STUDIES ON SCHISTOSOMIASIS. 10. DEVELOPMENT OF *SCHISTOSOMA MATTHEEI* IN SHEEP INFESTED WITH EQUAL NUMBERS OF MALE AND FEMALE CERCARIAE

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ABSTRACT

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The development of the female *Schistosoma mattheei* was significantly higher than that of the male ($P<0,0001$) in 12 sheep when each was exposed to equal numbers of male and female cercariae. Many more male than female worms usually develop after infestation with pools of cercariae of mixed sexes, a phenomenon which in the light of the present results seems to be due to a preponderance of male cercariae and not to the more efficient development of male than female cercariae.

The female worms recovered fell into 2 distinct population groups as regards length and pigmentation. Some overlap in the measurements of the breadths and in the numbers of ova in the uteri of the worms, however, makes the demarcation of the different populations less distinct in these respects. The female worms from 3 single-sex infestations contained either no ova (72 days after infestation) or fewer (after 134–137 days of development) than the small females from the 12 sheep.

The number of large females (602) recovered from the mesentery was approximately the same as that of the males (605) from this site. Similarly, although varying numbers of small female worms were recovered from the liver of every sheep, only 2 males and 2 large females were recovered, and these were from the same liver.

Because of the similarity between the numbers of male and large female worms, it is clear that, for *S. mattheei*, physical contact with male worms is essential for development to maturity of female worms, the mere presence of males in the host not being sufficient for this development to take place.

Résumé

ÉTUDES SUR LA SCHISTOSOMIASE. 10. *DÉVELOPPEMENT DE* SCHISTOSOMA MATTHEEI *CHEZ LE MOUTON INFESTÉ AVEC DES NOMBRES ÉGAUX DE CERCAIRES MÂLES ET FEMELLES*

Le développement de la femelle Schistosoma mattheei *fut significativement plus élevé que celui du mâle* ($P<0,0001$) *chez 12 moutons, quand chacun de ceux-ci fut exposé à des nombres égaux de cercaires mâles et femelles. Beaucoup plus de vers mâles que de femelles se développèrent habituellement après l'infestation avec un mélange de cercaires des deux sexes, un phénomène qui, d'après ces résultats, semble être dû à une prépondérance de cercaires mâles et non à un développement plus efficace des mâles que des femelles cercariae.*

Les vers femelles recouvrés tombent dans deux groupes de population distincts quant à leur longueur et à leur pigmentation. Un certain chevauchement dans les mensurations des largeurs et dans les nombres d'oeufs dans les uteri des vers, rend cependant la démarcation des différentes populations moins distincte dans ces domaines. Les vers femelles d'une infestation de sexe unique continrent, soit nul ova (72 jours après l'infestation) ou moins (après 134–137 jours de développement) que les petites femelles de 12 moutons.

Le nombre de grandes femelles (602) recouvrées du mésentère fut approximativement le même que celui des mâles (605) provenant de ce site. Similairement, et bien que des nombres variés de petits vers femelles furent recouvrés du foie de chacun des moutons, seulement 2 mâles et 2 grandes femelles furent recouvrés, ceux-ci provenant du même foie. A cause de la similitude entre les nombres de vers mâles et des vers femelles grandes il est clair que, pour S. mattheei, *le contact physique avec les vers mâles est essentiel pour le développement jusqu'à maturité des cercaires femelle, la seule présence de mâles dans l'hôte n'étant pas suffisante pour que ce développement puisse prendre place.*

INTRODUCTION

As a rule, more male than female worms develop in experimental infestations of *Schistosoma mattheei* (McCully & Kruger, 1969; Van Wyk, Heitmann & Van Rensburg, 1975; Lawrence, 1976; Van Wyk, Van Rensburg & Heitmann, 1976) and also of *Schistosoma mansoni* (Stirewalt, Kuntz & Evans, 1951; Rowntree & James, 1977). Van Wyk *et al.* (1975) suggested that the disparity in the sexes of worms which developed in their sheep was due to the presence of more male than female cercariae in the infective doses rather than to a higher percentage development of the male cercariae.

The following experiment was planned to ascertain whether the viability of male cercariae differed from that of female cercariae.

Since females recovered in the trial were of different sizes, worm measurements and intra-uterine egg counts were done. In addition, for the sake of comparison, worms from sheep infested with female cercariae of *S. mattheei* only were measured and examined for ova.

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MATERIALS AND METHODS

Twelve 2-tooth Merino wethers, which had not previously been exposed to schistosomes, were used in the study. The origin of the strain of *S. mattheei* used and its maintenance in the laboratory have previously been described (Van Wyk, 1973).

Unisexual cercariae were obtained by exposing snails to one miracidium each (Moore, Yolles & Meleney, 1954). The sex of the cercariae was determined by infesting *Praomys* (*Mastomys*) *natalensis* with some cercariae from each snail and examining the young worms 21 days later (Cort, 1921).

Cercariae for the 12 sheep were collected separately from 2 snails as described by Van Wyk (1973). One hundred male and 100 female cercariae, removed one at a time by pipette from the cercarial suspension, were placed in 12 plastic measuring cylinders, one for each sheep (Van Wyk *et al.*, 1975).

Sheep were infested by immersing a leg in the cercarial suspension for 30 minutes after the skin of the leg had been thoroughly cleaned with water (Van Wyk *et al.*, 1975).

After infestation the cercariae which remained in the measuring cylinders were counted under a stereoscopic microscope.

From 74–76 days after infestation the 12 sheep were killed and perfused for worm recovery (Van Wyk *et al.*, 1976). For comparative worm measurements, a further 3 sheep were infested with only female cercariae of an inbred strain of *S. mattheei* (maintained in hamsters in the laboratory), and killed 71 (Sheep 13), 127 (Sheep 14) and 134 days (Sheep 15) after infestation (Table 4).

The worms from all 15 sheep were relaxed with pentobarbital sodium, killed with heat and shaken vigorously. While the lengths of the female worms from all 15 sheep were measured on a transparent glass ruler after formalinization, their breadths were determined over the midpoint of the ovary, using a standard microscope fitted with an ocular scale.

Using the technique of Heitmann (unpublished data, 1973), the intra-uterine eggs of mature female worms were counted after the worm tissue and caeca had been cleared with 5% KOH solution for approximately 5–10 minutes. This technique was not necessary for underdeveloped worms, since the eggs, not being obscured by the caeca, were easily visible.

The numbers of male and female worms recovered from the sheep were compared statistically (5% level of significance) by means of the Wilcoxon matched-pairs signed-ranks test (Siegel, 1956).

RESULTS

A mean of 3,4% cercariae failed to penetrate the sheep and remained in the measuring cylinders after infestation (Table 1). A weak negative correlation found between the percentages of cercariae that failed to penetrate and the percentages of worm development was not significant ($0,05 > P < 0,1$).

