?

Studies on variation ~~in~~ of eggshape in

SCHISTOSOMA HAEMATOBIUM

(Bilharz, 1852)

(Schistosomatidae; Trematoda)

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ABSTRACT.

The relationships of members of the family Schistosomatidae Looss, 1899, emnd. Poche, 1907, are briefly described and the relevant literature on variation in parasitic helminths in general and in the schistosomes in particular is surveyed, together with some of the available methods of statistical analysis.

Techniques used in the maintenance of strains of Schistosome haematobium in the laboratory and the preparation of the material for examination are given in detail, together with the procedure adopted for the recovery and measurement of eggs of the parasites. A new method for the measurement of eggs is described.

Techniques employed in the observations on living worms and in their histological examination are described and the methods of statistical analysis used are given in detail.

Results of the histological examinations and observations on living worms are presented and discussed,

and the formation of malformed eggs is discussed in detail.

The results of the measurements of over 5,300 eggs from 70 laboratory hosts and from man are given and are analysed and discussed in detail, and the conclusion is drawn as a result that the material showed a great deal of non-random variation.

The reduction in size of adult worms and eggs recovered from experimental hosts is described and discussed.

Factors likely to affect egg shape and host-parasite relationships are suggested in general terms and the need for further work on the subject emphasised. Attention is drawn to the effect of the host upon the parasite, and the dangers of describing new species of parasites from material not comprehensively studied are emphasised.

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SECTION 1 - INTRODUCTION.

INTRODUCTION.

The schistosomes are exclusively parasites of the venous blood systems of their vertebrate hosts (either birds or mammals) during the adult stage, and are generally found in the abdominal vessels. They have various species of aquatic molluscs as intermediate hosts; the sexes are separate and there is considerable sexual dimorphism in the adult stages.

The male worm is generally much stouter than the female, although the latter is generally the longer. The female is retained in the gynaecophoric canal of the male, formed by the infolding of the lateral body margins to form a tube-like structure. Eggs of fairly characteristic size and shape are laid by the female, and escape through the walls of the host blood vessels to either the intestinal or bladder lumen or into the nasal cavity in the case of Schistosoma nasalis in cattle, whence they are passed out to the exterior. The eggs hatch in water to liberate a ciliated miracidium, the sex of which is already determined, and which must locate and penetrate the appropriate host

snail within about 24 hours. Development in the snail takes several weeks and results eventually in the liberation of large numbers of free-swimming, forked-tailed cercariae. These have a characteristic behaviour and appearance, and will actively penetrate the skin of any mammal or bird (as appropriate) frequenting that body of water (e.g. for drinking, feeding, or washing). The cercariae penetrate the skin of the mammal or bird but are only successful in entering the blood system of the correct definitive host, where subsequent development to the adult form (either male or female) takes place. The worms pair off and travel from the liver, where the final developmental stages occur, to the definitive site, whereupon the female begins to lay eggs. The whole cycle takes several months to complete.

A number of schistosomes is known from birds, and these are of little interest beyond the fact that the cercariae are capable of causing a characteristic form of dermatitis (swimmer's itch) in exposed persons; there is no development of the cercariae in the mammal, the cercariae being rapidly destroyed in the under-lying layers of the skin.

Although a number of schistosomes have been described during the past hundred years or so, there are probably less than twenty well authenticated species from mammals which have a wide acceptance, the remainder being regarded as synonyms.

The most widely accepted classifications of the mammalian forms includes all the latter in the genus Schistosoma. Within this genus there are three comparatively distinct groups, namely (1) that containing Schistosoma japonicum alone, (2) the Schistosoma mansoni group, and (3) the Schistosoma haematobium group. Of these, the latter is by far the most complex and contains the majority of the forms known to occur in Mammalia. Each group contains one or more forms of great importance to man, as well as others found in wild or "domestic" animals.

1) Schistosoma japonicum group. Schistosoma japonicum is the only member of this group and is confined to the Far East. It is widespread in Japan, Formosa, China and the Phillipine Islands, and it infects man, his domestic animals cattle, water buffaloes, pigs, rodents, etc. The Eggs are typically almost spherical, with or without a small

lateral spine. The intermediate hosts are snails of the genus Oncomelania. This form always produces intestinal symptoms, and is never associated with the blood vessels of the bladder. There is accumulating evidence that each of the four geographic regions has a morphologically and physiologically characteristic strain of schistosome but such differences are only slight. This species of schistosome is the only one occurring in man in which reservoir hosts are definitely known to occur as a regular part of the life cycle.

2) Schistosoma mansoni group. This group is more complex than the S. japonicum group, and its members occur in Central and South America, Africa, the Middle East, and India. Schistosoma mansoni itself is widespread in Africa and Central and South America, having man as its definitive host in these areas. The eggs are characteristically pear-shaped with a stout lateral spine. The intermediate hosts are snails of the genera Australorbis (S. America), and Biomphalaria (Africa). S. incognitum from India occurs in pigs and dogs but not so far as is known in man, and S. rodhaini and S. margrebowiei from Africa in rodents and wild ruminants respectively. The form S. mansoni var. rodentorum

from wild rodents in Africa is of doubtful validity. All the members of this group cause intestinal symptoms in their definitive hosts.

