SECTION 'D'.

LIFE CYCLE OF

COSMOCERCA KASHMIRENSIS.
The life-cycle of Nematodes presents far more variations than in Trematodes and Cestodes. It may be direct involving no intermediate host or indirect having one or several intermediate hosts. In both cases there are further variations in different genera and species of any Nematode group. Hyman (1951) has described as many as nineteen types of life-cycles in Nematodes. In both Cosmocerca kashmirensis, Fotedar, 1959 and Oxystrongyulus srinagarensis, Fotedar, 1960 the two common oxyurid parasites in toads in Kashmir, the life-cycle is typically zoo-parasitic. In Cosmocerca kashmirensis, the parasitic stage is in the intestinal tract of the toad, Bufo viridis. Part of its life-cycle is spent as free-living larvae or juveniles. In a zoo-parasitic cycle the life-cycle is direct, involving no intermediate host. The parasite must spend a part of its life-cycle outside as free-living juvenile. Finally an infective stage is reached which enters the host either by active penetration through skin or are ingested by the host. The juvenile may directly proceed to the site which is normally inhabited by the adult or it may undergo visceral migration for varying periods before settling down as sexually mature adult in the normal site.

The occurrence of moulting of which there are usually four, is a characteristic feature of Nematodes. Each moulting involves the shedding of old cuticle from body,
buccal capsule, excretory canal, rectum and even vagina. After a period of feeding and growth, the larva undergoes a period of inactivity or lethargus. This is followed by moulting. The young one may undergo one or two moults while inside the egg-membrane. Such juveniles after hatching accordingly pass through fewer stages. In *Cosmocerca kashmirensis*, there are only two moultings — first involving the hatching of first-stage larva and the second in the formation of a second-stage larva — the infective stage which has been observed here to penetrate actively the host-skin. After passing through host-body-tissue, it eventually reaches the lungs where it undergoes further development and probably another moulting. Finally it reaches the rectum for further growth and sexual maturity.

Present efforts have been made to study the different stages of development of eggs, hatching and development of larvae and their mode of infection to the host. Besides this the longevity of adults and larvae and viability of eggs under different environmental conditions have been studied.

The earliest account available to the writer for reference was on the life-cycle of *Oxysomatium variablis*, Harwood, (1930), which is an allied form of *Cosmocerca kashmirensis*.

Studies on the life-cycle of oxyurids have been made in other parts of the world, but as far as the writer is aware no such work has been done in India. The notable contributions on the oxyurid life-cycles are on *Blatticola*.
blatae by Bozeman, (1942); on Enterobius Vermicularis by Nolan and Reardon, (1936); on Probstmayria vivipara by Ransom, (1907); on Heterakis gallinae by Uribe, (1922); Dorman, (1928); Elpham (1933); and Baker, (1935, 1936); and on Subulura brumpti by Alicata, (1939).
MATERIALS AND METHODS

1. Naturally-infected toads were found in the vicinity of the University Campus and collected for laboratory use. Naturally-infected hosts were also collected from other areas of Kashmir.

2. Preliminary tests for the infection of Cosmocerca in toads was made by examining the host-faeces. In an infected host the faeces normally contain embryonated eggs of parasite.

3. The intestines of toads were opened in physiological salt solution and allowed to remain as such for some time till the worms moved out. They were next transferred to separate containers containing the salt solutions for further study.

COLLECTION OF EGGS

1. The eggs were collected by various methods.

   (1) EXAMINATION OF FaecES

      (a) SEDIMENTATION PROCESS

      The faeces of naturally-infected hosts were collected and placed in glass containers in which water was added. The material was thoroughly mixed with water and the solution was allowed to stand for some time. The supernatant fluid was poured off, and the container was refilled with water and again allowed to settle for some time. The process was repeated several times until the material was clear. Each sample of faeces from the host was checked for infection by examining a portion of sediment under the microscope.
(b) **CENTRIFUGATION.**

A solution of faecal matter was normal saline was also centrifuged to obtain eggs. The process was repeated several times removing each time the supernatant. The sediment was removed and examined for eggs.

(ii) The gravid females were kept in physiological saline solution or tap-water and within a few hours the eggs were laid.

(iii) The uterus of gravid females was cut open or macerated and eggs at different stages of development were obtained for study.