A total of 609 male and 800 female worms was recovered from the 1 200 cercariae of each sex used for infestation, a mean development of 58,6% (50,8% for the male and 66,7% for the female cercariae, Table 1). The difference in development of the 2 sexes is highly significant ($P < 0,0001$).

The worms recovered from the mesentery comprised 93,5% of the total (99,3% of the males and 89,1% of the females), while 5,2% (0,3% of the males and 8,9% of the females) were recovered from the liver and 1,3% from the lungs (0,3% of the males and 2,0% of the females).

One hundred and eleven of the 713 female worms recovered from the mesentery were small and resembled immature females in unisexual infestations. The remainder of the females and all the males were fully developed adults. The large female worms (602) were very similar in number to the males (605) from this site.

Worm distributions according to size and intra-uterine egg count distributions are given in Fig. 1–9.

The lengths of female worms from the mesentery fell into one of the 2 distinct groups and are designated for convenience as either "large" or "small" females. The length of only one worm was intermediate between the 2 groups; this worm was empirically grouped with the large females.

The worm measurements and intra-uterine egg counts are summarized in Table 2, and the numbers of worms measured in Table 3.

Worm lengths

The mean lengths of the "large" and "small" female worms from the mesentery of Sheep 1–12 (killed 74–76 days after infestation) were 21,8 mm (range 15–27) and 9,7 mm (range 4–13) respectively (Table 2, Fig. 1–3). Only small females were recovered from the mesentery of the remaining 3 sheep; those from Sheep 13 (killed 71 days after infestation) had a mean length of 7,5 mm (range 6–9 mm) and those from Sheep 14 and 15 (killed 134–137 days after infestation) had a mean length of 11,5 mm (range 4–15 mm).

TABLE 1 Development of cercariae and worm distribution

Sheep	Duration (days)*	Worm distribution: Mesentery ♂	Mesentery ♀ Large	Mesentery ♀ Small	Mesentery ♀ Total	Liver ♂	Liver ♀	Lungs ♂	Lungs ♀	Total worms ♂	Total worms ♀	Total worms % development	Cercariae failed to penetrate (%)
1.......	74	42	41	5	46	0	6	0	0	42	52	46,0	1,5
2.......	74	40	40	22	62	0	1	1	6	41	69	55,0	0,5
3.......	74	52	52	2	54	0	8	0	0	52	62	57,0	4,5
4.......	74	50	50	5	55	0	10	0	0	50	65	57,5	1,0
5.......	75	39	38	5	43	0	2	0	0	39	45	42,0	8,0
6.......	75	61	61	18	79	0	1	0	0	61	80	70,5	2,5
7.......	75	66	66	3	69	0	8	0	1	66	78	72,0	1,5
8.......	75	62	62	4	66	2	11	0	0	64	77	70,5	1,0
9.......	76	39	39	13	52	0	4	1	4	40	60	50,0	7,0
10.......	76	67	66	3	69	0	1	0	5	67	75	71,0	4,5
11.......	76	34	34	20	54	0	7	0	0	34	61	47,5	7,5
12.......	76	53	53	11	64	0	12	0	0	53	76	64,5	1,0
Mean....	75	50,4	50,2	9,3	59,4	0,2	5,9	0,2	1,3	50,8	66,7	58,6	3,4
S.D.***...	—	11,6	11,7	7,3	10,5	0,6	4,1	0,4	2,3	11,6	11,1	10,9	2,8
C.V. (%)**	—	23,0	23,3	78,5	17,6	300,0	69,5	200,0	176,9	22,8	16,6	18,6	82,4
Total...	—	605	602	111	713	2	71	2	16	609	800	—	—

* Days between infestation and death   ** Coefficient of variation   *** Standard deviation

TABLE 2 Comparative worm sizes and numbers of intra-uterine ova

Dimension and site of worms	Worm measurements and numbers of ova					
	Sheep 1–12 (infested with ♂+♀ worms for 74–76 days)		Sheep 13 (infested with ♀ worms only for 71 days)		Sheep 14+15 (infested with ♀ worms only for 134 days)	
	Mean	S.D.†	Mean	S.D.†	Mean	S.D.†
Lengths (mm)						
Mesentery (large ♀)	21,8	2,0	—	—	—	—
Mesentery (small ♀)	9,7	1,8	7,5	0,8	11,5	1,7
Liver	9,9*	1,9	6,1	0,7	9,4	2,3
Lungs	7,1	2,4	5,8	1,0	8,5	2,8
Breadths (μm)						
Mesentery (large ♀)	250	29	—	—	—	—
Mesentery (small ♀)	171	40	158	20	231	41
Liver	164*	23	174	19	227	54
Lungs	136	26	152	7	—	—
Ova in uterus (number)						
Mesentery (large ♀)	23,0	15,0	—	—	—	—
Mesentery (small ♀)	5,8	10,6	0	0	2,0	3,0
Liver	3,4*	6,1	0	0	0,7	1,4
Lungs	0,8	3,0	0	0	—	—

† Standard deviation

* Two large ♀ worms (one broken and the other 18 mm in length, 220 μm in breadth and containing 6 ova in the uterus) recovered from the liver of Sheep 8, were disregarded for calculation of these mean values

TABLE 3 The number of worms measured or examined for ova for the data summarized in Table 2

Locality	Sheep		
	1–12	13	14–15
Mesentery (large ♀)	369	—	—
Mesentery (small ♀)	99	53	94
Liver	50	46	92
Lungs	16	8	36

With one exception (Sheep 8), all females from the livers were small, having a mean length of 9,9 mm (range 6–13), 6,1 mm (range 4–8) and 9,4 mm (range 3–13) in Sheep 1–12, 13 and 14 & 15 respectively. One of the large females from the liver of Sheep 8 was 18 mm in length; the other was broken and could not be measured.

The mean length of worms from the lungs was 7,1 mm (range 4–12) in Sheep 1–12; 5,8 mm (range 4–7) in Sheep 13 and 8,5 mm (range 4–13) in Sheep 14 and 15.

Worm breadth

The mean breadth of the large female worms recovered from the mesentery of Sheep 1–12 was 250 μm (range 146–323, see Table 2 and Fig. 4–6), whereas that of the corresponding small females was 171 μm (range 73–322). The mean breadth of the females from Sheep 13 was 158 μm (range 117–205) and from Sheep 14 and 15 was 231 μm (range 117–307).

The mean breadth of female worms from the livers of Sheep 1–12, Sheep 13 and Sheep 14 and 15 was 164 μm (range 117–220), 174 μm (range 132–220) and 227 μm (range 88–337) respectively, while that of worms from the lungs was 136 μm (range 88–190) and 152 μm (range 146–151) for Sheep 1–12 and Sheep 13 respectively. The breadth of worms from the lungs of Sheep 14 and 15 could not be measured because the worms were damaged after the lengths had been determined.

