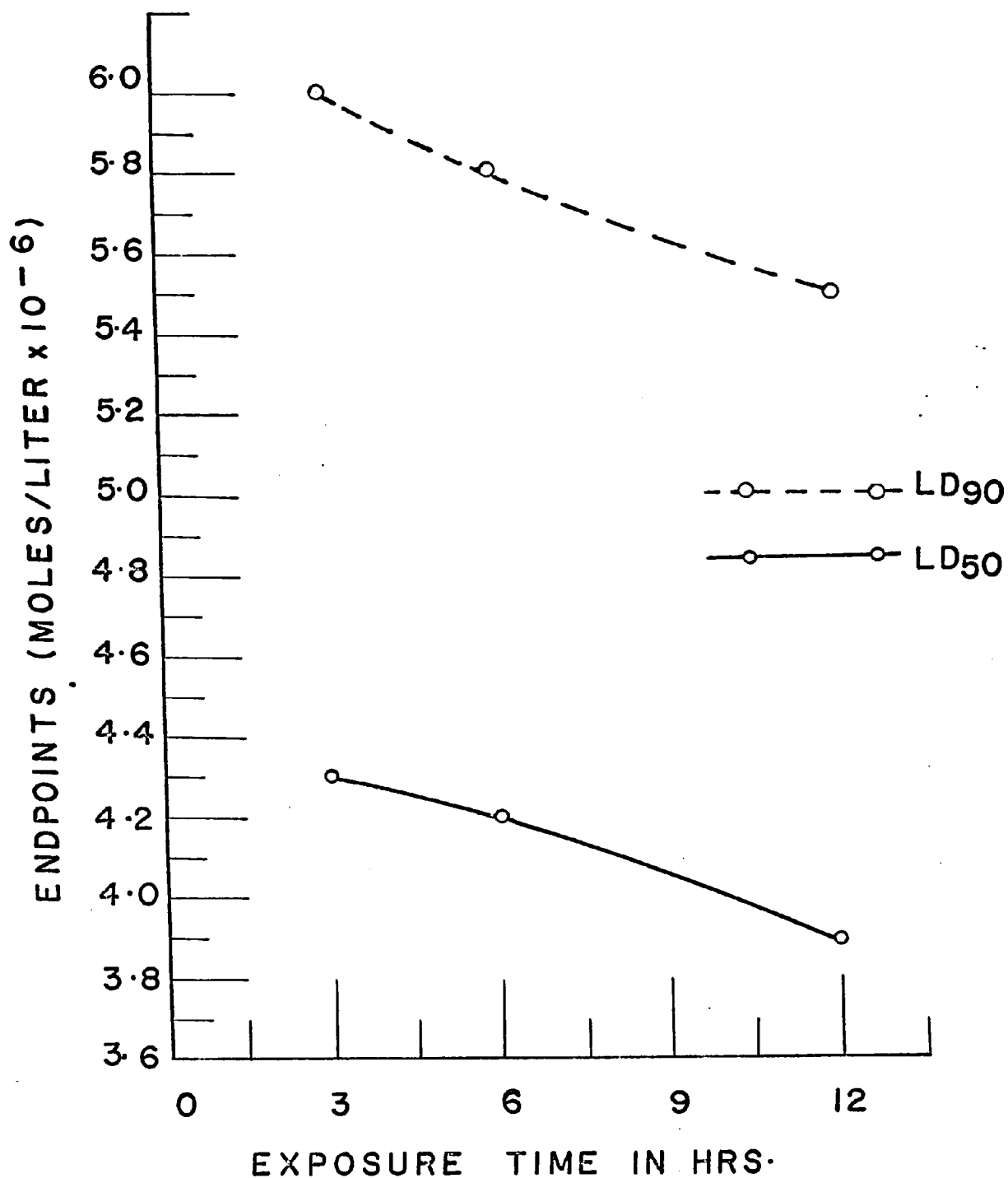


Figure 6.--Time-Dose Concentration Relationship for Thiabendazole in the Treatment of Hockworm Eggs at Room Temperature (26 C) and Neutral pH (6.9-7.0).

DISCUSSION

Because of the complex nature of the life-cycles of the intestinal soil-transmitted nematodes, as well as differences in educational and economic standards throughout the world, the creation of a single, well organized, successful control method has been impossible. Consequently, effective worm control by traditional methods has been limited mainly to those regions with high standards of sanitation.

According to Beaver (1961), a control program should aim at developing natural barriers to the transmission of the nematodes. However, the traditional programs which emphasized sanitation, treatment and wearing of shoes, although they have had some other values, had little lasting effect on the universal prevalence of the soil-transmitted nematodes (Hou et al., 1959).

An effort to sterilize feces containing parasitic eggs and/or larvae as a means of control has recently received special attention, and a large number of chemicals have been studied for ovicidal properties (Kozai, 1960, 1962; Thitasut, 1961; Kutsumi and Komiya, 1965; Chatterjee, et al., 1966). The chemical agents, sodium nitrite, iodine, carbon disulfide and thiabendazole, because of their superior ovicidal qualities, have been selected for further investigation in the present study.