3) Schistosoma haematobium group. This is both the largest and most complex of the three groups, both from the schistosome point of view and from that of the intermediate snail hosts. All the members of this group are characterised by more or less elongate eggs with a fairly to very conspicuous terminal spine; several members are known to infect man. The most important member of this group is Schistosoma haematobium itself, occurring in central and northern Africa, Arabia, and in some areas around the Mediterranean Sea. The intermediate hosts are snails of the genus Bulinus, except for a very small isolated focus on the west coast of India, where the ancylid snail Ferrissia tenuis has been reported to be the intermediate host, and another focus in southern Portugal where Planorbarius metidjensis apparently acts as intermediate host. Schistosoma haematobium causes typical urinary lesions in man, and inhabits the veins in the bladder wall. An apparently morphologically identical schistosome, also causing typical urinary symptoms, but having a different intermediate host (snails of the sub-genus Bulinus

(Physopsis)) is found in the western half of Africa. A form possibly slightly morphologically distinct from the above is found in the southern part of Africa. This form, S. capense, also has as its intermediate host B. (Phyopsis), and again gives rise to typically urinary symptoms. So far as is known these forms are confined to man. S. bovis, on the other hand is principally a parasite of cattle and other ruminants, and is found in Africa, Arabia, and India. This species is quite similar to the previous members of this group but has larger and more spindle-shaped eggs. There are somewhat doubtful records of its occasional occurrense in man, but it is primarily a parasite of cattle. Very similar to this form is S. mattheei, an intestinal parasite of sheep and cattle in central Africa, which is also believed to oocur occasionally in man, apparently causing vesioal symptoms. The eggs of this form are intermediate between the rounded shape of S. haematobium eggs and the larger spindle shaped eggs of S. bovis. Both S. bovis and S. mattheei have snails of the sub-genus Bulinus (Physopsis) as intermediate hosts; S. bovis also utilises Bulinus itself. The position of S. mattheei relative to S. bovis is similar in some respects to that of S. capense to S. haematobium, and parallels the situation found in the

snail hosts Bulinus and B. (Physopsis). A fifth species of more doubtful authenticity is S. intercalatum, which appears to lie somewhere between S. haematobium and S. capense on the one hand and S. mattheei on the other. This form is again B. (Physopsis) - borne, but the eggs are more elongate than those of S. haematobium and less spindle shaped than those of S. mattheei. S. intercalatum, if such a form really exists as a distinct species, causes intestinal bilharziasis in humans. S. leiperi is a small form with large elongate eggs from wild ruminants in Africa. Much detailed work is still needed to determine the relationship of these forms.

Such species as S. spindalis and S. indicum from India are parasites causing intestinal symptoms in cattle, sheep, horses, etc. and have characteristically large eggs which render them reasonably distinct from the above group of African forms.

The species of schistosome, their intermediate hosts, distribution, and definitive hosts are set out in Table 1, pages 23, and 24.

Table 1.

SPECIES OF SCHISTOSOMES OCCURRING IN MAMMALS.

Strain	Hosts (intermediate)	Distribution	Definitive hosts
1. S. haematobium (Bilharz, 1852)	B. (Physopsis) africanus globosus Bulinus	Africa C. Africa Middle East	Man
2. S. capense (Harley, 1864)	B.(Physopsis)	S. Africa	Man
3. S. bovis (Sonsino, 1876)	B. (Physopsis) Bulinus	Africa, India.	Cattle
4. S. japonicum (Katsurada, 1904)	Onoomelania	Far East	Man & domestic animals.
5. S. indicum (Montgomery, 1906)	Indoplanorbis	India	Cattle
6. S. spindalis (Montgomery, 1906)		India Cen. Africa	Cattle, horses wild ruminants
7. S. mansoni (Sambon, 1907)	Australorbis Biomphalaria	S. America Africa	Man
8. S. incognitum Chandler, 1926	Indoplanorbis (probably)	India	Pigs, dogs.
9. S. matthesi Veglia & LeRoux, 1929	B. (Physopsis) africanus & globosus	S. & Cen. Africa	Cattle, sheep ? man
10. S. curassoni Brumpt 1931 (? = S. matthesi or S. bovis)			Cattle
11. S. rodhaini Brumpt, 1931	Biomphalaria pfeifferi	Cent. Africa	Rodents ? man

Strain	Intermediate hosts	Distribution	Definitive hosts
12. S. margrebowiei LeRoux, 1933	Unknown at present	Africa	Cattle, wild ruminants
13. S. nasalis Rao, 1932 Or 1935		India	Cattle
14. S. suis Rao & Ayyer, 1933 (= S. incognitum Chandler.)			
15. S. intercalatum Fisher, 1934	B. (Physopsis) africanus globosus	Central Africa	Man (?)
16. S. mansoni var. rodentorum Schwetz, 1953	Biomphalaria pfeifferi & sudanica	"	Rodents
17. S. leiperi LeRoux, 1955	Bulinus (Physopsis)	"	Cattle, sheep wild ruminants

The distribution and variation of the schistosomes themselves and of their intermediate hosts is still far from well defined, and much work is needed to determine the relationships of this very important complex of parasites and their hosts. This is especially true of the S. haematobium group.

