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STUDIES ON *DICTYOCAULUS FILARIA*
IV. THE MORPHOGENESIS OF THE PARASITIC STAGES IN LAMBS

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ABSTRACT

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In males the parasitic 3rd stage, 3rd moult, 4th stage, 4th moult and 5th stage are reached on Days 1 to 2, 3, 4, 5 to 6 and 8 respectively. The 4th stage is swollen in the anal region and accessory genitalia differentiate rapidly during the 4th moult. In females development from 4th stage larvae through the 4th moult to the early 5th stage is delayed; these stages are present from Days 4 to 6, 7 and 8 respectively. There is little differentiation in the 3rd stage. In the 4th stage the tail has become tapered and by the 4th moult the vulva lips have formed; they become patent after moulting. The genital ducts start showing evidence of patency only 7 days after the 4th moult completing this process 10 days later. The uterus contained embryonated eggs on the 28th day.

INTRODUCTION

The developmental stages of *Dictyocaulus filaria* (Rudolphi, 1809) were first described by Hobmaier & Hobmaier (1919) who recovered parasites 4, 5 and 10 days old from an experimentally infested lamb. In the mesenteric lymph nodes they found larvae in the 3rd and 4th moults as well as 5th stages while immature males and females were recovered from the bronchi. Subsequently its development was studied by Kauzal (1933) in lambs and guinea pigs and by Soliman (1953) in guinea pigs, rabbits and white mice.

MATERIALS AND METHODS

The experimental design is summarized in Table 1. The experimental animals were Dorpers (Dorset Horn × Blackheaded Persian) or Merinos from 5 to 12 months old. Parasites are also described from two animals which did not form part of this investigation: the 28 day old worms were collected from a 4 month old Dorper infested with 15 000 larvae and the 45 day old specimens from a goat infested with 2 000 larvae.

The techniques used in this investigation are those described by Anderson & Verster (1971a, 1971b).

The developmental stages were mounted in formalin, Lugol's iodine solution or Cotton blue and sealed with Gurr's Glyceel. Adult worms were cleared in lactophenol.

At least 20 worms from each infestation were examined. Each specimen was then classified according to its stage of development, and if 3 days old or more, was also sexed. The range of variation, the mean and the standard deviation were determined for the developmental stage of the parasite irrespective of its age; when less than 10 readings were recorded only the mean was calculated.

TABLE 1 *Experimental design*

Sheep No.	No. of larvae dosed	Age of infestation at autopsy in days
1	2 990	1
2	3 060	2
3	2 975	3
4	3 080	3,5
5	2 992	4
6	3 087	5
7	2 998	6
8	3 087	7
9	2 992	8
10	3 186	8
11	3 240	14
12	3 120	24

RESULTS

The total length of the infective larvae, as well as those of parasites 1 to 28 days old, are summarized in Table 2.

The sizes of various organs of the infective larvae and of the parasitic 3rd stage larvae are given in Table 3, while those of male and female parasites 3 to 8 days old are summarized in Tables 4 and 5 respectively.

TABLE 2 Total length (μ) of developmental stages of *D. filaria*

Developmental stage	Age of infestation (d)	Male		Female	
		Range	Mean ± **s.d.	Range	Mean ± s.d.
Infective larvae*	—	524 – 610	556,9 ± 23,0	—	—
Parasitic third*	1 – 2	472 – 592	537,2 ± 29,5	—	—
Third moult	3	476 – 560	518,1 ± 26,0	488 – 568	537,2 ± 25,6
Fourth stage	4	480 – 576	523,2 ± 25,4	512 – 576	537,8
Fourth moult	5 – 6	480 – 560	522,8 ± 23,2	—	—
	7	—	—	520 – 576	557,6 ± 20,7
Fifth stage	8	576 – 656	612,8 ± 29,5	576 – 672	617,6 ± 36,5
	14	1 344 – 2 060	1 626,4	1 575 – 2 400	1 943,2 ± 276,7
	24	6 720 – 11 455	9 709,5 ± 1 757,0	6 060 – 13 160	10 099,8 ± 2 380,0
Adult	28	28 000 – 39 000	30 000,6 ± 3 000,3	27 000 – 44 000	34 000,0 ± 5 000,2

*Sexes cannot be differentiated
**s.d. = Standard deviation

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TABLE 3 *Dimensions (μ) of infective and exsheathed parasitic third stage larvae*