The breadth of one of the 2 large female worms from the liver of Sheep 8 was 220 μm.

TABLE 4 Infestation and worm recovery data of sheep 13–15 infested with ♀ worms only

Sheep No.	Interval between infestation and autopsy (days)	Cercariae* (No.)	Worm development		Worm distribution (%)		
			Number of worms	%* development	Mesentery	Liver	Lungs
13	72	1 500	742	50	10,7	88,1	1,2
14	137	960	732	76	61,4	36,1	2,5
15	134	1 040	930	89	19,0	75,4	5,6

* Because small numbers of cercariae were available, the infective doses could not accurately be determined (Van Wyk & Groeneveld, 1973)

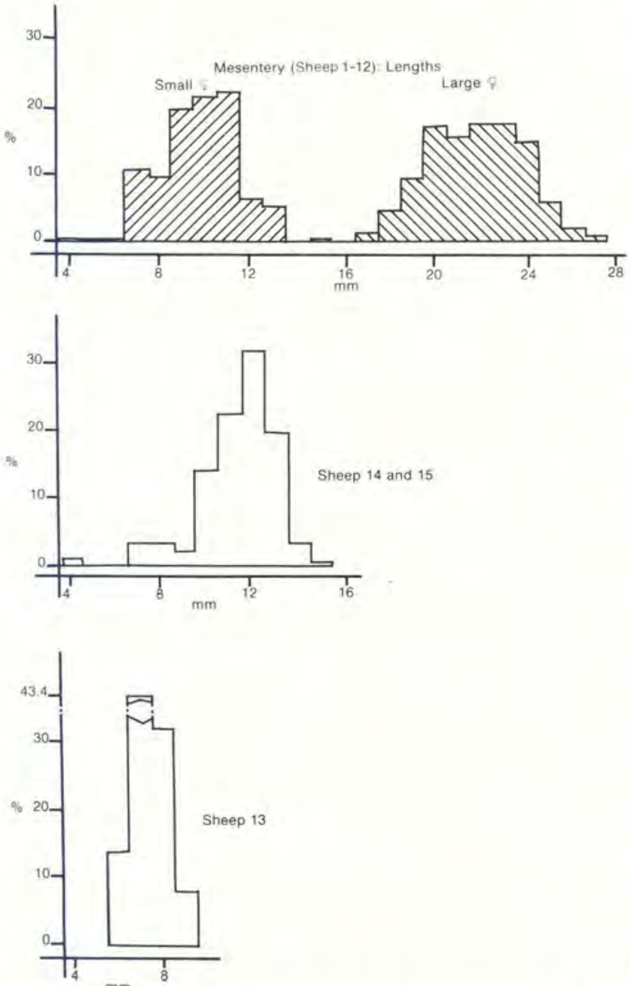

FIG. 1 Frequency distributions of the lengths (mm) of the female (♀) worms recovered from the mesentery. The numbers of worms measured are as follows:

Sheep 1–12............. small ♀ 111; large ♀ 602
Sheep 13............... total ♀ 53
Sheep 14+15........... total ♀ 94

Intra-uterine ova

The mean number of ova in the large females from the mesentery of Sheep 1–12 was 23,0 (range 0–72), while that of the corresponding small females was 5,8 (range 0–83, Table 2 and Fig. 6–9). All the females from the liver and lungs of these sheep were small and contained a mean number of 3,4 (range 0–22) and 0,8 (range 0–12) ova respectively. The mean intra-uterine egg counts of worms from the various organs of Sheep 13 and Sheep 14 and 15 (infested with female worms alone) ranged from 0–1,8 (range 0–13). Only 1 ovum was found in 107 females from Sheep 13, infested 71 days before autopsy, and a total of 227 ova was counted in 186 females from Sheep 14–15, infested 134–137 days before autopsy.

Sheep 8

In addition to 9 small female worms, a single intact large female and another that was broken, were recovered from the liver of this sheep; it was also the only sheep which had any male worms (2) in the liver (Table 1).

Details of worm recovery from Sheep 13–15 are listed in Table 4. The percentage of cercariae that developed to worms was approximately 50 % for Sheep 13, 76 % for Sheep 14 and 89 % for Sheep 15 (Table 4).

DISCUSSION

In contrast to most infestations with *S. mattheei* where the sexes of cercariae are not determined before infestation (Van Wyk *et al.*, 1975; Van Wyk *et al.*, 1976), in this trial highly significantly more female than male worms developed when sheep were exposed to equal numbers of cercariae of each sex. The overall percentage development (58,6 % ± 10,9 %) was similar to that of previous trials in this laboratory where 63,8 % ± 13,8 % (Van Wyk *et al.*, 1975) and 57,2 % ± 3,4 % (Van Wyk *et al.*, 1976) *S. mattheei* cercariae developed.

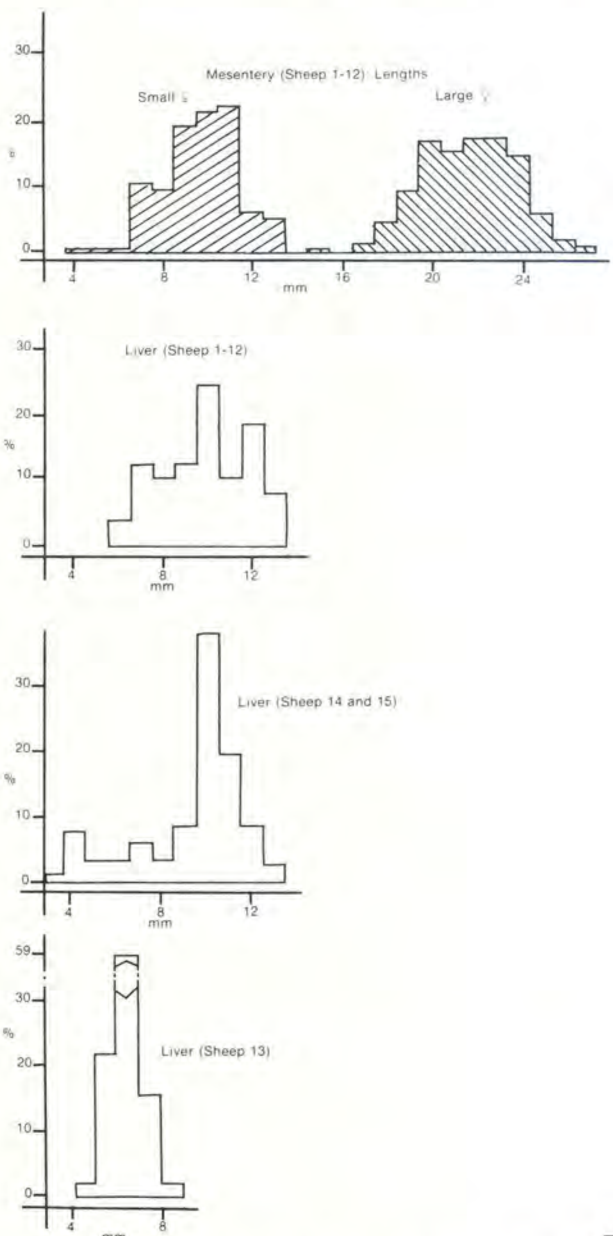

FIG. 2 Frequency distributions of the lengths (mm) of the ♀ worms recovered from the liver, compared with those from the mesentery of Sheep 1–12. The numbers of worms measured are as follows:

Sheep 1–12 (mesentery)... small ♀ 111; large ♀ 602
Sheep 1–12 (liver)........ total ♀ 50
Sheep 13 (liver).......... total ♀ 46
Sheep 14+15 (liver)...... total ♀ 92

There is a possibility that this significant difference in development of male and female cercariae may be due to differences in the physiological condition of the 2 host snails, as surmised by Stirewalt & Fregeau (1968), and not to the sex of the cercariae *per se*. Nevertheless, in the light of an overall percentage development similar to that of previous trials, these results support the hypothesis of Van Wyk *et al.* (1975) that the usual preponderance of *S. mattheei* males is probably due to a higher percentage of male cercariae in collections from a large number of snails rather than to a greater infectivity of male cercariae. A more satisfactory test could utilize male and female cercariae pooled from a large number of snails to minimize the effect of possible variations in the infectivity of cercariae from different snails.

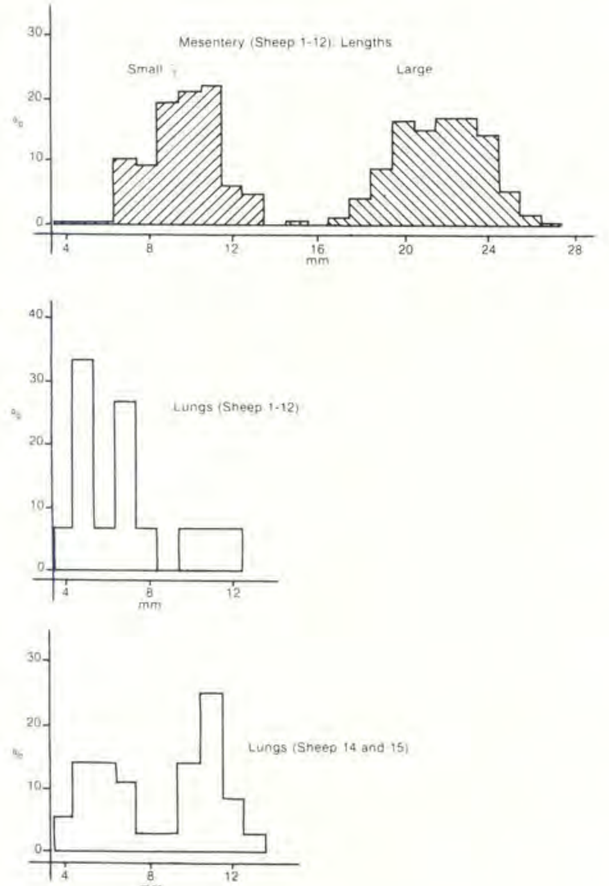

FIG. 3 Frequency distributions of the lengths (mm) of the ♀ worms recovered from the lungs, compared with those from the mesentery of Sheep 1–12. The numbers of worms measured are as follows:

Sheep 1–12 (mesentery)... small ♀ 111; large ♀ 602
Sheep 1–12 (lungs)....... total ♀ 16
Sheep 13 (lungs)......... total ♀ 8
Sheep 14+15 (lungs)..... total ♀ 36

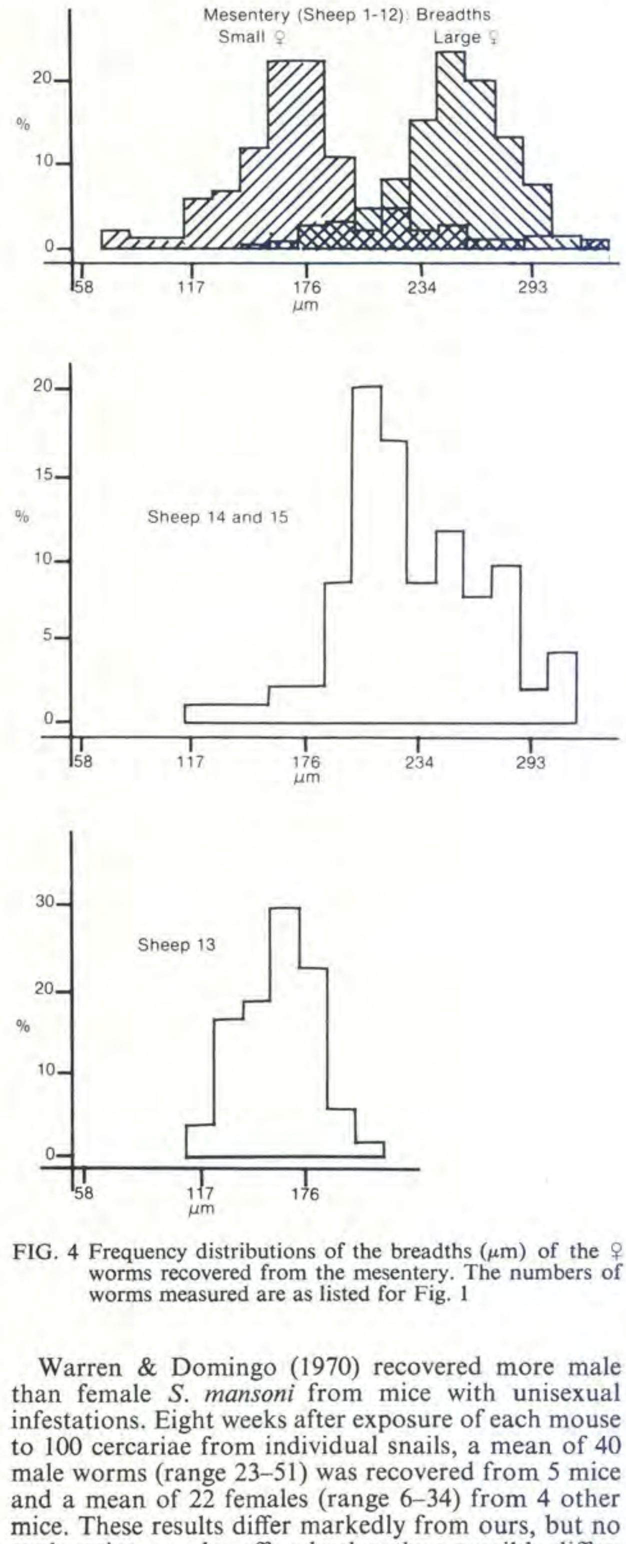

FIG. 4 Frequency distributions of the breadths (µm) of the ♀ worms recovered from the mesentery. The numbers of worms measured are as listed for Fig. 1

Warren & Domingo (1970) recovered more male than female *S. mansoni* from mice with unisexual infestations. Eight weeks after exposure of each mouse to 100 cercariae from individual snails, a mean of 40 male worms (range 23–51) was recovered from 5 mice and a mean of 22 females (range 6–34) from 4 other mice. These results differ markedly from ours, but no explanation can be offered other than possible differences between the 2 species.