There is a pressing need for a detailed, critical analysis of eggs and their shape in the schistosomes because the worms themselves cannot be obtained in human infections until after death, and the eggs are the only accessible direct evidence of the presence of the parasite. There is thus a need for the experimental investigation of egg shape in order to help all who deal with this disease to understand the nature of the material with which they are dealing. As LeRoux (1961) has remarked, too much reliance cannot be placed on egg morphology until more is known about the range of variation of egg shape and factors affecting it. The relationships of the various members of the Schistosoma haematobium complex must also be elucidated and an attempt made to determine whether the newer species found in wild and domestic animals, especially in Africa (e.g. S. mattheei, S. intercalatum, etc.) are of importance in human infections.

Statistical methods of distinguishing between possibly morphologically distinct taxonomic groups can be used where other more conventionsl techniques can not be applied. This is particularly the case where the groups under investigation show an apparently continuous range of variation in features which cannot be described accurately in a purely qualitative manner yet can be measured and are possibly of importance. The object of the work described was to determine whether populations of the same species of schistosomes drawn from different geographical areas vary in morphology, and if so, under what conditions, if any, alteration of these variations could be induced. Attempts were also made to define the limits within which these intra-specific variations occurred.

SECTION 2 - HISTORICAL.

HISTORICAL

In a recent paper LeRoux (1961) stated that "the identification of Schistosoma species with absolute certainty is wellnigh an impossibility if the criteria employed are the shape and size of the eggs recovered from faeces". Similar views were expressed by Buchanan (1937) when he said that "reliable diagnosis cannot be made on egg form and measurements as such may show marked variation even in the same individual", and more recently Nelson et al (1962) and Hairston (1962) are among those who have drawn attention to and described the effects of the host environment ("physiological environment") on the schistosomes, and particularly the eggs. Nelson et al (1962) gave a warning that much more caution is required in giving names to terminal-spined eggs. It appears at least theoretically possible for the range of variation of one so-called species to overlap that of another.

Cram (1955) and Wright (1962) have summarised the evidence of strain differences in the schistosomes parasitising man and in their intermediate hosts, and Wright (1960 & 1962) has discussed the probable mechanisms involved in the production of these strain differences. Hsu and co-workers have demonstrated in a series of papers (Hsu & Li, 1957; Hsu and Hsu, 1958a & 1958b)

that four geographic strains of S. japonicum exist, each corresponding to a different species of snail host, and that each of these four strains has developed its own characteristics. McCullough (1959) has reported a most striking example of strain-specificity in S. haematobium. Wright (1962) has amplified this work and has described experiments on the cross-susceptibility of S. haematobium and strains of Bulinus spp.

Forsyth (1958) has ascribed variation in the results of drug treatment of S. haematobium and S. mansoni in Kuwait to variations in the parasites themselves.

There appears to be no reason why variations should be confined to the three principal schistosomes parasitising man, and it may well be found on further investigation that the same principle applies in other schistosome-snail relationships.

In a long paper written in 1923, Chandler stated that the speciation of parasites and their relationships have presented some of the most difficult and most interesting problems in the field of parasitology, and that these problems are of prime importance in connexion with the control and prophylaxis

of a number of important infections of man and domestic animals. Chandler continued by expressing the opinion that all parasites undergo slight physiological changes in order to become acclimatised to different environments, and that such changes may have to be present before the new environment can be invaded, or alternatively may be developed in response to exposure to the new environment. He considers that physiological changes are inevitably connected with morphological changes, although the latter may not necessarily be obvious. In considering the possible mechanism by which these physiological and morphological changes are brought about Chandler emphasises that it is necessary to distinguish between somatic acclimatisation and genetic modification. He suggests that it is possible that not all modifications of parasites in different habitats are due to segregation and survival of favoured genetic races. Nevertheless, the segregation of adaptable races stands out as the most probable and most frequent factor involved in the formation of the "hostal" races.

Chandler is of the opinion that whether this explanation is accepted or not, the known facts favour an extensive recognition of the widespread impurity of species in nature,

of the physiological and morphological instability of parasites and other animals and of the resultant variability of infectivity and pathogenicity. For this reason Chandler thinks that it is wiser to await positive evidence as the result of searching investigations into the constancy and nature of the so-called specific difference between proposed species of parasite living in different environmental conditions before they are recognised as distinct, well founded species. However, he adds the warning that the converse tendency to lump together parasites from different hosts or habitats without nomenclatorial distinction is just as serious a mistake.

In a more recent paper on intraspecific variation in parasitic flatworms, Stunkard (1957) elaborated on Chandler's (1923) conclusion with special reference to flatworms, and stated that "members of a single species of parasitic flatworms may develop in a number of different species of final hosts.... The size and morphology of these parasites are profoundly affected by the host-species in which the worm develops. Specimens (of Fasciola hepatica) from a cow, a rabbit and a guinea pig may manifest differences far greater than those usually employed to distinguish between species".

Stunkard is more specific in his conclusions than Chandler, and concluded that there were no adequate means of measuring intraspecific variation in parasitic flatworms, and until factors which influence the developmental and morphological features of these parasites are worked out, the precise limits of specificity would remain uncertain.