2. **PREPARATION OF CULTURE MEDIA FOR DEVELOPMENT OF JUVENILES.**

Media are generally used for studying the hatching and development of larvae from eggs. Media serve the purpose of providing the growth requirements of individuals. The media prepared from rat-faeces and host-faeces proved highly successful in present experiments. The medium from rat-faeces was first used by Yekogawa (1922) for studying early larval stages of *Nippostrongylus muris*. Rat-faeces medium was prepared by boiling one tea-spoonful of faeces with hundred cc of water and filtering the mixture. Similarly medium from the faeces of donor animals was made. The filtrates obtained from two samples of faeces were diluted several times before using. Besides some cultures were made in tap-water and physiological salt-solutions where development was studied. The development of bacteria and fungi in a culture was prevented as far as possible by changing media twice daily. The medium was also aerated before use.
The infective-stage juveniles were allowed to infect the uninfected hosts as described herein. The experiments were carried out in the laboratory at room-temperature varying from 18-35°C during summer months.

3. OBSERVATION OF LARVAE AND PREPARATION OF PERMANENT MOUNTS.

A speck of Mayer's albumin was applied to the cavity slide, a drop of water containing embryonated eggs and larvae was dropped in the cavity for examination. Luke warm 70% alcohol was dropped over the larvae. Slide was left as such for a moment and by means of pipette alcohol was removed. Later lactophenol as a clearing agent was added and as such the slide was ready for observation. The permanent preparations were made in glycerine jelly. The specimens were directly transferred from glycerine solution to mounting medium. Some of the larvae and eggs were preserved in glycerine and alcohol. Cotton-blue stain was also used for staining larvae but study of unstained specimens was found more useful. The microphotographs of larvae and eggs were taken of both living and mixed material. Before fixing the larvae they were examined in living condition also.

DEVELOPMENT OF EGGS & EMBRYOS

Only the early stages of segmentation of eggs were studied. In a gravid worm the uteri are completely filled in with embryonated eggs. As many as hundred to hundred fifty eggs were counted in a single worm. The stages described herein were obtained by macerating the uterus. Starting from the germinal zone of ovary in the worm, the cells of that region become separated which are formed by repeated divisions and
come to growth zone. These cells, which represent the future eggs, are small in size in early part of growth zone and later grow in size and become rounded in hinder part. There are usually one-called stages in this part of ovary which are oval in shape. The one-celled eggs measure 90 x 57-60 /im in size, bearing a delicate shell (Figure 1). Granular protoplasm fills the egg. Segmentation starts in the proximal part of uterus where eggs are in early stages of segmentation. One-to four-celled stages are usually seen in this region. Two-celled eggs measure 90 x 50 /im in size, each cell being 40 /im (Figure 2). Four-celled stage measures 90 x 60 /im (Figure 3). After this stage the size of cells becomes small, but their division takes place rapidly, as many eggs are seen at morula stage, each measuring 70 - 100 x 60 - 70 /im (Figures 4 & 5). The morula stage is followed by the gastrula stage which is marked by the invagination of cells from a point in the shape of notch. The notch goes on increasing and ultimately a vermiform stage is reached.

The embryonated eggs at the time of their release by the gravid females measure 75-150 x 60-100 /im. (Fig. 6-14). The embryos inside the egg-shell are full of granules and show active movements. Their size increases, measuring 275-300 x 23-26 /im(Fig. 15,16). The excysted embryo measures 240 x 23 /im(Fig. 14). The movements of embryos inside the eggs were studied and it was noted that as soon as embryonated eggs were about to be hatched, the larvae showed active movements. The embryonated eggs hatched in all media including tap-water. Normally embryonated eggs after release from the gravid females hatch within a few
hours, ranging from 3-5 hours at room-temperature of 30-35°C. With the decrease in temperature the time taken in hatching increases and may be prolonged to about 24 hours at temperatures from 15-20°C. Fully-developed embryonated eggs developed further and some of the larvae which were incapable of coming out of egg-shell soon degenerated.
FREE-LIVING STAGES IN THE LIFE CYCLE