Bisexual infestations were established in the present trials (Sheep 1–12) to counter any possible effect that the absence of one sex may have on the establishment and development of the other. Nevertheless, this precaution does not appear to have played a role in the trials of Warren & Domingo (1970) and Rowntree & James (1977) where the respective mean development of approximately 30% and 34–47% cercariae of *S. mansoni* in unisexual infestations was better than a mean of 22,1% obtained by Stirewalt *et al.* (1951) with bisexual infestations in 489 albino mice.

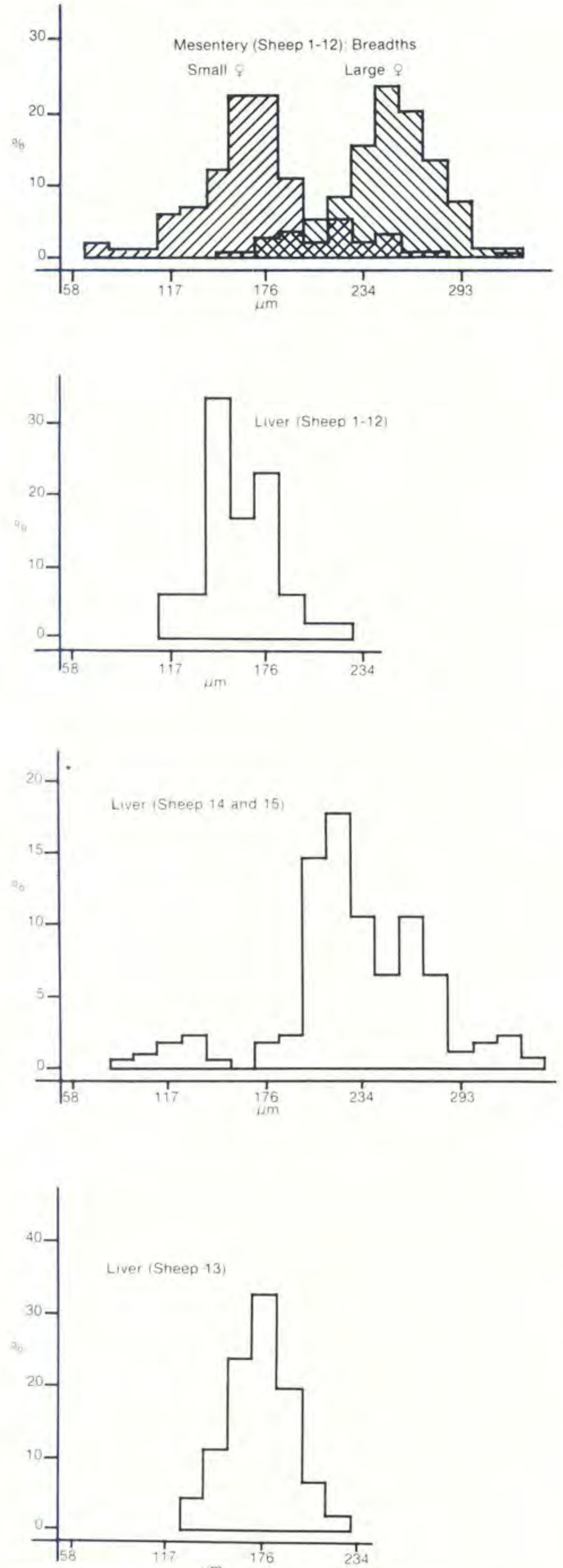

FIG. 5 Frequency distributions of the breadths of the ♀ worms recovered from the liver, compared with those from the mesentery of Sheep 1–12. The numbers of worms measured are as listed for Fig. 2