Sneath (1957) discusses the general problems and principles of classification and gives suggestions as to their approach from a more mathematical point of view, with especial reference to the bacteria, while in a later paper Sneath & Sokal (1962) review the general topic of numerical taxonomy, and give an outline of some of the methods available to workers in these fields. These authors discuss in detail many aspects of numerical taxonomy in a recent book (Sokal and Sneath, 1963). Of the three methods suggested by these authors, (Sneath & Sokal, 1962), that of co-efficient of association is not applicable here as it is generally used to refer to characters which manifest themselves as "present or absent". The second method is the correlation co-efficient technique as described by Sneath (1961), while the third method, which has been adopted here, measured the "taxonomic distance". This method uses the convention of a multi-dimensional space, with one dimension for each character under study, and although this is impossible to visualise or represent graphically for more than three characters,

the distances between the taxonomic entities can be calculated. Sokal (1961) gives the distance method in greater detail, including a worked example. There are several variations on this technique, two of which have been used in the present work. The first method involved the computation of generalised distances between the taxonomic entities and the construction of an appropriate model. In this method, the generalised distances are vectors, i.e. they have both length and direction relative to each other, and represent the relationships of the groups of organisms under study in a model space of n dimensions (in this case 4 dimensions corresponding to the four characters measured). Albrecht and Blackith (1957), Blackith (1958) and Blackith and Albrecht (1959) are among those who describe actual experiments using this method.

The second method used in the present work has been the calculation of canonical variates or vectors. The relationships of the discriminant function (generalised distance) analysis and the canonical variate approach have been outlined by Blackith (1960), who points out that although "the canonical variate approach may not bear so readily identifiable an interpretation as do the discriminant functions, a test for

the number of significant dimensions of variation is then available". The canonical variates are used to calculate the co-ordinates for a multi-dimensional graph, each dimension corresponding with a character in the sample, and to determine the number of significant sources of variation which underlie the observed values of the measured variates (dimensions).

Both methods have been used in the present work but the generalised distance approach was adopted for the majority of the work. The computations involved in both these methods are rather involved; both sets of calculations were based on a small sample of material, approximately 180 eggs being used. The recalculation of the generalised distances and the canonical variates using the complete set of over 5,300 sets of measurements would give a sharper discrimination between the groups. However, the use of a sub-optimal technique has been shown by Lubischew (1962) to be quite acceptable in the absence of a more accurate analysis.

SECTION 3 - MATERIALS AND METHODS.

MATERIALS & METHODS.

a) Materials.

Species of snails used were Bulinus truncatus (Audouin) from Shambat (Sudan), Cairo, Israel, Seyd Moshen (Iran), Tehran, Baghdad and Basrah, Bulinus truncatus rohlfsi (Clessin) from Lagoa Cabemba, Angola and from Ibadan, Nigeria, B. senegalensis Müller from the Gambia, West Africa, and B. (Physopsis) globosus (Morelet) from Kisumu (Kenya) and Amani (Tanganyika). These snails were maintained as described by Claugher (1960). The general relationships of these species are shown in Figs. 2 and 3, pages 43 & 45, and their geographical origins in Fig. 4, page 47. However, Wright (1962) has pointed out that at the specific level large numbers of forms have been named which have been shown to be spurious by recent work and he advocates the use of Mandahl-Barth's (1958) species-group concept of snail taxonomy which is biologically more realistic. Therefore the subdivisions of the genus Bulinus at the species level (Fig. 3 page 45) should not be regarded as definite and the species-group concept is largely adopted hereafter.

Schistosome haematobium strains were used from Ghana,

Dutsi (near Kano, Nigeria), Cairo, Shambat (near Khartoum, Sudan), Kisumu (Kenya), Baghdad, Tehran, Kuwait, Dirgag and Said (Aden). The geographical locations of these strains is shown in Fig. 1. page 41.

The mammalian hosts used were laboratory white mice, golden hamsters, multimammate mice (Mastomys natalensis), African grass or spiny rats (Arvicanthis abisynicus) and jirds (Meriones shawi). These were kept in shoe-box type cages either individually or as breeding pairs. They were maintained on a cubed rat diet (diet 41 of Bruce & Parkes) as supplied by Messrs. Oxoid. Oxoid diet 41B was later used to try to stimulate breeding. Greenstuff, generally cabbage and lettuce hearts, and carrots were given to all the mammals weekly. Water and rat cake were supplied ad libitum.

b) Techniques used in passaging the parasites

i) Intermediate hosts.

Methods for hatching schistosome eggs and for infecting the snail intermediate hosts followed those described by Standen (1949, 1951. and 1952)

Figure 1. Geographical origins of the schistosome strains.