	Infective larvae		Parasitic third stage (1 – 2 d)	
	Range	Mean ± s.d.	Range	Mean ± s.d.
Total length: With sheath	524 – 610	556,9 ± 23,0	—	—
Without sheath	513 – 601	550,1 ± 24,9	472 – 592	537,2 ± 29,5
Width: Anterior end	8 – 14	11,4 ± 2,2	8 – 17	11,2 ± 1,7
Oesophageal bulb	22 – 28	24,3 ± 1,8	14 – 26	19,0 ± 2,9
Genital primordium	19 – 25	23,5 ± 2,5	14 – 24	17,6 ± 2,8
Anus	14 – 20	17,9 ± 1,6	10 – 18	13,8 ± 1,8
Oesophagus: Length	155 – 182	169,4 ± 8,7	142 – 187	167,3 ± 11,9
Width of bulb	7 – 14	12,4 ± 1,7	6 – 14	10,5 ± 2,0
Distance of excretory pore from anterior end	94 – 118	108,2 ± 21,5	90 – 123	106,7 ± 7,8
Distance from excretory pore to nerve ring	12 – 26	18,4 ± 4,0	15 – 38	23,1 ± 5,9
Genital Primordium: Length	8 – 17	13,8 ± 2,2	13 – 26	17,1 ± 3,2
Width	6 – 14	8,8 ± 2,0	6 – 14	8,8 ± 1,5
Distance from genital primordium to tail	200 – 235	216,1 ± 14,6	178 – 248	212,7 ± 15,8
Distance from anus to tail	48 – 75	64,2 ± 6,4	40 – 76	56,4 ± 8,5
Distance from anus to sheath	60 – 80	69,9 ± 6,5	—	—

TABLE 4 *Dimensions (μ) of the male developmental stages of* D. filaria

Stage of Development	Third moult		Fourth stage		Fourth moult		Fifth stage	
Age of infestation	3 Days		4 Days		5–6 Days		8 Days	
	Range	Mean ± s.d.	Range	Mean ± s.d.	Range	Mean ± s.d.	Range	Mean ± s.d.
Total Length: With sheath	476–560	518,1±26,0	—	—	480–560	522,8±23,2	—	—
Without sheath	468–550	499,0	480–576	532,2±25,4	474–541	570,7±20,6	576–656	612,8±29,5
Width: Anterior end	10–15	12,7±2,0	11–15	13,6±1,4	10–14	11,7±0,9	11–18	13,7±2,2
Oesophageal bulb	15–19	17,8±1,2	17–21	19,4±1,2	16–20	18,8±1,0	14–24	19,0±2,9
Genital primordium	15–19	17,0±1,1	15–20	18,6±1,4	17–20	18,5±1,0	15–22	18,2±2,1
Anus	14–18	15,8±1,2	16–20	18,5±1,5	16–20	18,7±1,3	17–23	19,6±2,7
Oesophagus: Length	152–183	170,2±8,7	148–175	163,3±7,1	136–187	154,8±12,0	133–176	157,9±15,0
Width	10–14	12,0±1,5	11–14	13,2±1,1	11–14	12,0±0,7	9–16	12,7±2,1
Distance of excretory pore from anterior end	88–116	101,5±9,1	90–104	97,0	92–104	97,5	86–106	98,8
Distance from excretory pore to nerve ring	16–24	21,0	16–22	20,2	18–24	20,8	14–18	15,2
Length of genital primordium	14–38	24,1±6,8	16–60	32,6±5,4	80–140	112,4±17,6	124–210	162,2±24,0
Distance from genital primordium to tail	168–225	200,3±14,5	186–250	211,8±19,5	176–205	189,5±12,8	200–252	219,7±21,1
Anus to tail	33–54	46,3±6,9	48–58	51,6±3,7	16–40	35,0±2,1	14–18	16,3±1,3
Spicules: Length							20–44	32,4