Experiments with these chemical agents aimed at revealing their lethal capacities, stability in solution,

and the influence of temperature and pH on their activity were conducted using the eggs and/or larvae of Ancylostoma caninum, Strongyloides fülleborni, Ascaris lumbricoides var. suis and Trichuris vulpis. In addition to the criteria given above, certain information pertaining to the practical use of these chemical agents was needed. Therefore, throughout this investigation questions that were aimed at revealing the value and feasibility of a given chemical as an ovicide were emphasized. As briefly described in the introductory section, in order that a chemical agent be considered a satisfactory ovicide, it should be effective against the parasites in question at low concentrations. The chemical should be of such a nature that variations in the temperature and pH of the medium do not easily influence its action and also that the presence of organic matter, for instance, feces, does not inhibit or reduce the ovicidal activity of the agent to such an extent that it can no longer kill the parasites. The chemical ought to be easily available at low prices so that it permits widespread use. While the chemical should have a fast killing ability, it should not have harmful effects to the user, his domestic animals, plant crops or the soil; in fact it should be able, if possible, to contribute to the fertility of the soil rather than destroy it; furthermore, it should not damage the fertilizing qualities of feces. Last, but not least, the chemical compound should be easy to prepare, handle and apply under field conditions. Based upon all these factors, it appears

possible to analyze and evaluate the chemical agents as to their ovicidal and larvicidal values.

Preliminary findings on the effects of temperature and pH on the survival of the test organisms in the absence of the chemicals showed that temperature 8 C - 37 C and the pH range 5.8 - 9.0 had negligible lethal effects. Therefore, subsequent results obtained from exposing organisms to the chemical agents were assumed to be independent of other environmental and physical conditions. The only exception was the 8 C temperature which was found to inhibit the development and hatching of the hookworm eggs. The unexpected faster rate of *Strongyloides* growth at 8 C than at other temperatures might be attributed to the fact that the eggs were at different development stages when collected so that larval development was not regular, since some of the eggs might have been fully developed when cultured. The latter probably gave rise to the apparent rapid growth.

The possibility of using sodium nitrite as an ovicide was first recognized by Nagano (1952). This author also reported that he had observed NaNO_2 to be ineffective against *Ascaris* eggs in media with high pH values. This observation was confirmed by Kozai (1960, 1961) whose results based on experiments with *Ascaris* and hookworm eggs in feces indicated that eggs in feces where pH had been lowered by adding calcium superphosphate were killed, but those in feces with high pH (6.9-8.8) were not.

The relative susceptibility of the various parasites

is indicated in Table XXXVI. The data show that the hookworm eggs were the most susceptible to NaNO_2 . Lethal doses in terms of LD_{50} and LD_{90} ranged from 1.04 to 1.70×10^{-3} M and 1.61 to 2.04×10^{-3} M at between 13.0 - 37.5 C treated for 12 hours. On the extreme end of the susceptibility scale were the Ascaris eggs which were susceptible only to doses in the range of 0.84 M to 1.38 M (LD_{50}) and 1.28 M to 1.66 M (LD_{90}) at a similar temperature and 60 hours of treatment.

The results obtained from treating the hookworm eggs in feces with NaNO_2 show an unexpected departure from the usual pattern observed in all of the experiments in this series in which parasitic eggs were exposed to NaNO_2 . The data in Table XXIV show that the doses of NaNO_2 which were lethal to hookworm eggs were about ten times and 100 times (LD_{50} and LD_{90}) less than those required to kill the larvae of hookworms (Table XVII) and Strongyloides (Table XVIII). It would be expected that the presence of organic matter, particularly feces, should have had a suppressing effect on the action of the chemical agent. However, remotely, if not directly, related to this unusual observation is the report by Yamaguti and Kimura (1955). These co-workers suggested that human feces contained a chemical substance which was lethal for Ascaris eggs. In addition to this observation it might also be concluded that dog feces contained a similar substance, and that perhaps this chemical substance and NaNO_2 together had a greater ovicidal activity than either of them acting

individually.

The results obtained from the exposure of hookworm and *Strongyloides* larvae to NaNO_2 (Tables XVII and XVIII) show that the larvae of *Strongyloides* (0.04 M to 0.12 M) were more susceptible than those of hookworms (0.11 M to 0.41 M) and that the effect of temperature was not noticeable. There is no obvious explanation for this. However the data show that at higher pH values (8.6-9.2), nearly twice as much NaNO_2 was required to give the same lethal effect as that at lower pH values (5.8-7.4). These findings confirm the reports by Nagano (1952) and Kozai (1960). On the whole, the data in these two tables show little variation in the effects at the different temperature and pH ranges. The only significant exceptions were the data at room temperature (24.0-25.6 C), which showed a lower mean endpoint at all three pH values as compared to those at 13.5-15.5 C and 35.0-37.0 C. This activity probably indicated that NaNO_2 at the concentrations used here was very stable at room temperature, at which it was most active, and that the activity was slightly reduced when the temperature was raised or lowered. Further examination, of the data on the effect of NaNO_2 on hookworm eggs (Table XXIV) reveals a definite influence of pH and temperature in that there was increased demand for NaNO_2 with rise in pH and decrease in temperature. As mentioned earlier, the influence of temperature on the action of NaNO_2 on the hookworm and *Strongyloides* larvae (Tables XVII and XVIII) was not so obvious. On the other hand,

the effect of pH was consistent, in that at high pH values higher concentrations of NaNO_2 were used. The opposite was true at lower pH values.