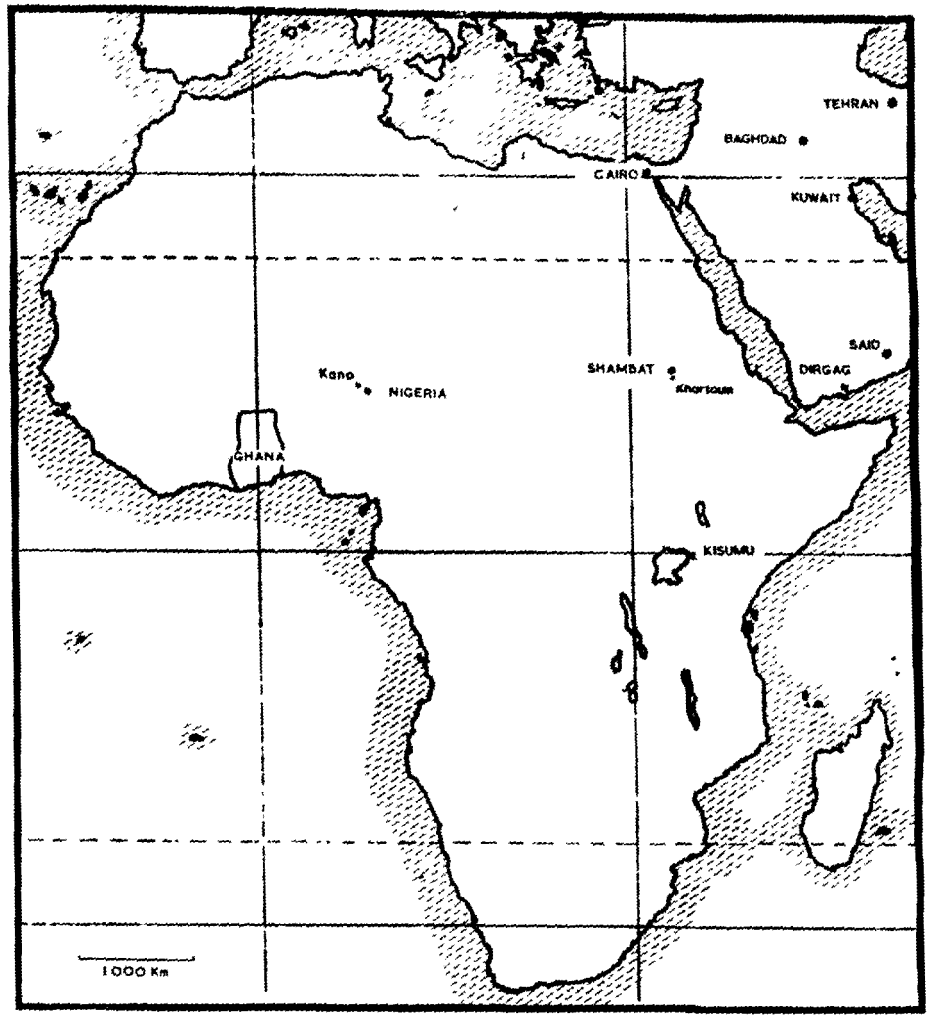

TEHRAN
BAGHDAD
CAIRO
KUWAIT
SAID
DIRGAG
SHAMBAT
Khartoum
Kano
NIGERIA
GHANA
KISUMU
1000 Km

FIGURE I

Figure 2. Relationships of some of the higher taxa of the phylum Mollusca.