FIRST LARVAL PHASE

The emergence of first stage juvenile from the embryonated egg is an interesting observation. It tries to put its way out of the egg-shell usually through a weak spot at the end. Freshly-hatched larva measures 310 /µin length. The body is comparatively wider at oesophageal-bulb region and narrows towards both ends (Fig. 17). The anterior end is more or less rounded and posterior end terminating in a pointed tail. The larva goes on increasing in size and measurements of several larvae were taken which varied from 370-410 /µin length and 12-25 /µ in width. The larva gradually becomes cylindrical in shape. The newly-hatched larva has thin and unmarked cuticle and continues to be so after further development. The buccal capsule is short and cylindrical measures 10 /µin length. The oesophagus is typically rhabditiform type. The total length of oesophagus is 85-95 /µ. The lumen shows a clear line through the centre. The chyle intestine of a newly-hatched larva is thickly dotted with refractile granules which increase later in number. In a newly-hatched larva the lumen of intestine is very clear and can be seen as a wavy line. The intestine measures 200-230 /µin length. The anal opening is visible. The tail measures 50-60 /µin length. The nerve-ring is near the upper part of oesophageal constriction, being at a distance of 50-60 /µ from the anterior end. The genital primordium could not been seen in a newly-hatched larvae, but as the larvae increase in size the genital primordium starts making...
appearance slightly posterior to the middle of chyle intestine. It is about 5 \text{min size} and is at a distance of 150-180 \text{\mu m} from anterior end. When first hatched the larvae are rather sluggish, but soon they begin to feed and show active movements. This activity continues to be there for entire duration of the first larval phase. After a period of three to four days of active movement, the first-stage larvae become sluggish and enter a brief period of lethargy. This is followed by moulting. The larvae are seen in sheaths due to the loosening of cuticle. The medium is soon full of sheathed larvae.

The sheathed larvae measure 400-460 x 25-35 \text{\mu m size} (Fig. 13-2). At this moment there is general lengthening of body, while the cuticle has become loose for moulting. This moulting is completed in liquid medium. The unsheathed larvae transferred to the filter-paper also shed their cuticle. The larvae thus emerged are second stage (infected stage) larvae.

**INFECTION-PHASE LARVA**

After the first moulting of the larva, the infective phase is developed. This type is distinguished from the first type by changes in body-sizes and anatomy (Fig. 22). The infective larva is slender and cylindrical measuring 470-580 \text{\mu m} length and 16-35 \text{\mu m} width. The larva continues to increase in size till it reaches 300 \text{\mu m} length. The tail is long and measures 80-90 \text{\mu m}. The cuticle shows striations. Buccal capsule is reduced and prominences of lips are seen. The
oesophagus is slender, measuring 100-120 μm in length. Here
the oesophagus is again of rhabditiform type but is more
elongated. The lumen shows a clear line in anterior third
of oesophagus. The intestinal cells are arranged in two
rows. The intestine is further elongated here and measures
310-330 μm in length. The nerve-ring is at a distance of
70-75 μm from anterior end. The genital primordium can
usually be found on the ventral side of body between two
rows of intestinal cells, slightly posterior to the middle
of intestine. It is at a distance of 265 μm from the anterior
end. The infective larvae are active and resemble filariform
larvae in general appearance and movements. They were seen
raising their body on their tails and moving their anterior
end. If the anterior end does not touch any object they
drop back and start crawling. They were also observed to
penetrate the sheaths left by them.
PARASITIC STAGE (Experimental infection)

SUB-CUTANEOUS INFECTION

The lung stages were recovered after sub-cutaneous infection of infective-stage juveniles by injection method described later.

The oesophagus of the juveniles at this stage resembles that of the adult. This is true of lips and other morphological features. Probably these stages undergo moulting, but was not actually observed. Following measurements were made:— (in mm.)

- Length of worm — 2.4 - 2.8
- Maximum width — 0.11 - 0.12
- Length of Oesophagus including oesophageal-bulb — 0.35

Young adults were obtained after cutaneous, sub-cutaneous and oral infections. They resemble adults. The sexually mature adults are developed from this stage.

Main Measurements (in mm.)

- Body length — 3.3 - 4.0
- Maximum width — 0.25 - 0.45
- Length of oesophagus including oesophageal-bulb — 0.36 - 0.45
- Length of pharynx — 15 - 20
- Tail length — 0.45 - 0.50
MODE OF INFECTION

Previously number of workers tried different methods to obtain the infection of oxyurid worms by introducing larvae cutaneously, sub-cutaneously and by mouth. Harwood (1930) in his experiments on *Oxysomatium variablis* used various methods. He obtained a lung-stage of the parasite after introducing larvae sub-cutaneously and through mouth. In another series of experiments he exposed the skin of toad to infective-stage larvae but results were negative. In yet another series of experiments he introduced large number of infective-stage larvae by injecting them sub-cutaneously and obtained some immature stages in lungs and intestines. While his results, as expressed by him, were inconclusive, it may be pointed out here that penetration through skin of infective-stage larvae could be the natural method of infection in *Oxysomatium*. Early, Goodey (1923) experimented skin-penetration methods but failed to obtain any stage.