Although the results of tests on the effect of NaNO_2 on the eggs of A. lumbricoides var. suis and T. vulpis (Tables XXV and XXVI) indicate that in both cases the lethal doses (LD_{50} and LD_{90}) differed only slightly, it should be pointed out that while *Trichuris* eggs were exposed to NaNO_2 for 12 hours, those of *Ascaris* took 60 hours of exposure. Judging from the periods of exposure and the individual differences in NaNO_2 concentrations at different temperature and pH conditions it can be concluded that *Trichuris* eggs were less resistant to the action of NaNO_2 than *Ascaris* eggs were. Furthermore, the data in Tables XXV and XXVI show that increase in the pH resulted in an increase in the required concentration of NaNO_2 and that increase in temperature resulted in a decrease. The influence of pH was not as obvious in the experiments with *Trichuris* eggs as in those with *Ascaris* eggs. The NaNO_2 concentrations needed for *Ascaris* and *Trichuris* eggs were about 10, 100 and 1000 times greater than those for *Strongyloides* and hookworm larvae (Tables XVII and XVIII) and hookworm eggs (Table XXIV) respectively. The chemical was stable at all the concentrations used, and it was fast-acting except in the case of *Ascaris* eggs, where it required up to 60 hours of exposure.

Even though iodine has had a long history of use as a disinfectant (Fair et al., 1944; Hitchens, 1922; Pond

and Willard, 1937) and as a germicide against a large variety of bacteria and viruses (Beckwith and Moser, 1933; Gershenfeld, 1948, 1952, 1954, 1955; Chambers et al., 1955) it is only recently that its effects as an ovicide have been investigated (Dozanska and Iwanczuk, 1959; Thitasut, 1961). Dozanska and Iwanczuk (1959) reported that a 0.13 per cent of solution of I_2 was ovicidal to *Ascaris* while Thitasut (1961) observed that in vitro, aqueous iodine solution at a concentration of 70 ppm to 250 ppm was able to destroy the eggs of *Ascaris*, *Toxocara* and *Trichuris* and the larvae of *Strongyloides* at 15 C to 30 C in less than 30 minutes, and that at a concentration of 500 ppm it could be used as a disinfectant for leafy vegetables under the same conditions. However, the investigation of I_2 as a disinfecting agent for feces infected with parasitic eggs and/or larvae, including the influence of temperature and pH on its activity, had not been fully studied. The results from all of the experiments with I_2 indicate that the chemical agent was active at fairly low concentrations over short exposure periods.

Examination of the data in Table XXXVII reveals that the larvae of *Ancylostoma* were the least resistant to the action of I_2 , and that those of *Strongyloides* were the next least resistant. This order was reversed in experiments with $NaNO_2$. However, in both cases the indication was that the larvae of *Ancylostoma* and *Strongyloides* are less resistant than the eggs of *Ascaris* or *Trichuris*. One of the most significant observations in these data

(Table XXXVII) is the resistance of the *Ancylostoma* eggs in feces to I_2 as compared with other forms of parasites which were treated in aqueous suspensions, as well as with the results of the action of $NaNO_2$ on the *Ancylostoma* eggs in feces (Table XXVI).

This phenomenon shows that while the organic matter in feces apparently had a suppressing effect on the action of I_2 , it had none on $NaNO_2$, and also that it took about ten times as much I_2 to kill hookworm eggs in feces as it did to give similar effects on other parasitic forms in aqueous media.

Although it required slightly stronger solutions of I_2 to kill *Trichuris* eggs than it did those of *Ascaris* (Tables XXVII and XXVIII), it should be noted that *Ascaris* eggs had to be treated for 24 hours in comparison to three hours for *Trichuris* eggs. This also indicates the resistance of *Ascaris* eggs over those of *Trichuris* as already observed in experiments with $NaNO_2$.

The results obtained from treatment of hookworm and *Strongyloides* larvae (Tables XIX and XX) show that there was no influence on the activity of I_2 by pH. However, in the experiments with *Ascaris*, *Trichuris* and hookworm eggs, at alkaline pH values (8.6-9.2), stronger concentrations of iodine were required. Inhibition of the action of I_2 at high pH values was in accordance with the report by Chang (1958) which showed that I_2 loses its activity with rise in the pH of the medium, as illustrated in Table XL below.

TABLE XL

THE EFFECT OF pH ON THE STATE OF
IODINE IN AQUEOUS SOLUTIONS*

Acid Solutions	Neutral Solutions	Alkaline Solutions
$\begin{array}{c} \text{I}_2 + \text{HOH} + \text{H}^+ \\ \downarrow \uparrow \\ \text{I}^- + \text{HIO} + 2 \text{H}^+ \end{array}$	$\begin{array}{c} \text{I}_2 + \text{HOH} \\ \downarrow \uparrow \\ \text{HIO} + \text{I}^- + \text{H}^+ \\ \downarrow \uparrow \\ \text{H}^+ + \text{IO}^- \end{array}$	$\begin{array}{c} 3 \text{I}_2 + 6 \text{OH} + \text{Water} \\ \downarrow \uparrow \\ 3 \text{I}^- + 3 \text{IO} + \text{Water} \\ \downarrow \uparrow \\ 2 \text{I}^- + \text{IO}_3^- \end{array}$

*Germicidal activity of I_2 decreases in the order of $\text{HIO}^- > \text{IO}^- > \text{IO}_3^-$ (Chang, 1958)

The fact that pH did not affect the action of I_2 in experiments on *Ancylostoma* and *Strongyloides* larvae (Tables XIX and XX) may be related to the fact that treatment of the larvae was done in aqueous suspensions without any interfering organic matter. The relative susceptibility of the larvae and the fast-killing action of I_2 may also have overshadowed the effect of pH.