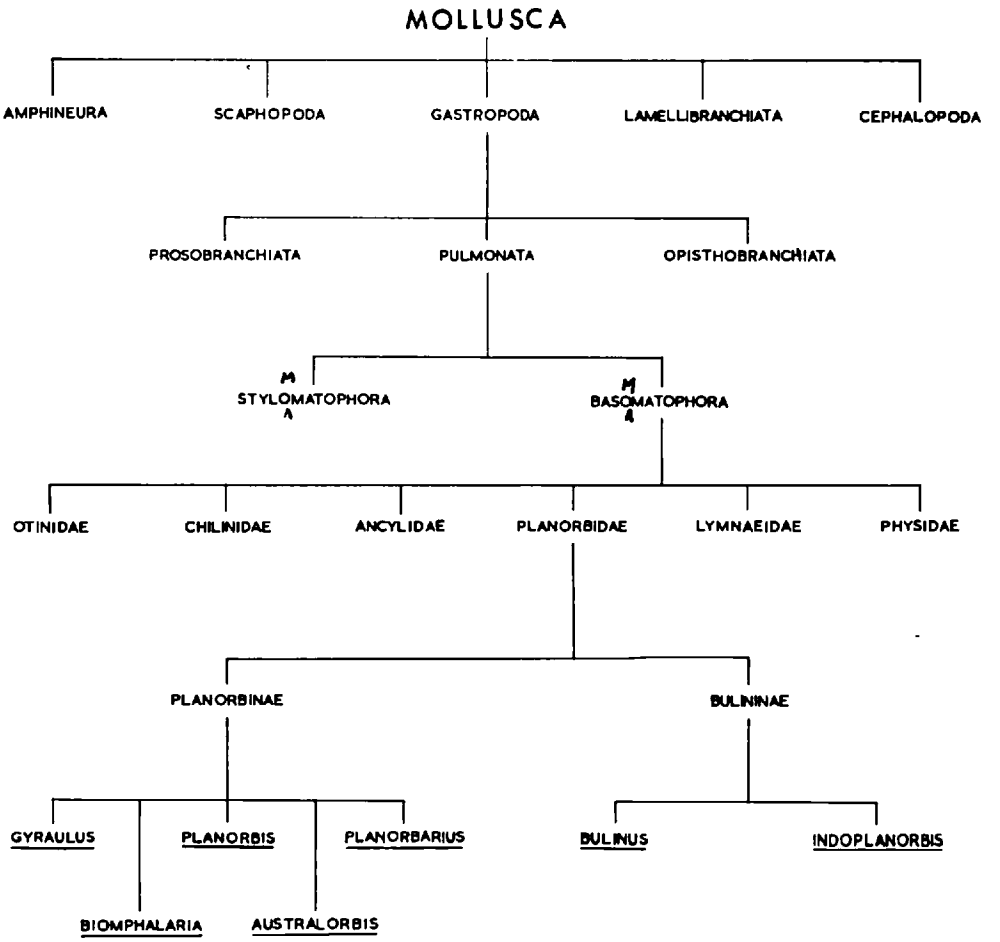

MOLLUSCA
AMPHINEURA
SCAPHOPODA
GASTROPODA
LAMELLIBRANCHIATA
CEPHALOPODA
PROSOBRANCHIATA
PULMONATA
OPISTHOBRANCHIATA
STYLOMATOPHORA
BASOMATOPHORA
OTINIDAE
CHILINIDAE
ANCYLIDAE
PLANORBIDAE
LYMNAEIDAE
PHYSIDAE
PLANORBINAE
BULININAE
GYRAULUS
PLANORBIS
PLANORBARIUS
BULINUS
INDOPLANORBIS
BIOMPHALARIA
AUSTRALORBIS

FIGURE 2

Figure 3. Relationships of some of the more important species of the molluscan genus Bulimus Müller.

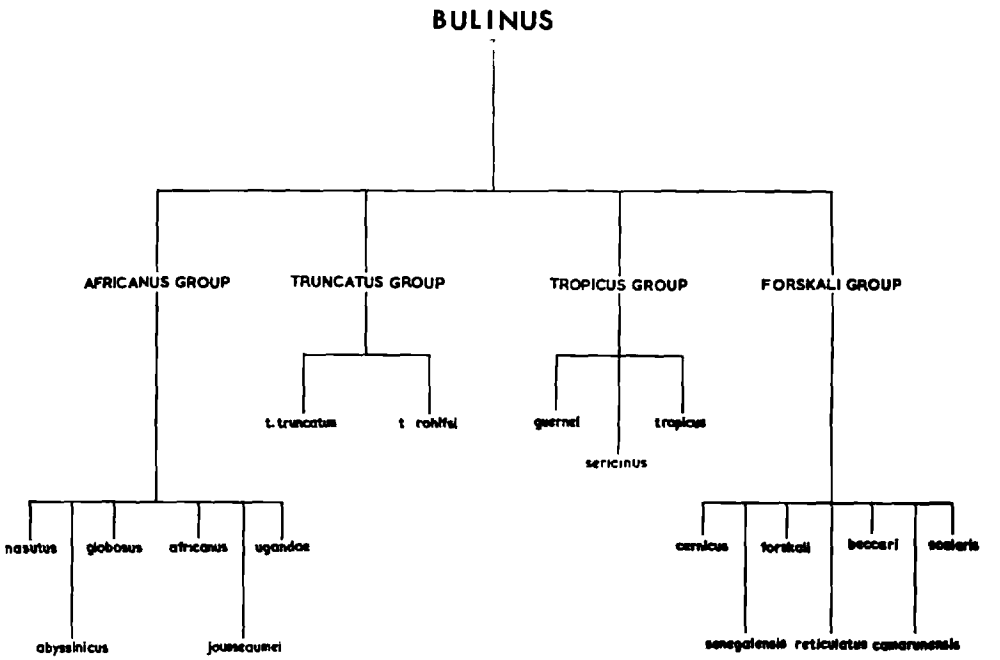

BULINUS
AFRICANUS GROUP
TRUNCATUS GROUP
TROPICUS GROUP
FORSKALI GROUP
t. truncatus
t. rohlfsi
guernei
tropicus
sericinus
nasutus
globosus
africanus
ugandae
abyssinicus
jousseaumei
cernicus
forskali
beccari
scalaris
senegalensis
reticulatus
camerunensis

FIGURE 3

Figure 4. Geographical origins of the snail intermediate hosts.