In our present series of experiments all methods were repeated/some useful conclusions drawn.

1. To begin with infective-stage larvae were introduced in the buccal cavity of toads which were previously tested for negative infection. A few young adults were obtained from the rectum from some toads dissected after eighth and tenth day. No lung stage was collected during the interval from the toads infected in this manner. It was then presumed that the infection was direct, involving no visceral migration and larvae reached directly to the normal site i.e. rectum.
2. Subsequently the infective-stage larvae were directly introduced into stomach by a dropper but results were negative. Not a single toad showed any parasite when dissected after the interval of three days each. This created some doubts on the said presumptions of direct mode of infection.

3. Since the infective-stage larvae showed visible tendency for raising their body and penetration into sheaths which were left by them behind in the medium, experiments on skin penetrations were also tried. The toads were fastened over shallow watch glasses containing hundreds of infective-stage larvae in a small amount of medium and allowed to remain as such for a period lasting up to three hours. Such toads were again dissected at intervals of three days each and their intestines and viscera thoroughly examined for parasitic stage. While the lung stages could not be traced, young adults were recovered from rectum and intestines in the toads dissected after twelve to fifteen days of their infection by skin.

4. The infective-stage larvae were injected sub-cutaneously to the toads. In one of these toads the lung stages were traced which had been injected five days earlier. In several other toads young adults were obtained after two weeks or more period of infection.
CONCLUSION

The conclusions drawn are that the toads are infected by infective-stage larvae by active penetration through skin. The larvae may wander about in the viscera reaching there either through blood or by physical migration through the body tissues and organs and ultimately may reach lungs where they undergo a period of growth and probably moulting. It cannot be said with certainty that the larvae necessarily migrate through blood but the chances cannot be ruled out.

Experiments were performed to find their presence in blood which was centrifuged at different intervals after skin infection. It appears that the larvae if by any chance reach buccal cavity of toad, also penetrate through mucous membrane and reach lungs.

This might probably have been the case in Harwood's experiments who obtained a lung stage in toads which were fed orally. From our experiment (No. 1) in which the young adults were recovered in the intestines, the lung stages were apparently missed. There is also a chance that the larvae after penetration may simply wander about in body tissues and viscera, not necessarily lungs and ultimately reach the intestine. That the larvae failed to develop if ingested directly into the intestine is evident from our experiment (No. 2).

In nature the toads can be infected by active penetration of infective-stage larvae while resting on moist soil or water. The first-stage larvae can develop and grow in small
amount of water in the fields in which faeces containing embryonated eggs have been dropped by the host. In this medium as experimentated in the laboratory, the infective-stage larvae can conveniently develop and infect the toads which happen to rest on those infective patches in the fields.
EFFECT OF TEMPERATURE, MOISTURE AND DESSICATION ON EGGS & LARVAE

Temperature:- Temperature has an important effect on hatching of eggs, the activity of both first and second stage larvae, their time of moulting and longevity.

Eggs were kept at different temperatures for different periods and their hatching time was recorded. Effect of freezing temperatures was also observed. Following chart gives an idea of the time taken by the eggs to hatch under different temperatures:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Minimum time</th>
<th>Maximum time</th>
</tr>
</thead>
<tbody>
<tr>
<td>21°C</td>
<td>8 hours</td>
<td>21 hours</td>
</tr>
<tr>
<td>25°C</td>
<td>6 &quot;</td>
<td>20 &quot;</td>
</tr>
<tr>
<td>30°C</td>
<td>5 &quot;</td>
<td>18 &quot;</td>
</tr>
<tr>
<td>35°C</td>
<td>3-4 &quot;</td>
<td>16 &quot;</td>
</tr>
</tbody>
</table>

The eggs placed in a chamber at temperature above 43°C did not hatch and in fact died within 6-12 hours.