The effect of temperature was very noticeable in all experiments at all levels. The data (Table XXXVII) show that I_2 was more effective at lower temperatures than at higher temperature ranges. These results are in agreement with the report by Wilson (1960). The period of exposure with best results were found to be not more than three hours for all the parasitic forms except *Ascaris* eggs.

which required up to 24 hours of treatment. This again also indicates the relative resistance of *Ascaris* eggs. An attempt to lengthen exposure periods failed because of the instability of I_2 from evaporation when in solution. Laboratory observations from titrations showed that starting with test-suspensions containing I_2 at a concentration of 7.0×10^{-3} M, after 24 hours of exposure the concentration of I_2 had decreased to just about 1.0×10^{-6} M. This loss in strength was also accompanied by gradual loss in the color of the iodine solution. At lengthened exposure periods (up to 24 hours) the best results were obtained by covering the sample tubes and keeping them in dark surroundings.

Bartlett and Schmidt (1957) suggested that the application of I_2 in the field of sanitation could be improved by preparation and use of nonionic surfactant-iodine complexes. These investigators reported from their experiments that while the major part of iodine in the non-ionic surfactant-iodine complexes remained free and titratable, its properties were modified in such a way that there was increased stability in dilute aqueous solutions, elimination of staining, and reduction of skin and eye irritation.

Of all the chemicals thus far reported, preparation of CS_2 solutions was by far the most difficult. The highest practical concentration was a 1 M solution, prepared by dissolving CS_2 liquid in ethyl alcohol and diluting it with water. In experiments where stronger solutions would

have been preferred it was found that the amount of ethyl alcohol used in the controls had lethal effects similar to those of CS₂ itself. This problem was solved by using moderate CS₂ concentrations and lengthening the exposure periods.

Unlike the results on iodine, there are no indications that the action of carbon disulfide was suppressed by the presence of feces during the treatment of hookworm eggs (Tables XXXVII and XXXVIII). On the other hand, there are also no indications that the presence of feces enhanced CS₂ activity as was the case with NaNO₂ (Table XXXVI).

While treatment of the hookworm and Strongyloides larvae took a maximum of three hours, it required up to 12 hours of exposure in treating hookworm eggs and 84 hours for Ascaris and Trichuris eggs. The CS₂ concentrations needed for the Ascaris and Trichuris and hookworm eggs were about 100 times as strong as those for hookworm and Strongyloides larvae (Table XXXVIII).

Regarding the influence of pH on the action of CS₂, the data (Table XXXVIII) show that at high pH values (8.6-9.3) there was a greater demand for CS₂ than at the neutral and acid pH values. The reduced activity of CS₂ in the presence of feces and urine to form ammonium dithiocarbamate, which in turn was a much weaker ovicide than carbon disulfide ($\text{CS}_2 + 2 \text{NH}_3 \longrightarrow \text{H}_2\text{N} - \text{CS}_2\text{NH}_4$).

However, in this study, reduced activity of CS₂ was found not only in media with high pH as a result of feces

or urine, but in both aqueous and fecal media. It may therefore, be concluded that in these experiments the action of CS₂ was generally slowed in media at alkaline pH.

The influence of temperature on the treatment of hookworm and *Strongyloides* larvae (Table XXXVIII) was not regular. However, the data on the treatment of the eggs of hookworm, *Ascaris*, and *Trichuris*, show that the required dose decreased with increase in temperature. Similar observations were made by early investigators, namely, Komiya et al. (1957); Kutsumi and Ote (1955) and Tomomatsu (1961).

The early in vitro experiments with thiabendazole on the survival of the eggs of *Ascaris*, *Trichuris*, *Strongyloides*, *Trichostrongylus* and hookworms, and on a variety of saprophytic and pathogenic fungi (Leland and Bogue, 1964; Tiner, 1964; Stone et al., 1964; Robinson et al., 1964; Kutsumi, 1964; and Chatterjee et al., 1965, 1966) revealed that thiabendazole was lethal at concentrations of about 1.5 to 25 ppm at temperatures of 5 C to 30 C. While the dose of thiabendazole was found to decrease with increase in temperature, the effect of pH was not found to influence its ovicidal activity (Kutsumi and Komiya, 1965).

Treatment of hookworm and *Strongyloides* larvae with thiabendazole did not kill the 5 - 10 day-old larvae (non-feeding third-stage, filariform larvae). More elaborate experiments on growing hookworm larvae, starting with the eggs, were carried out in order to determine at what point in the development the larvae acquired resistance to

thiabendazole.