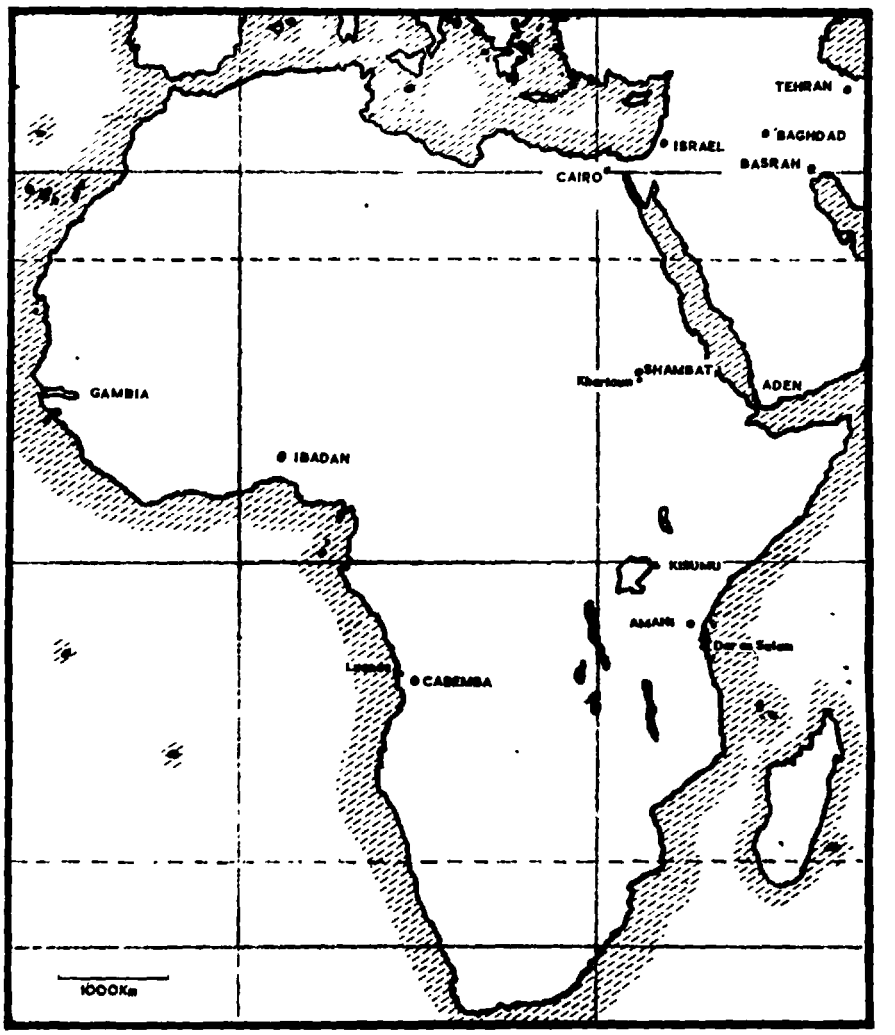

TEHRAN
ISRAEL
BAGHDAD
CAIRO
BASRAH
GAMBIA
SHAMBAT
ADEN
IBADAN
KISUMU
AMANI
CABEMBA
1000Km

FIGURE 4

In the present work snails were exposed to miracidia of human origin in the areas from which they originated and were immediately dispatched by air mail packaged as described by Claugher (1960) or were exposed to miracidia hatched from faeces or portions of heavily infected liver as described by Standen (1949).

More recently a method suitable for the transport of live eggs from the field to the laboratory was developed and has been used with considerable success (Jewsbury, 1964). Eggs were recovered from the urine of infected persons by sedimentation and centrifugation, and were washed twice in 0.9% saline solution with added penicillin at a concentration of 125 i.u. per ml. They were then re-suspended in further penicillin-saline in stoppered vessels and sent by air in a chilled vacuum flask containing a little ice. Considerable numbers of miracidia have been obtained on numerous occasions by this method.

Cercariae were obtained from infected snails by the methods described by Standen (1949).

Infection rates among the exposed strains of Bulinus were found to vary considerably, although generally 5% to 20% were successfully infected. The remainder either

did not survive the infection or else were refractive to exposure. Similarly the number of cercariae obtained from each snail varied from a few to several hundreds.

ii) Definitive hosts.

The potentialities of a wide range of mammals to infection with S. haematobium and S. mansoni have been investigated by Moore, Yolles and Meleney (1949), Azim and Cowper (1950) Stirewalt, Kuntz and Evans (1951), Moore and Meleney (1954), Kuntz and Malakatis (1955a, 1955b, and 1955c), and Kuntz (1961). From this work it appeared that white mice, hamsters, Arvicanthis abisynicus, Meriones shawi, and possibly Mastomys natalensis, were the best available laboratory hosts. All these species have been used in the present work (see Table 2, Page 50) and although none was completely satisfactory under the conditions of the experiments, hamsters have been found to be most suitable. Although it is possible to keep somewhat larger numbers of mice in the same space, they were not found to be altogether satisfactory hosts, very few being capable of carrying a reasonably heavy infection. Arvicanthis abisynicus were found to be only tolerable hosts despite more hopeful earlier reports. These animals became excessively fat and this gave rise to considerable

Table 2 - Summary of exposures.

Host animal	Number exposed	No. failing to come to postmortem	Number at postmortem	Number positive	No. +ve hosts as % of total exposed	Total No. of cercariae used.	Total No. worms recovered	Av. No. worms per +ve host	No. worms as % of total cercariae	No. worms as % of +ve cercariae
Hamster	75	40	35	29	38.5	27,150	1,133	41.1	4.25	9.4
Arvicanthis	29	8	21	14	48.0	13,650	521	30.8	4.75	7.5
Mouse	35	12	23	19	54.0	11,785	425	15.2	2.75	4.0
Meriones	6	1	5	5	83.0	3,500	111	22.2	3.15	3.7
Mastomys	6	3	3	3	50.0	1,850	58	19.3	3.13	5.8
Rhesus monkey	1	-	1	-	-	2,000	-	-	-	-
152	64	88	70	46.0	59,935	2,248	32.0	3.75	7.25	

practical difficulties in the recovery of worms at post mortem. Infection rates were also often low, only a few worms being recovered in most cases; all-male infections were also quite common. The jird Meriones shawi was also disappointing; only six animals were used, and less than 3½% of the cercariae were recovered as adult worms and one animal was completely negative 100 days after infection. Mastomys natalensis likewise yielded less than 3½% of the cercariae as adult worms and were not nearly as satisfactory as hamsters. One Rhesus monkey was exposed but surprisingly found to be apparently negative on post-mortem - no eggs were recovered from the faeces either. The sources of material are given in Table 3, pages 52, 53 ~~& 54~~.