With an exposure of 1 hour at 0°C and then placed for 10-24 hours at temperatures ranging from 15-21°C, the eggs showed no response towards hatching. But these eggs after 1 hour exposure at 0°C did revive when placed at 28°C or up to 35°C. Such eggs started hatching within three to five hours at 28°C. The eggs exposed for two hours or more at 0°C did not revive when transferred to chamber at higher temperature.

Similar experiments were performed for the first- and second-stage larvae. The first-stage larvae could be revived after placing them for one hour to maximum of five hours at 0°C. The larvae exposed for six hours ultimately died.
Similar experiments when performed on freshly-moulted second stage larvae showed that these were slightly more resistant.

An exposure at 0°C up to 8 hours and in one case up to 12 hours did not prove fatal. The exposure beyond 12 hours killed the larvae. Similarly, two hours at 50°C were lethal to larvae.

**MOISTURE**

Water is found essential for the hatching of eggs. The eggs placed in moist containers did not hatch. Water is also found to be essential for the first-stage larvae.

The larvae when kept in containers containing moist blotting paper died within 6-8 hours. The second-stage larvae sheathed and unsheathed larvae however remained alive for more than 48 hours, when kept in a moist container with places of filter paper.

The infective-stage larvae also possess greater viability particularly those with sheaths. They could withstand complete dessication for up to six hours, whereas eggs and first-stage larvae died almost immediately.

**LONGEVITY**

Experiments were also performed to study the longevity of parasitic females and males outside the host in different media under a particular range of temperature. Similar observations were made on the longevity of larvae. In all these experiments, the medium was replaced after every four to six hours by fresh-aerated medium. The adults removed from
the host were placed in cavity blocks containing different media like tap-water, normal saline and solutions made from host-faeces and rat-faeces.

Following table shows the maximum period for which the worms and larvae survived in different solutions:

**Longevity of Parasitic Adult Worms**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Adult</th>
<th>Maximum period of survival (in hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tap water</td>
<td>♂</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>24-48</td>
</tr>
<tr>
<td>Normal Saline</td>
<td>♂</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>24-32</td>
</tr>
<tr>
<td>Host faecal soln.</td>
<td>♂</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>60</td>
</tr>
<tr>
<td>Rat faecal soln.</td>
<td>♂</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>♀</td>
<td>56</td>
</tr>
</tbody>
</table>
## Longevity of Larvae

<table>
<thead>
<tr>
<th>Medium</th>
<th>Stage</th>
<th>Minimum period (days)</th>
<th>Maximum period (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tap Water</strong></td>
<td>I</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td><strong>Normal Saline</strong></td>
<td>I</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td><strong>Host faecal Soln.</strong></td>
<td>I</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td><strong>Rat faecal soln.</strong></td>
<td>I</td>
<td>96 hours</td>
<td>100 hours</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>
EXPLANATION TO LETTERING IN FIGURES

a. -- Amph.
b.c. -- Buccal Capsule.
e.p. -- Excretory pore.
i.c. -- Intestinal Cells.
g.p. -- Genital primordium.
n.r. -- Nerve Ring
oes. -- Oesophagus.
PLATE NO. LXXXIV—XCV

PLATE NO. LXXXIV
Fig. 1-5  Developmental stages of egg.

PLATE NO. LXXXV
Fig. 6-13  Embryonated Eggs.
Fig. 14  Excysted embryo.
Fig. 15-16  Developed embryonated eggs.

PLATE NO. LXXXVI
Fig. 17  First Larval stage.
Fig. 18-21  Ensheathed larval.

PLATE NO. LXXXVII
Fig. 22  Infective stage larva.

PHOTO MICROGRAPHS

PLATE NO. LXXXVIII
1  Embryonated eggs in hest faeces.
2  Embryonated eggs passed by female worm.

PLATE NO. LXXXIX
3  Culture of first stage larvae and Embryonated eggs.

PLATE NO. XC
4  Ensheathed larva.
5  Second stage Larva.

PLATE NO. XCI
6  Lung-stage after sub-cutaneous infection.

PLATE NO. XCVI
7  Young form after cutaneous infection.

PLATE NO. XCVII
8  Young adult after oral infection.

PHOTOGRAPHS

PLATE NO. XCVI
1  Showing the penetration of larvae cutaneously to the host.
2  Showing the injection of larvae sub-cutaneously to the host.

PLATE NO. XCV
3  Showing the injection of larvae orally to the host.
PLATE NO. LXXXIV:

0.1 mm.

100 μ
PLATE NO. XC:
PLATE NO. XCI