The data in Table XXIII show that the mortality rates at thiabendazole concentrations 5.0×10^{-5} M and 5.0×10^{-3} M were 85.5 per cent and 90.0 per cent on the second day of larval development (1st stage larvae). However, they show that the rates declined sharply (13.8 per cent and 26.7 per cent) on the third day of development (2nd stage larvae). On the fourth day, the mortality rates were nearly the same as in the controls, that is, 4.7 per cent at 5.0×10^{-5} M and 7.0 per cent at 5.0×10^{-3} M, while it was 4.3 per cent in the controls. Figure 7 is a graphical presentation of the resistance of the developing hookworm larvae to thiabendazole.

The results of this experiment show that developing hookworm larvae acquire resistance to thiabendazole on about the third day of development. According to Matsusaki (1962) the larvae of *Ancylostoma* reach the non-feeding third stage between 64 and 72 hours. However, a few larvae have their mouths closed and are not feeding as early as 52 hours of development. Consequently, the data in Table XXIII indicate that the third stage filariiform larvae are resistant to thiabendazole.

Similar observations on the resistance of the third stage larvae of *Ancylostoma caninum* to thiabendazole were made by Hitch (1964). An attempt by Stone et al. (1965) to treat exsheathed larvae failed. The latter experiment proved that larval resistance to thiabendazole was not dependent on the larval sheath. The most likely explanation

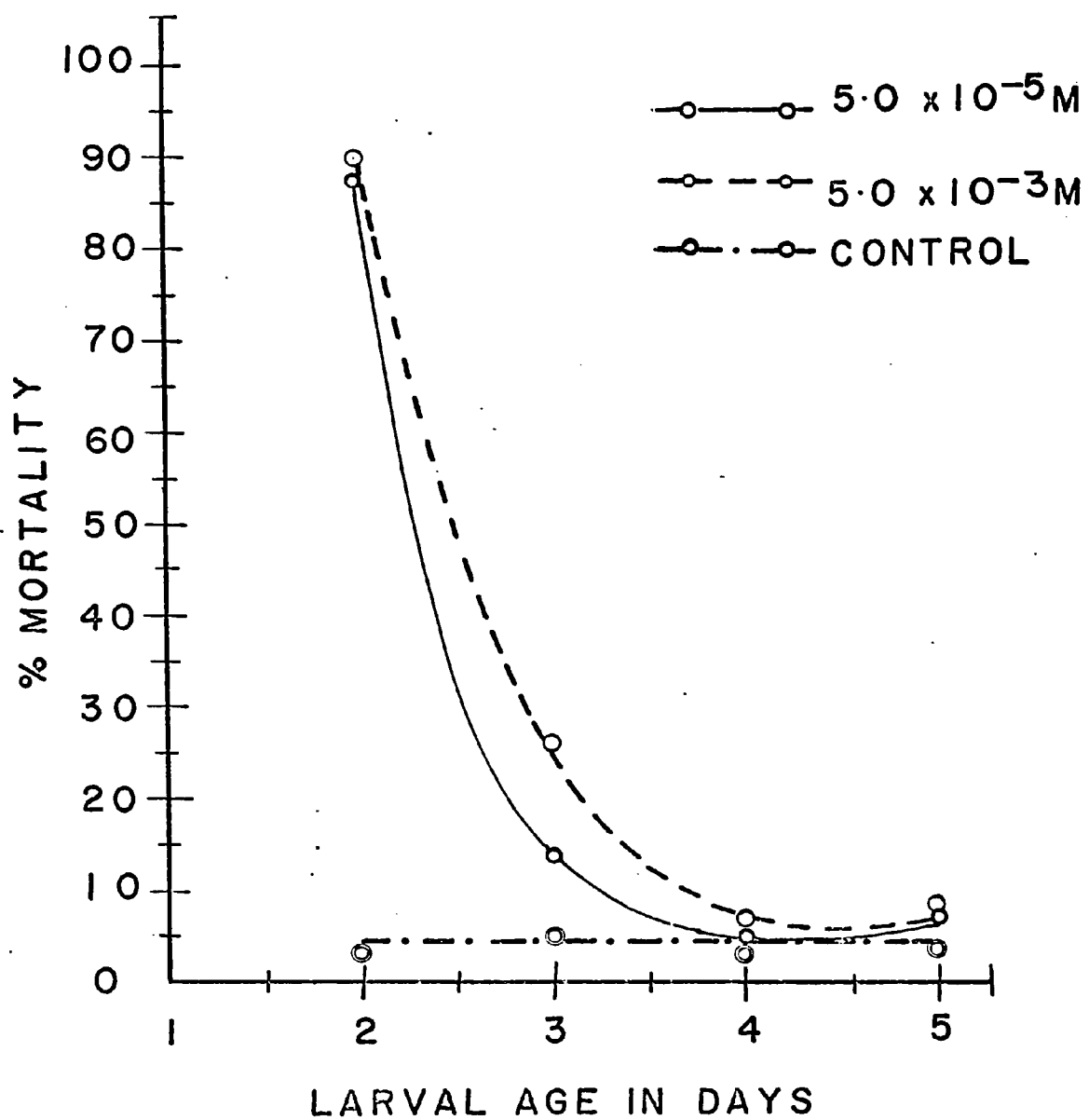


Figure 7.--Susceptibility of Developing *Ancylostoma caninum* Larvae to Thiabendazole at Room Temperature (24.5 C) and Neutral pH (7.2).

for this might be the non-feeding condition of the larvae because of the closed mouths. In addition to this it is also possible that, even if the chemical substance entered the worm by diffusion through the surface of the body, it would not have any effect, because the larva is physiologically or metabolically inactive, and the drug would therefore, not be incorporated in the body of the worm.