TABLE 5 *Dimensions (μ) of female developmental stages of* D. filaria

Stage of Development	Third moult		Fourth stage		Fourth moult		Fifth stage	
Age of infestation	3 Days		4 Days		7 Days		8 Days	
	Range	Mean ± s.d.	Range	Mean ± s.d.	Range	Mean ± s.d.	Range	Mean ± s.d.
Total length: With sheath	488–568	537,2±25,6	—	—	520–576	555,5		
Without sheath	—	—	512–576	537,8	517–574	552,4	576–672	617,6±36,5
Width: Anterior end	10–14	11,5±2,0	10–14	11,2	10–13	11,9±0,7	12–16	12,8±1,4
Oesophageal bulb	18–21	19,0±1,3	18–20	19,0	17–22	19,0±2,1	16–23	18,2±2,6
Genital primordium	16–19	17,2±1,2	14–21	17,8	16–22	18,6±1,8	16–23	18,2±2,3
Anus	10–14	12,2±1,2	13–15	13,8	12–14	13,4±0,9	12–18	13,0±1,8
Oesophagus: Length	164–180	170,6	150–172	162,3	147–170	162,1±8,6	128–176	155,0±7,9
Width	12–14	12,9	12–15	12,9	11–14	12,8±1,1	12–17	13,1±1,7
Distance of excretory pore from anterior end	93–108	103,4	92–100	95,2	80–89	85,4	76–94	83,5
Distance from excretory pore to nerve ring	16–21	18,9	17–22	19,5	24	24,0	—	—
Length of genital primordium	28–56	40,7±13,8	38–84	57,0	104–132	116,0±13,1	106–134	117,8±9,2
Distance from genital primordium to tail	144–246	214,7±27,1	220–240	232,8	238–292	269,0±19,2	243–322	285,7±25,0
Distance from genital primordium to vulva	—	—	—	—	42–82	59,0±15,4	44–74	58,7±8,8
Distance from vulva to anus	—	—	—	—	130–188	155,8±17,0	150–220	184,8±21,3
Distance from anus to tail	40–58	51,0±5,7	48–60	53,0	43–60	50,4±5,9	48–56	54,6±3,5

Third stage larvae

The mouth opening of the infective larva is surrounded by six papillae and there is another papilla on the ventral surface of the tail near its tip (Fig. 1). The cuticle bears fine cross-striations less than 1 μ apart throughout its length.

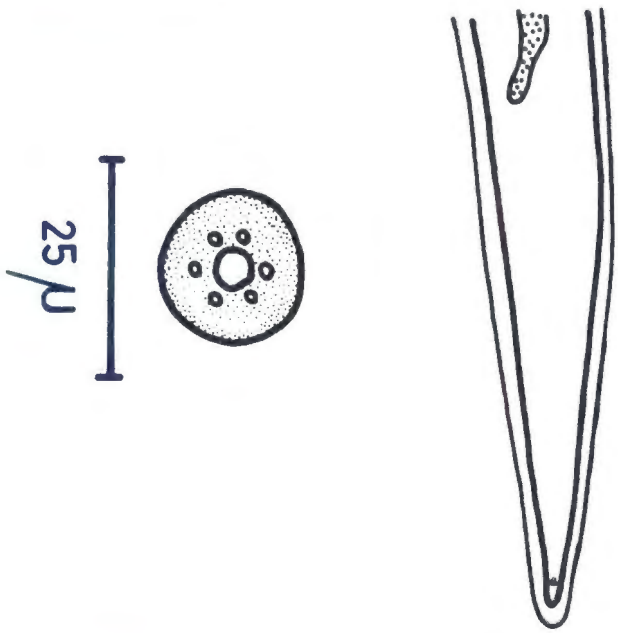

FIG. 1 Infective larva - *En face* view of the head (left) and ventral view of the hind end

Parasitic 3rd stage larvae 1 and 2 days old, resemble infective larvae but are unsheathed. Their genital primordia are slightly longer (13 to 26 μ) than those of infective larvae (8 to 17μ). It is impossible to differentiate between males and females at this stage.

On Day 3 when the larvae are in their 3rd moult the sexes can be distinguished from one another. In males the body width across the anus (14 to 18μ), is almost the same as that across the genital primordium (15 to 19μ), but in females the body tapers towards the posterior end, being 16 to 19μ at the genital primordium and 10 to 14μ at the anus (Fig. 3).

A few larvae were still moulting on Day 4 and in them the sheath was loose and clearly visible. In an attempt to obtain further specimens at this stage, a lamb was killed 84 hours after infestation but all its parasites had already developed to the 4th stage.

Morphogenesis of males

On Day 4 the male parasites are 4th stage larvae (Fig. 2). At this stage of development they differ from the 3rd moult in that they are not sheathed and the genital primordium is somewhat larger, viz. 16 to 60μ vs. 14 to 38μ.

On Days 5 and 6 the majority of the males are in the 4th moult (Fig. 2). At this stage the genital primordium has extended posteriorly and varies from 80 to 140μ in length while the distance from the anus to the tip of the tail has decreased. The differentiation of the copulatory bursa is rapid and all stages of development may be seen on Days 5 and 6 (Fig. 4).

First the tail narrows and tapers dorsally and then its tip branches as the bursal rays begin forming. The two ventral rays develop first, followed by the laterals and finally the dorsal rays. During the early stages in the differentiation of the bursa there is no evidence of spicule formation. The spicular pouches are first visible in those specimens in which the primordia of the bursal rays can be seen and towards the end of the 4th moult weakly sclerotized spicules 16 to 36μ in length have formed.