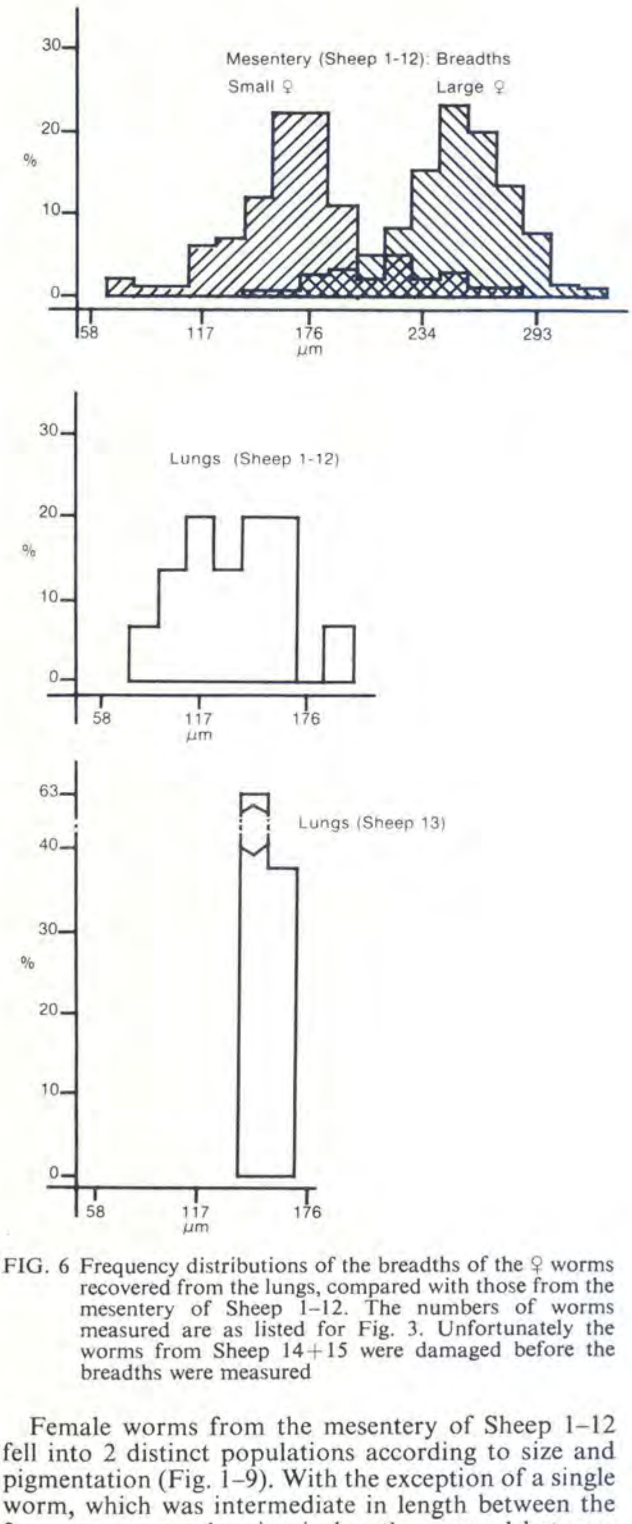

FIG. 6 Frequency distributions of the breadths of the ♀ worms recovered from the lungs, compared with those from the mesentery of Sheep 1–12. The numbers of worms measured are as listed for Fig. 3. Unfortunately the worms from Sheep 14+15 were damaged before the breadths were measured

Female worms from the mesentery of Sheep 1–12 fell into 2 distinct populations according to size and pigmentation (Fig. 1–9). With the exception of a single worm, which was intermediate in length between the 2 groups, no overlapping in length occurred between the 2 female worm populations from this site. On the other hand, although the mean breadth and numbers of intra-uterine ova differed markedly between the groups, there was considerable overlap in the ranges of these values.

James & Webbe (1975), working with *Schistosoma haematobium*, reported that: "Unpaired female worms of the South African strain were consistently stunted." A similar situation therefore possibly exists in this worm species. Unfortunately, however, no worm measurements of the "stunted" worms were reported by these authors, nor did they mention their criteria for classifying these worms as such.

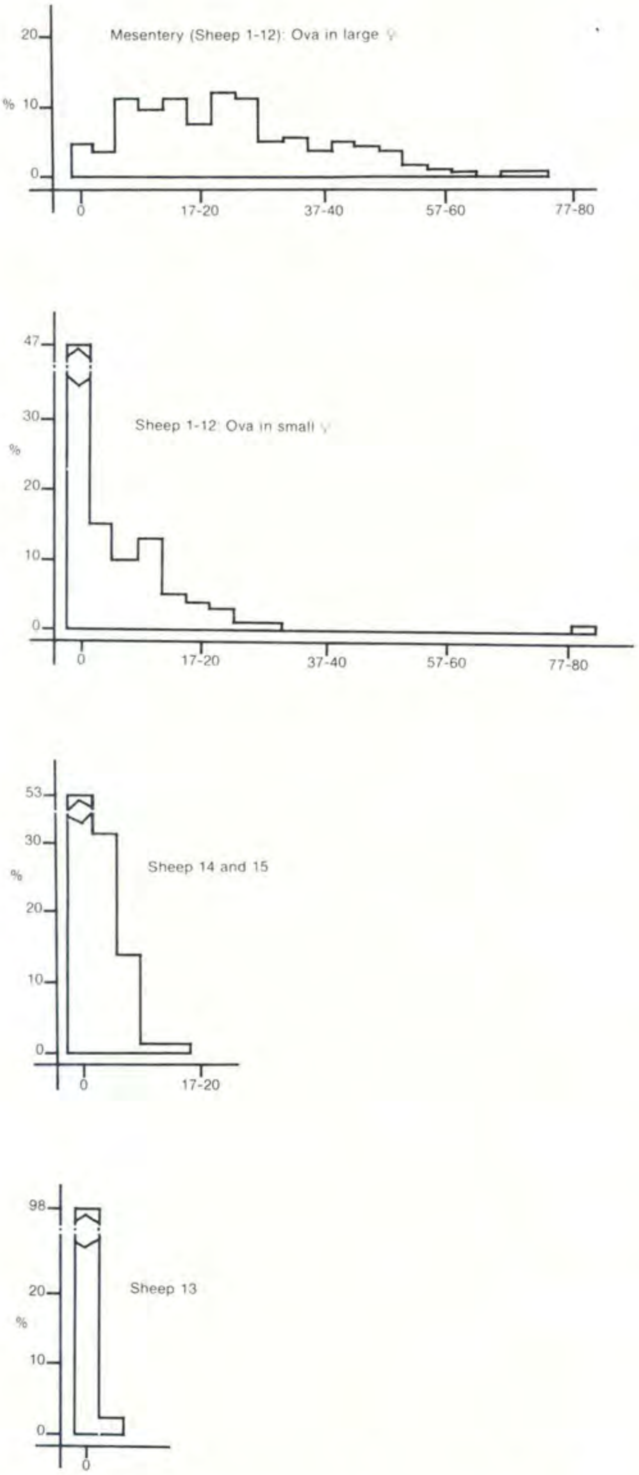

FIG. 7 Frequency distributions of the numbers of ova in the ♀ worms recovered from the mesentery. The numbers of worms examined are as listed for Fig. 1