This experiment further reveals a physiological relationship between developing hookworms and *Strongyloides*, in that they react to thiabendazole in the same way. On the other hand, thiabendazole experiments on the eggs of hookworm, *Ascaris* and *Trichuris*, the results of which are given in Tables XXX, XXXI, and XXXII respectively, show the relative susceptibility of these parasitic eggs as compared to the larvae. The data on the fifty per cent endpoints appear in Table XXXIX. In all the experiments only the influence of temperature and not that of pH was noticed. The required concentration of thiabendazole decreased with rise in temperature.

If it were not for the insusceptibility of the filariform larvae of hookworms, thiabendazole would otherwise have been a more favorable ovicide and larvicide than iodine (Tables XXXVII and XXXIX).

The following observations and comparisons of the data on the action of the chemicals obtained in this study can now be compared with those by some of the previous investigators. The work of earlier investigators is summarized in Table XLI. In this table, thiabendazole appears

to be the best ovicide in so far as concentrations are concerned. However, it should be pointed out that in this study thiabendazole had the disadvantage of being effective against only the eggs and the first and second stage larvae of hookworms and not against the third stage filariform larvae.

The data in Table XLII represent projected or estimated doses of each chemical (in this study) which will kill as many as 50 per cent or 90 per cent of the various parasitic forms at indicated temperature and pH ranges and exposure periods. From the data, it can be seen that when all factors, pH, temperature and exposure time and concentrations, are considered, iodine is by far the best of the chemicals as an ovicide and larvicide. The effectiveness of the chemicals on the various parasites is summarized in Table XLIII for comparison, based on the data obtained from experiments at room temperature and neutral pH. Only the LD₅₀ doses with their confidence limits are given.

Iodine, although rather unstable in solution, satisfies most of the other conditions as an outstanding ovicide compared with the rest of the chemical agents studied here (Table XLIII). In so far as cost is concerned, Table XLIV, thiabendazole and iodine are the cheapest of the chemicals; however, failure of the former chemical to kill filariform larvae of hookworm leaves iodine as the most practical ovicidal chemical resulting from this study.

TABLE XLI

PREVIOUS FINDINGS ON OVICIDAL ACTIVITIES OF EXPERIMENTAL CHEMICALS

Chemical	Parasite	Findings*	Investigator
NaNO_2	Ascaris and hookworm eggs	1:1000-1:2000 resulted in 100% mortality at 28 C after 3-8 days in acid medium	Kozai (1960, 1962)
I_2	Hookworm and Strongyloides larvae. Eggs of Ascaris, Toxocara & Trichuris	70 ppm against hookworm and Strongyloides larvae resulted in 100% kill in 5 minutes at 15-30 C. 100, 120 and 250 ppm resulted in 100% mortality in Ascaris, Toxocara and Trichuris eggs	Thitasut (1961) Dozanska et al. (1959)
CS_2	Ascaris and hookworm eggs	1% emulsion resulted in 100% kill of eggs in feces at 10 C in 7 days. 0.1% resulted in 100% kill at 5-20 C in 7 days. 1:2000 resulted in 100% kill in 14 days.	Matsumura (1953) Kutsumi et al. (1955) Komiya et al. (1957) Tomomatsu (1961)
$\text{C}_{10}\text{H}_7\text{N}_3\text{S}$	Ascaris, Trichuris and hookworm eggs	1.56 to 6.25 ppm at 25 C for 7 days resulted in 100% kill at pH 5-8. 400 ppm - 100% kill at 5 C in 3 days 50 ppm - 100% kill at 15 C in 3 days 6.25 ppm - 100% kill at 25 C in 3 days	Kutsumi (1963, 1964, 1965) Chatterjee et al. (1965, 1966) Stone et al. (1965)

*moles/liter = moles x molecular weight in gm x 1000 ÷ 1 liter = mg/liter = ppm

1:1000 NaNO_2 = 1 g NaNO_2 in 1000 ml H_2O = 1 g/69 g/mole = 1/69 moles or 1/69 moles/liter

TABLE XLII
CALCULATED OVICIDAL DOSAGES KILLING 50-90%
OF PARASITIC FORMS**

Chemical	Temperature/ pH Ranges	Hookworm Larvae	Strongyloides Larvae	Hookworm Eggs (in feces)	Trichuris Eggs	Ascaris Eggs
NaNO ₂	$\frac{13.0-37.5\text{ C}}{5.8-9.3}$	0.11-0.36 (3 hrs)	0.04-0.13 (3 hrs)	1.0-2.0 $\times 10^{-3}$ (12 hrs)	0.84-1.66 (12 hrs)	0.84-1.66 (60 hrs)
I ₂	$\frac{13.5-38.0\text{ C}}{5.7-9.1}$	1.3-3.1 $\times 10^{-4}$ (3 hrs)	1.3-3.2 $\times 10^{-4}$ (3 hrs)	5.6-10.0 $\times 10^{-3}$ (3 hrs)	3.0-7.9 $\times 10^{-4}$ (3 hrs)	3.0-6.8 $\times 10^{-4}$ (24 hrs)
CS ₂	$\frac{14.0-37.8\text{ C}}{5.8-9.3}$	2.0-4.7 $\times 10^{-3}$ (3 hrs)	1.8-4.0 $\times 10^{-3}$ (3 hrs)	0.23-0.48 (12 hrs)	0.44-0.84 (84 hrs)	0.62-0.83 (84 hrs)
C ₁₀ H ₇ N ₃ S	$\frac{14.4-37.5\text{ C}}{5.7-9.2}$	*selective effect	Selective effect	2.6-6.7 $\times 10^{-6}$ (12 hrs)	0.86-1.5 $\times 10^{-4}$ (36 hrs)	1.7-3.0 $\times 10^{-4}$ (36 hrs)