Fifth stage males are present for the first time on Day 6 and all the males reach this stage by Day 8 (Fig. 2). These worms differ from those in the preceding stage in that they are unsheathed and their genital primordia have increased in length (124 to 210μ). The spicules are more heavily sclerotized and vary from 24 to 44μ in length; a gubernaculum was not observed at this stage.

Very little growth takes place while the worms are in the mesenteric lymph nodes. Migration to the lungs commences on Day 8 and active growth sets in soon after this is completed (Table 2). On Day 14 the spicules vary from 40 to 76μ in length and on Day 24 From 160 to 248μ. The gubernaculum was first noted on Day 24 when it varied from 27 to 32μ in length. By Day 28 the worm is adult and the copulatory bursa as described by Boev (1957) is fully developed. The spicules, which vary from 248 to 384μ in length and from 40 to 62μ in width proximally, are reticular in structure throughout. The distal third of each has relatively light sclerotized lateral expansions and curves towards the ventral surface. The gubernaculum, which is also reticular and only lightly sclerotized, is 32 to 67μ in length.

Although the worms are sexually mature and patent at 28 days they have not yet attained their maximum length nor is the development of the spicules complete. In worms 45 days old the spicules are 440 to 554μ long and 48 to 88μ wide proximally. The gubernaculum is 64 to 84μ in length and lies close to the lateral expansions of the spicules; it is not easy to see as both these structures are lightly sclerotized (Fig. 5).

Morphogenesis of the female

Male and female parasites develop at the same rate until Day 4 when both are in the 4th larval stage. Thereafter the development of the female is slower by 1 to 2 days than that of the male.

Fourth stage females differ from those in the 3rd moult in that they are unsheathed and their genital primordia are somewhat larger, (38 to 84μ vs 28 to 56μ). The vulvar primordium is visible by Day 6. This structure is 160 to 198μ anterior to the anus and 42 to 56μ posterior to the proximal end of the genital primordium. At this stage the genital primordium is 88 to 134μ long and it shows no further development until the worms have reached the 5th stage. The 4th moult occurs on Day 7 and 5th stages are present on Day 8 when the vulva becomes patent but the rest of the genitalia are still a solid mass of cells.

On Day 14 both the pars ejaculatrix and pars haustrix are present. The opening of the vulva extends into the pars ejaculatrix but does not reach the sphincters. Simultaneously openings develop in both horns of the pars haustrix adjacent to the sphincters. These are not joined to the lumen in the pars ejaculatrix but are separated from it by solid tissue (Fig. 6). The two parts of the genital primordium which will eventually differentiate into the ovaries are reflexed and grow towards the vulva.

By Day 24 the lumen of the pars ejaculatrix is continuous with that of the pars haustrix and differentiation of the rest of the genitalia is complete (Fig 7). On Day 28 and Day 45 the parasites are larger and contain embryonated ova but otherwise resemble those on Day 24.