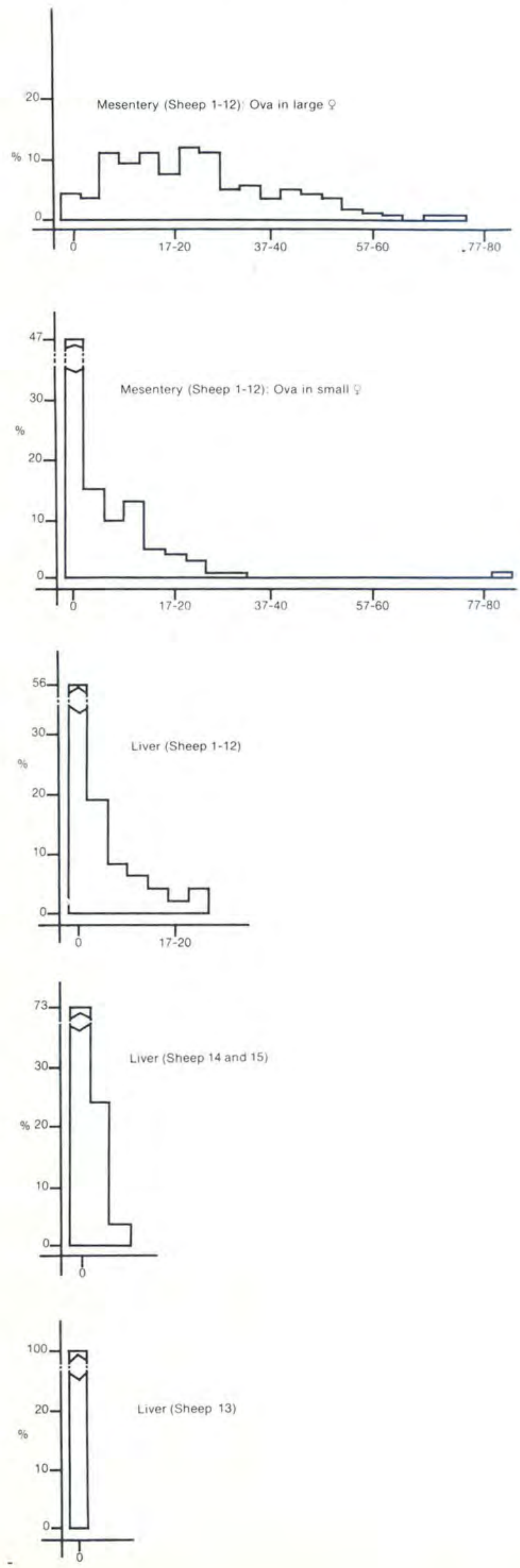

FIG. 8 Frequency distributions of the numbers of ova in the ♀ worms recovered from the liver, compared with those from the mesentery of Sheep 1–12. The numbers of worms examined are as listed for Fig. 2 →

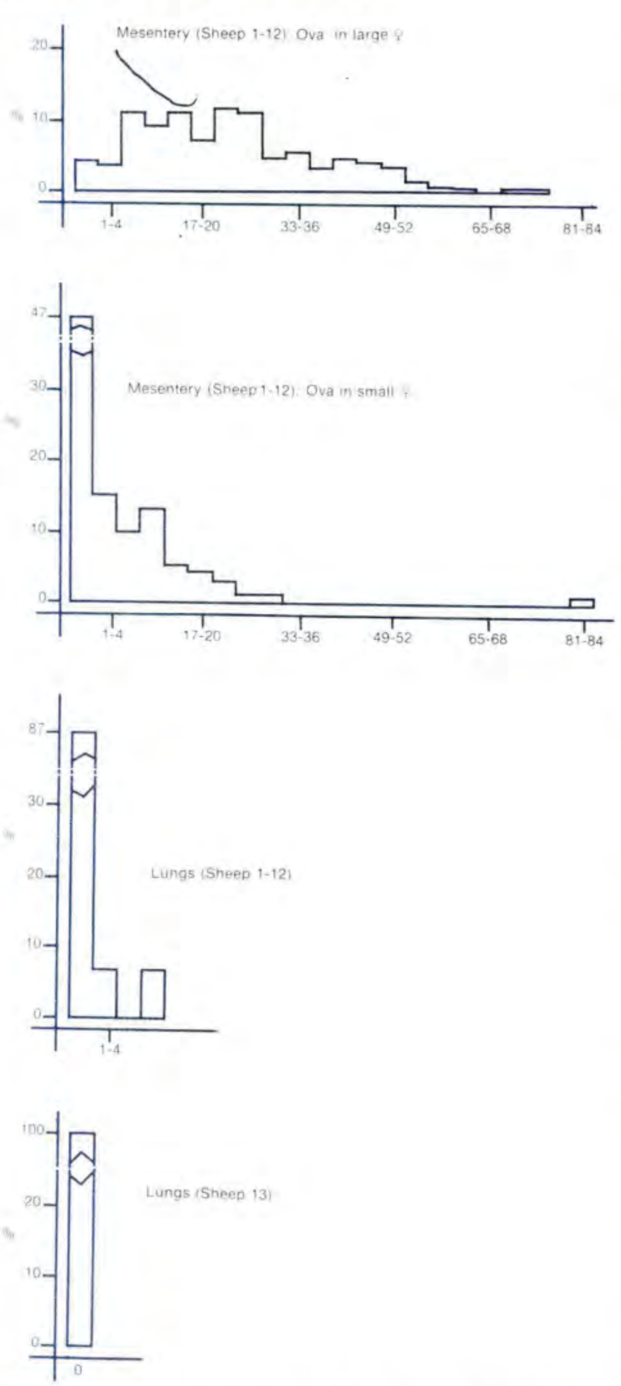

FIG. 9 Frequency distributions of the numbers of ova in the ♀ worms recovered from the lungs, compared with those from the mesentery of Sheep 1–12. The numbers of worms examined are as listed for Fig. 3

It is interesting that the number of fully-developed females from the mesentery closely approximates that of the males from this site. Not all worms are removed by perfusion (Foster, Cheetham & Mesmer, 1968; Van Wyk *et al.*, 1975; Van Wyk *et al.*, 1976) and males are very difficult to see through the vessel wall, since, unlike the female worm, they have no prominent dark grey caeca. Considering these difficulties, the similarity in the numbers of worms of each sex probably means that the number of female worms that become fully developed depends directly on the number of males present. Supportive evidence is the recovery of 2 large females from the liver of the only sheep which had any male worms (2) in the liver.

It seems possible, therefore, that the fully-developed females were more closely associated with the males than the underdeveloped females recovered from the same sites of these sheep. In this respect, it is well known that female schistosomes do not develop fully unless or until the host animal is infested with male schistosomes as well (Moore *et al.*, 1954; Taylor, 1971).

A comparison of these large and small females from the mesentery of Sheep 1–12 with those from the liver and lungs of the same sheep and those from the unisexual infestations in Sheep 13–15 casts more light on this matter.

Lengths

Worms from all the unisexual infestations as well as those from the liver and lungs of Sheep 1–12 were similar in length to the group of small mesenteric females (Fig. 1–3). In only 1 case (old unisexual infestation, Sheep 14 and 15) was there any overlap with the large mesenteric females. The mean length of the small females from the mesentery was intermediate between that of the young and old unisexual infestations (Table 2).

Breadths

The breadths of the different worm populations overlapped much more than the lengths. While the mean breadth of the small mesenteric females was very similar to that of the younger unisexual infestation (mesentery), the mean breadth and range of breadths of worms from the older unisexual infestations were very similar to those of the large mesenteric females (Fig. 4–6).

The breadths of worms originating from the livers of the sheep were similar to those from the mesentery.

While no worms from the lungs of the older unisexual infestation were measured or examined for ova, the few worms recovered from the lungs in the younger infestation were similar in size to the females from the liver and to the small females from the mesentery of the 12 sheep.

Intra-uterine ova

Worms from bisexual infestations (mesentery, liver and lungs) had much higher mean egg counts and ranges in egg counts (Table 2, Fig. 7–9) than their counterparts from unisexual infestations. Similarly, large females had many more eggs per worm than small females. Mean egg count per small female decreased in the following organ sequence: Mesentery, liver, lungs. Females from the livers of Sheep 1–12 contained more intra-uterine eggs than the worms from the mesentery in the unisexual infestations.