*5.0 $\times 10^{-3}$ M to 5.0 $\times 10^{-5}$ M effective against only first and second stage larvae, and not third stage.

**Based on combined concentrations (M) for LD₅₀ and LD₉₀.

TABLE XLIII
COMPARISON OF THE EFFECTIVENESS OF CHEMICALS (LD_{50})* ON
EXPERIMENTAL PARASITES AT ROOM TEMPERATURE AND NEUTRAL PH

Chemical	Temp/pH	Strongyloides Larvae	Hookworm Eggs	Hookworm Eggs	Trichuris Eggs	Ascaris Eggs
$NaNO_2$	$\frac{23.0-26.0\ C}{6.8-7.2}$	$\frac{0.04}{+5.8 \times 10^{-3}}$ (3)**	$\frac{0.11}{+6.1 \times 10^{-2}}$ (3)	$\frac{1.2 \times 10^{-3}}{+1.8 \times 10^{-4}}$ (12)	$\frac{1.1}{+9.0 \times 10^{-2}}$ (12)	$\frac{1.3}{+1.5 \times 10^{-3}}$ (60)
I_2	$\frac{23.5-26.4\ C}{6.6-7.2}$	$\frac{1.5 \times 10^{-4}}{+2.3 \times 10^{-5}}$ (3)	$\frac{1.4 \times 10^{-4}}{+6.9 \times 10^{-5}}$ (3)	$\frac{6.5 \times 10^{-3}}{+4.2 \times 10^{-4}}$ (3)	$\frac{3.2 \times 10^{-4}}{+3.6 \times 10^{-5}}$ (3)	$\frac{3.8 \times 10^{-4}}{+7.3 \times 10^{-5}}$ (24)
CS_2	$\frac{23.0-26.0\ C}{6.9-7.3}$	$\frac{2.2 \times 10^{-3}}{+3.6 \times 10^{-4}}$ (3)	$\frac{2.1 \times 10^{-3}}{+3.4 \times 10^{-4}}$ (3)	$\frac{0.23}{+3.1 \times 10^{-2}}$ (12)	$\frac{0.46}{+6.7 \times 10^{-2}}$ (84)	$\frac{0.66}{+4.9 \times 10^{-2}}$ (84)
$C_{10}H_7N_3S$	$\frac{24.5-26.0\ C}{6.9-7.4}$	N.E.***	N.E.***	$\frac{3.9 \times 10^{-6}}{+8.9 \times 10^{-7}}$ (12)	$\frac{1.2 \times 10^{-4}}{+5.6 \times 10^{-6}}$	$\frac{2.0 \times 10^{-4}}{+2.0 \times 10^{-5}}$ (36)

*Expressed as (M)

**Exposure periods in hours

N.E.***No effect on third stage filariform larvae

TABLE XLIV

DAILY FAMILY EXPENSES FOR PARASITIC CONTROL:
 BASED ON 45.4 LITERS* OF
 SEWAGE TREATMENT

Chemical			Cost/Unit (U.S. \$)**	Total Cost (U.S. \$)**
Kind	Requirement (g)	Concentration (moles/l)		
NaNO_2	5,437.2	1.6	0.44/lb	7.06
I_2	60.9	1.0×10^{-2}	1.76/lb	0.23
CS_2	4,215.9	0.84	0.41/pt	3.61
$\text{C}_{10}\text{H}_7\text{N}_3\text{S}$	2.0	3.0×10^{-4}	2.70/60g bottle	0.10

*Fair and Geyer (1956).

**Calculated from catalog prices by local chemical companies.

The strongest iodine concentrations used in these experiments were 5.6×10^{-3} M to 1.0×10^{-2} M applied in the treatment of hookworm eggs in feces. An assumption can be made here that the above I_2 concentration range will kill any kind of parasitic eggs and/or larvae present in feces. Therefore, an estimated I_2 concentration of 6.5×10^{-3} M $\pm$ 5.0×10^{-4} M based on the activity of I_2 on hookworm eggs in dog feces at room temperature (25.4 C) and neutral pH (6.7-7.1) can be regarded as a practical iodine concentration to be used in the control of the intestinal soil-transmitted nematodes. However, slight

variations in the temperature and pH of the medium would not greatly affect the I_2 dosages recommended here. This is because these I_2 concentrations were selected based on laboratory temperature and pH conditions of 13.5 - 38 C and 5.7 - 9.1 respectively. The pH of normal feces varies from 6.8 to 8.8 (Kozai, 1960). Therefore, variation in the pH of the media should have little influence on the action of I_2 on parasitic eggs and/or larvae.