FIG. 2 Developmental stages of the male

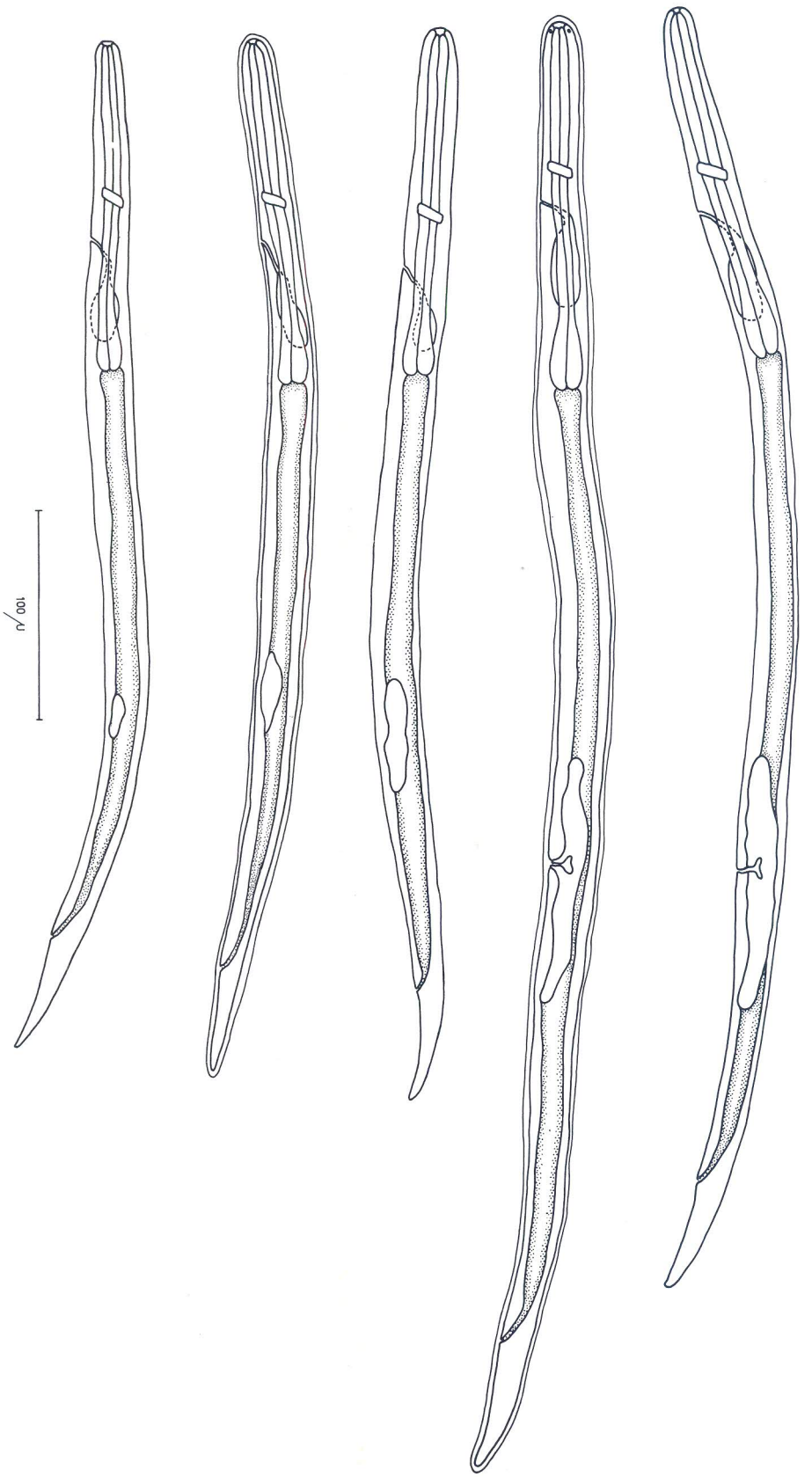

FIG. 3 Developmental stages of the female

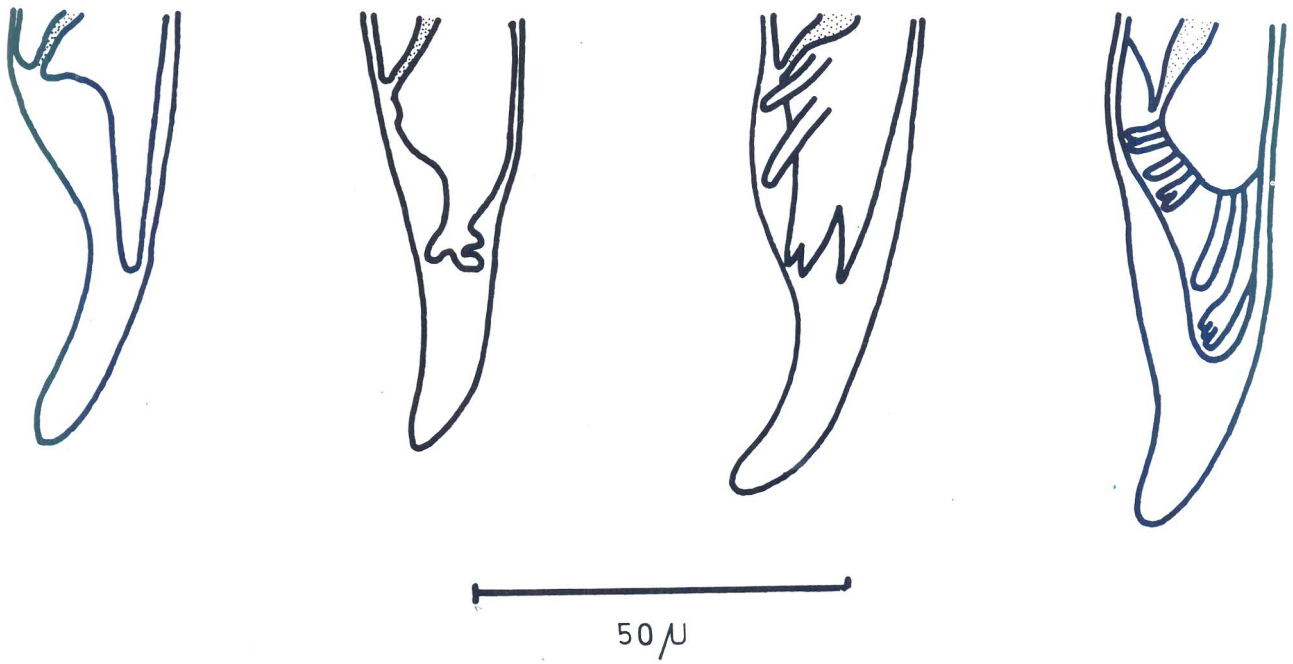

FIG. 4 Development of the copulatory bursa of the male

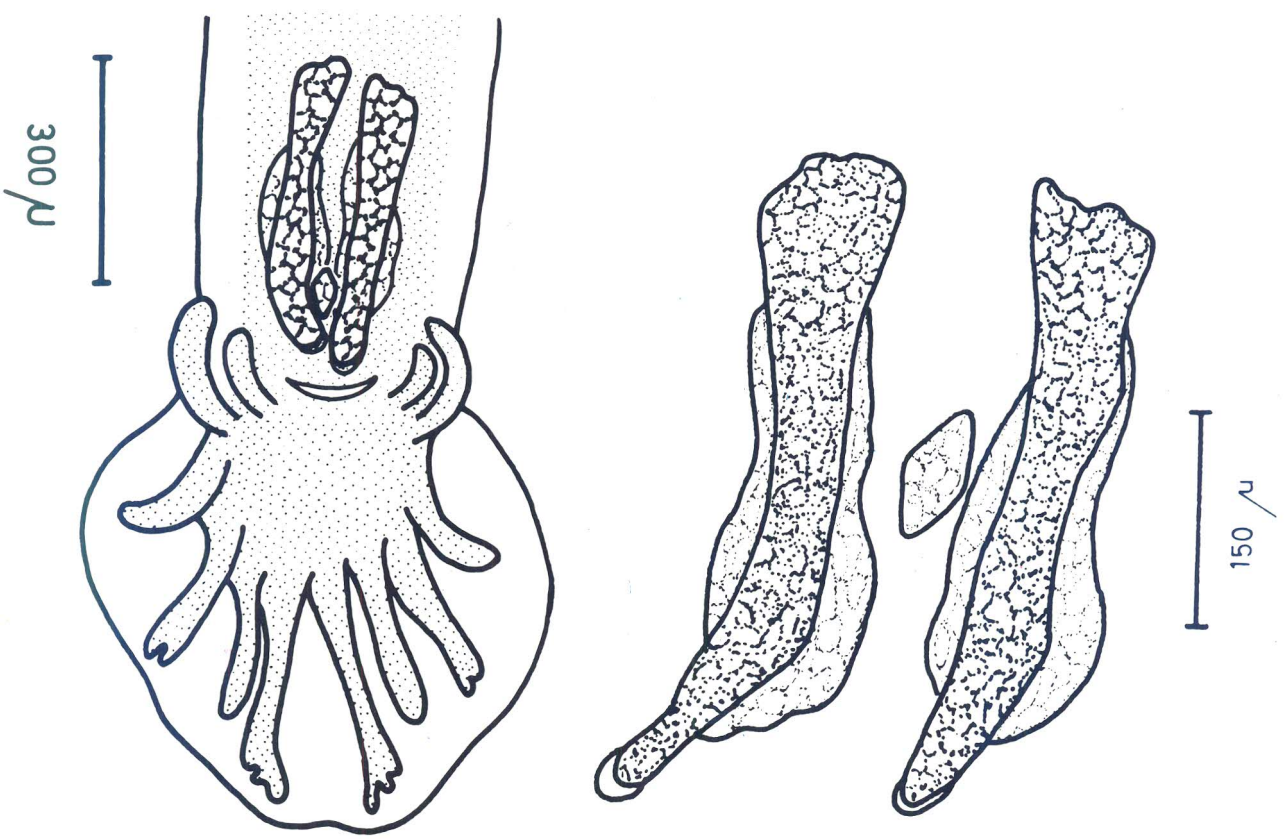

FIG. 5 Copulatory bursa (left) and spicules (right); 45 days old

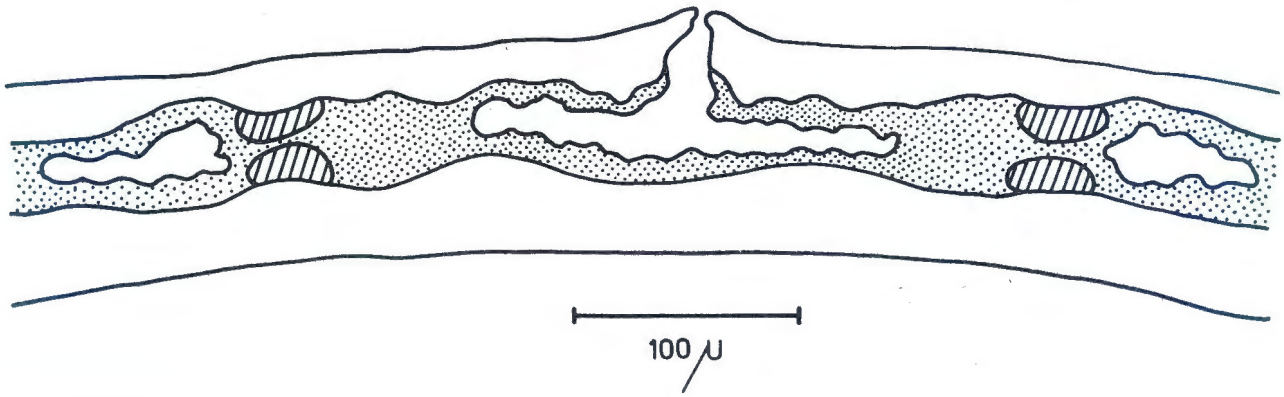

FIG. 6 Ovejector showing development of the lumen in the pars ejaculatrix and pars haustrix; 14 days old

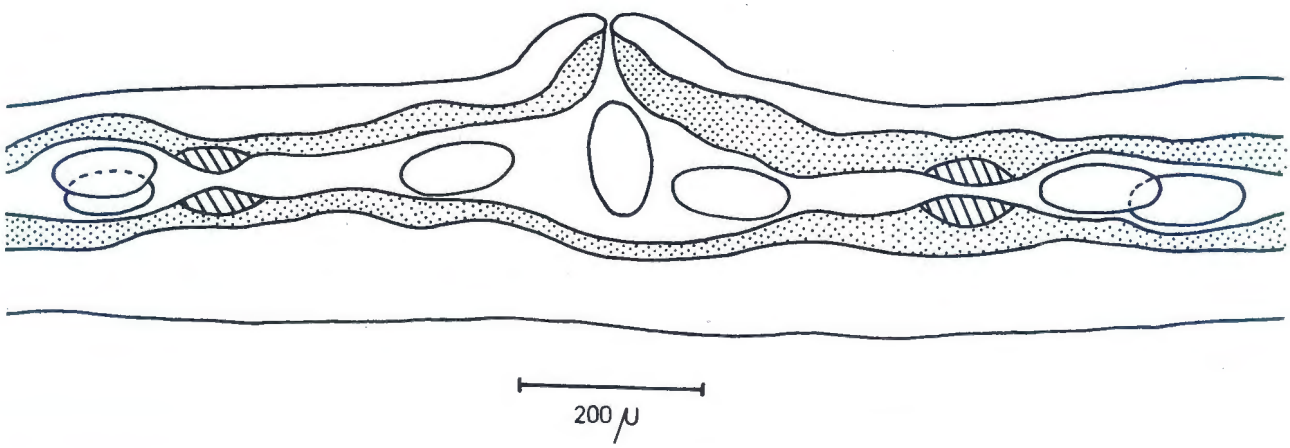

FIG. 7 Ovejector showing continuous lumen in the pars ejaculatrix and pars haustrix; 24 days old

DISCUSSION

The development of *D. filaria* to the 5th stage is remarkable not only for its rapidity but also because it develops to this stage without any significant increase in size. Hobmaier & Hobmaier (1929) recovered 5th stage worms from a lamb which was infested successively 4, 5 and 10 days earlier. The