With the exception of most of the ova from the large females in Sheep 1–12, the intra-uterine ova did not appear viable. These results, including the occurrence of ova in female *S. mattheei* from unisexual infestations, confirm the findings of Taylor, Amin & Nelson (1969) and Taylor (1971).

It seems that male worms in these sheep either did not change partners for their calculated copulation period of 40 days or exchanged only for females of similar maturity; otherwise a group of females of "intermediate" size would have been found in the mesenteries of the 12 sheep. Furthermore, the stimulus for female worms to develop to full maturity appears to be physical contact with the male worms and not merely contact with their secretory and/or excretory

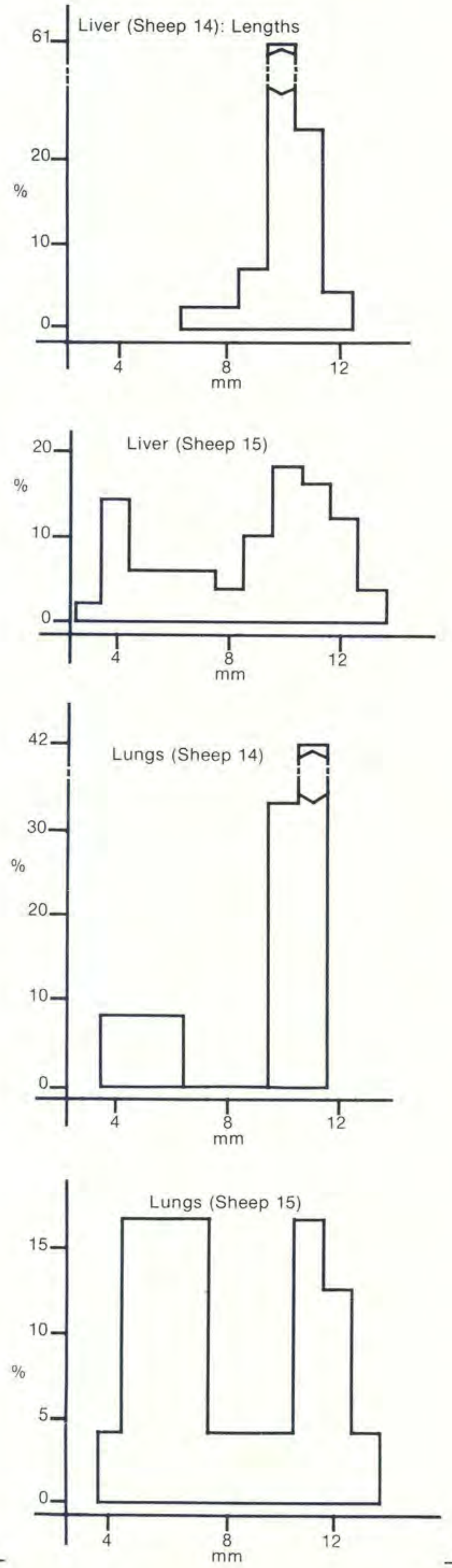

FIG. 10 Comparative frequency distributions of the lengths (mm) of ♀ worms recovered from the livers and lungs ← of Sheep 14 and Sheep 15. The numbers of worms measured are as follows:

Sheep 14 (liver).........	43
Sheep 14 (lungs)........	12
Sheep 15 (liver).........	49
Sheep 15 (lungs)........	24

products, otherwise the (unpaired) worms in the liver would have been more mature in development at the time of slaughter, as were most of the females from the mesentery. Fan (1971) and Erasmus & Shaw (1977) drew similar conclusions from observation of worms from experimental infestations and Shaw (1977) from *in vitro* experiments. Problems in verifying this in the present trials are:

1. Since, unfortunately, the similarity in numbers of large female and male worms was noticed only after they had been separated, it was impossible to know which worms were *in copula* when recovered.
2. The possibility that at least some of the small females in the mesentery did copulate at times cannot be excluded.

Perhaps some of the underdeveloped females shared male worms with the large females at times. Fan (1971), for instance, reported the occurrence of up to 4 mature *S. mansoni* females in the gynaecophoric canal of a male and often encountered both large and small females concurrently in one and the same male worm. The relatively large numbers of intra-uterine ova recorded from the small females from the mesentery of the 12 sheep compared with the counts from the unisexual infestations support this possibility. On the other hand, secretory and/or excretory products from the male worms in the hosts' blood circulation may have played a role without any direct physical contact having taken place.

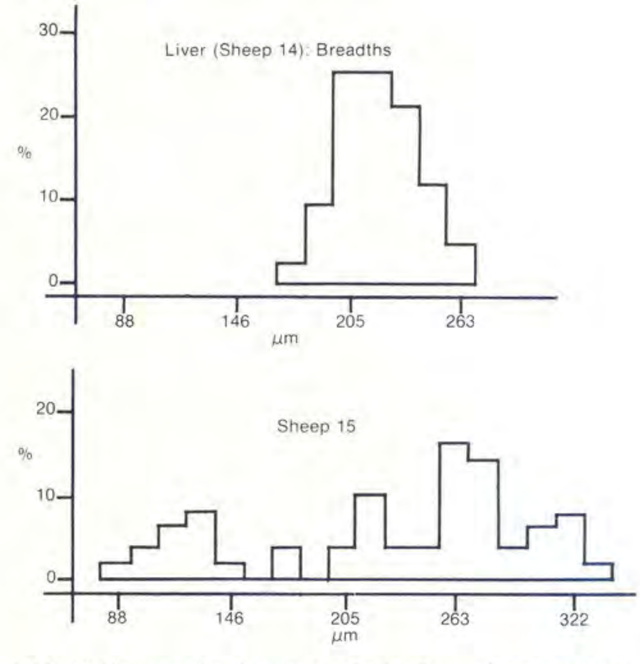

FIG. 11 Comparative frequency distributions of the breadths (μm) of the worms recovered from the livers of Sheep 14 and Sheep 15. The numbers of worms measured are 43 and 49 from the livers of Sheep 14 and 15 respectively

There appear to be 2 size populations of female worms in the unisexual infestations in Sheep 14 and 15 (Fig. 2 & 3). Comparison of the lengths of the worms from the livers and lungs of Sheep 14 and 15 (Fig. 10) shows that 2 size populations occurred only in Sheep 15. A biphasic histogram representing the breadths of females from the livers of these 2 sheep (Fig. 11) also has its origin in the variation in the breadth of the worms of Sheep 15. No explanation can be suggested for this.

Our data confirm the findings of Foster *et al.* (1968) and Fan (1971) that small female worms may be encountered in the mesentery, since they were found by us in this site in both single sex and bisexual infestations.

Male worms recovered in the present investigations were measured for comparison with worms from other trials. However, no comparable differences in size were noticed as with the females and their dimensions are disregarded in this paper.

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