In field conditions where large volumes of sewage are involved, spreading of the sewage in shallow treatment pans, or frequent stirring if treated in deep barrels, is recommended to allow for penetration of the chemical.

SUMMARY

The need for chemical substances which could be used to interrupt the exogenous stage of the life-cycles of parasitic nematodes led to this investigation. The action of selected chemical agents, NaNO_2 , I_2 , CS_2 and thiabendazole, on the survival and development of common intestinal soil-transmitted nematodes was studied under controlled conditions of temperature and pH.

Intestinal nematodes of domestic animals used in the experiments to represent the human forms, included Ancylostoma caninum and Trichuris vulpis from the dog, Ascaris lumbricoides var. suis from the domestic pig and Strongyloides fülleborni from the monkey. The eggs and/or larvae of the parasites were exposed to each of the chemicals at approximate temperatures of 15 C, 25 C or 37 C in media with pH values of 6, 7 or 9. The influence of temperature and pH on the lethal concentrations of the chemicals were studied. The lethal doses were calculated and reported as LD_{50} and LD_{90} . Only the concentrations for the final of the three exposure periods were reported. In addition to the influence of temperature and pH, other factors, such as stability, ease of handling and preparation, irritation and cost of the chemicals, were also considered.

Observations of NaNO_2 as a larvicide or ovicide indicated that, it was more effective on the hookworm eggs in feces than on Trichuris or Ascaris eggs in aqueous media. The effect on hookworm or Strongyloides larvae

falls in between. In the treatment of the eggs of hookworm, *Trichuris* and *Ascaris* the NaNO_2 lethal concentrations were seen to decrease with rise in temperature. There was little or no influence of temperature in experiments with the larvae of hookworm and *Strongyloides*. Rise in the pH of the media resulted in increase in the required concentration of NaNO_2 . Media of pH 8.6 to 9.2 were quite suppressive to NaNO_2 action.

The required I_2 concentrations for the parasitic forms were found to be from 10 to 1000 times less than those of NaNO_2 . The effect of I_2 on the parasites was nearly equally similar except that on hookworm eggs in feces which required slightly stronger solutions. The periods of exposure were also considerably shorter in I_2 experiments as compared to those in NaNO_2 . The I_2 concentrations required for the parasites increased with rise in temperature, especially more so in experiments with the parasitic eggs. The same trend was also evident in the case of pH.

Thiabendazole concentrations required for parasitic eggs were ineffective against the third stage filariform larvae of hookworms and *Strongyloides*. However, they were effective on the growing stages less than three days old. Less amounts of thiabendazole were required for the parasitic eggs than those of either NaNO_2 or I_2 at similar temperatures and pH values. The pH of the media had no influence on the activity of thiabendazole. The rise in temperature resulted in decrease in the concentration of

the chemical.

The effect of CS₂ on the parasites was least remarkable in comparison to the other chemicals, NaNO₂, I₂ and thiabendazole. This is especially more so, when the time of exposure in CS₂ experiments is considered; it took the latter chemical up to 84 hours to kill the parasites as compared to 3 - 24 hours with most other chemicals to give the same effect. On the whole the required CS₂ lethal concentrations on parasitic larvae were nearly 100 times less than those for parasitic eggs (Ascaris and Trichuris). The influence of temperature in CS₂ experiments on Strongyloides larvae was not so remarkable, but in hookworm larvae and eggs, as well as Ascaris and Trichuris eggs, rise in temperature resulted in decrease in the concentration of the lethal CS₂ dose. Rise in the pH of the media resulted in increase in the concentration of the chemical. From the foregoing observations it was concluded that:

1. The order of parasitic resistance from the most resistant was: Ascaris eggs, Trichuris eggs, hookworm eggs, hookworm larvae and Strongyloides larvae.
2. Iodine was the most practical chemical agent as an ovicide. It could be used in the field at the cost of \$0.23 a day by a family of five to treat the daily household sewage (feces and urine) of 45.4 liters in the amount of 60.9 g and concentration of 1.0×10^{-2} M.
3. Thiabendazole, although very effective against parasitic eggs, was not effective against Strongyloides and hookworm larvae after their second day of development.

4. Sodium nitrite and thiabendazole were stable, while carbon disulfide and iodine because of their volatility were unstable.
5. It was found that the growing Strongyloides larvae (2 days and older) were similarly insusceptible to the effect of thiabendazole like those of hookworm, and that required thiabendazole doses for parasitic eggs were effective on the larvae before their third day of development.
6. Iodine was partially suppressed by feces; there was no effect by feces on CS_2 , while thiabendazole and sodium nitrite gave the best results in the presence of feces.
7. Chemical effects on parasitic larvae were better than on the eggs, except in the case of thiabendazole, which had no effect on the larvae, and NaNO_2 which had better results on the hookworm eggs than on parasitic larvae.
8. On the whole, rise in temperature resulted in decrease in the required chemical concentration. Iodine was the main exception to this phenomenon, and here the reverse was true.
9. Generally, alkaline pH values (8.6-9.3) had suppressive effects on chemical activity, resulting in high concentration demand.
10. Field applications could be done by adding a chemical solution in a deep container with sewage and stirring the contents at intervals, or by spreading the sewage

in shallow containers and applying the chemical solution over it to permit penetration.

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