infective larvae used to infest this animal were 560μ long; both the male and female 5th stages from the mesenteric lymph nodes were 800μ long, while immature females in the bronchi varied from 800 to 1 200μ in length.

In the present investigation the mean length of parasites 8 days old is somewhat longer than that of the infective larvae, but there is little difference in the range of variation of these stages (Table 2).

The development of the parasitic stages of *Dictyocaulus viviparus* (Bloch, 1782) in the guinea pig was studied by Douvres & Lucker (1958). They found that its rate of development is slightly faster than that of *D. filaria* in lambs in this investigation (Table 6). In the present investigation, however, the female parasites of *D. filaria* remain in the fourth stage longer than the males, and thereafter their development is slower by 1 to 2 days than that of the males.

Kauzal (1933) assigns parasites recovered on Day 8 from the bronchi of a lamb and a guinea pig to the 4th stage. In his description of these parasites he states: "In the bursa of the males the ventral rays appeared to be lacking. The spicules were also lacking, as in the case of larvae from Guinea Pig No. 3. The females were up to 925μ long by 32,2μ wide. The site of the vulva could be seen but the opening was not patent, this confirming the opinion that the parasites were fourth stage larvae and not young adults". The ventral rays are, however, clearly visible in his Fig. 2 illustrating the male parasite from Guinea Pig. No 3. At this stage of development the spicules are difficult to distinguish as they are lightly sclerotized. This investigation has shown that the copulatory bursa develops rapidly during the 4th moult and, as the larva illustrated by Kauzal is unsheathed, it must be a 5th stage rather than a 4th stage larva.

TABLE 6 *Comparison of the rate of development of* D. viviparus *and* D. filaria

Stage of development	Period after infestation (in hours)	
	D. viviparus (Douvres & Lucker, 1958)	*D. filaria* (This paper)
Parasitic third	18	24
Third moult	23	72
Fourth	43,5	96
Fourth moult	144	♂ 120–144 ♀ 168
Fifth	154	♂ 168 ♀ 192

It is more difficult to evaluate the stage of development of the female he described. Female parasites are classified as 5th stages when the opening of the vulva is patent. It is, however, not clear whether this includes the vagina or ovejector. In the present investigation the external opening is patent on Day 8 when the cuticle of the 4th stage is lost, but the lumen of the vulva does not become continuous with that of the ovejector until some days later. Female worms are, therefore defined as 5th stage when the vulva opening to the outside becomes patent.

In studies on the morphogenesis of parasitic nematodes the 4th stage larvae are often classified as early, middle or late 4th stages. Denham (1969), however, is of the opinion that these categories are inadequate to describe the extensive morphological changes occurring in these parasites and divided the parasitic life cycle of *Ostertagia circumcincta* (Stadelmann, 1894) into phases, based on the development of the gonads. Parasitic 3rd stage larvae and those in the 3rd moult represent Phases 1 and 2 respectively. The development of the 4th stage male is divided into six further phases, then worms in the 4th moult are classified in Phase 7 and 5th stages in Phase 8. The development of the female consists of a further nine phases, depending on the development of the ovary, uterus and ovejector; the 4th stage female passes through seven of these phases and the 5th through another two. Denham's classification not only defines each phase accurately but can also be used to describe the structure of a given worm population. An attempt was therefore made to divide the development of *D. filaria* into similar phases but this proved unsatisfactory. In this species the female genitalia undergo very little detectable differentiation prior to the 5th stage, probably because they do not increase in size before the worms have migrated from the lymph nodes to the lungs, i.e. after Day 8 (Table 2). This delay in growth also distinguishes *D. filaria* from other nematodes which develop rapidly and have a short prepatent period. Denham (1969) found that the parasitic 3rd stage of *O. circumcincta* was 0,68 mm long. Within 9 days the fifth stages of this worm had a mean length in males of 3,8 mm and in females of 4,9 mm. In *D. filaria,* however, the worms had grown but little by the time they had reached the 5th stage on the 8th day when the males had increased by 75,6μ and the females by 80,4μ when compared with the 3rd parasitic stage. The failure of this worm to grow is possibly due to the compact tissue of the mesenteric lymph nodes when compared with that of the lung and bronchi.

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REFERENCES

ANDERSON, P. J. S. & VERSTER, ANNA, 1971a. Studies on *Dictyocaulus filaria*. I. Modification of laboratory procedures. *Onderstepoort J. vet. Res.* This issue.

ANDERSON, P. J. S. & VERSTER, ANNA, 1971b. Studies on *Dictyocaulus filaria*. II. Migration of the developmental stages in lambs. *Onderstepoort J. vet. Res.* This issue.

BOEV. S. N., 1957. Pulmonary nematodes of ungulates in Kazakhstan. (In Russian). Alma-Ata: Izdatel. Akad. Nauk. Kazakh SSSR.

DENHAM, D. A., 1969. The development of *Ostertagia circumcincta* in lambs. *J. Helminth,* 43, 299–310.

DOUVRES, F. W. & LUCKER, J. T., 1958. The morphogenesis of the parasitic stages of the cattle lungworm, *Dictyocaulus viviparus,* in experimentally infected guinea pigs. *J. Parasit.,* 44 (Sect. 2), 28–29.

HOBMAIER, ADELE & HOBMAIER, M., 1929. Die Entwicklung des Lungenwurmes des Schafes, *Dictyocaulus filaria* Rud., ausserhalb und innerhalb des Tierkörpers. *Münch. tierärztl. Wschr.,* 80, 621–625.

KAUZAL, G., 1933. Observations on the bionomics of *Dictyocaulus filaria*. With a note on the clinical manifestations in artificial infections in sheep. *Aust. vet. J.,* 9, 20–26.

SOLIMAN, K. N., 1953. Migration route of *Dictyocaulus viviparus* and *D. filaria* infective larvae to the lungs. *J. comp. Path. Therap.,* 63, 75–84.

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