In general the methods of infecting the vertebrate hosts follow those used by Standen (1949). Methods such as those described by Olivier and Stirewalt (1952) and Pan, Kaufman & Hunter (1951) were too lengthy for the present work. Watson and Abdel-Azim (1949) record the results they obtained with nine different methods of exposure; that of partial immersion in "infested" water was the easiest and gave the best results (50% recovery of cercariae as adults and the greatest uniformity of

Table 3 - Sources of material.

Refs. No.		Animal No.	Tissue l	Tissue g	Uterus l	Uterus g	No. of worms recovered
Hamster							
1	Ghana	74	-	faecal sample		4	-
2		76	20	20		72	30
3		75	-	faecal sample		1	-
4	Nigeria	64	-	"		14	2
5		73	-	"		2	3
6	Shambat	4	-	-	84		26
7		3	-	-	16		36
8		7	150	210	81	97	42
9		10	199	100	33	-	48
10		15	99	13	44	-	31
11		19	100	100	106	-	133
12		5	100	100	5	-	14
13		12	100	100	-	346	80
14		28	-	-	15	-	22
15		23	38	4	-	-	20
16	Cairo	42	44	50	3	333	178
17		38	-	47	-	208	107
18		43	-	-	1	19	66
19		35	-	-	65	39	198
20	Kisumu	46	-	-		19	49
21		56	-	-			38
22		54	-	-	13		53
23		45	-	-	-	26	45
24	Baghdad	62	16	40	40	44	37
25		63	5	-	2	21	27
26		68	-	50	49	4	26
27		69	20	50	12		14
28		70		faecal sample		9	-
Mouse							
29	Shambat	16	-	-	-	14	40
30	Cabemba	12	-	-	11	-	10
31		14	-	-	59	31	46
32		9	-	-	6	16	41
33		19	100	15	-	-	13
34		26	30	-	-	3	5
35	Cairo	23	6	1	-	3	19
36		30	4	-	-	49	44
37		20	4	1	8	139	54
38		27	11	1	-	159	42
39		21	50	50	-	-	50

l = liver g = intestine
No. in l and g columns = no. of eggs measured

Table 3 - Sources of material.

Refs. No.	Animal No.	Tissue l	Tissue g	Uterus l	Uterus g	No. of worms recovered
Arvicanthis						
40 Cairo	18	1	-	-	79	61
41	7	-	-	92	-	42
42	8	-	-	60	51	39
43	9	-	-	-	49	80
44	12	-	20	2	-	28
Meriones						
45 Shambat	3	-	-	20		30
46	2	-	-	12		9
47	1	-	-	37	13	25
48	4	8	7	-	96	26
49	6	-	-	1	13	21
Mastomys						
50 Cairo	3	2	-	1	3	23
51	1	1	-	-	-	10
52	6	6	3	-	43	25
Man						
53 Ghana			Urine	50	-	-
54 Nigeria				50		
55 Cairo	1			25		
56	2			25		
57	3			25		
58	4			20		
59 Dirgag	13			8		
60	16			17		
61	17			3		
62	20			25		
63	23			25		
64	39			1		
65 Said	14			25		
66	34			25		
67	6			12		
68	7			1		
69 Kuwait	2			13		
70 Baghdad				34		

recovery). Methods involving the shaving of the animal or the injection of cercarial suspensions subcutaneously, intraperitoneally, or into the stomach were not so successful and were more difficult to perform. In the present work the host animal was placed in a bath of conditioned water (water kept for three days in aquarium tanks containing fish and water weed to remove any harmful dissolved heavy metals) at about 26° C. for about 30 to 45 minutes to stimulate the evacuation of faeces and urine immediately prior to exposure to S. haematobium cercariae, by the partial immersion method. Cercariae were counted and picked out of the styrene pots containing the stimulated shedding snails using a Pasteur pipette and viewed with a dissecting microscope and a strong horizontal beam of light. Cercariae were pooled, i.e. cercariae from several snails of the same strain and carrying the same strain of parasite were used for each exposure to minimuse the chance of unisexual infections. Exposures were usually made in glass Kilner-type jars or breffits, according to the size of the mammal. A small quantity of conditioned water was put in the jar, the cercariae added carefully so as to avoid as much damage to them as possible, and the animal carefully introduced.

Animals were unshaved; cercariae penetrated through the fur and on the tail and feet. The animal was exposed for 60 minutes as a rule; only on very exceptional occasions was an exposure made for a longer period. Dosage rates varied during the course of the experiments; initially a standard dose of 500 cercariae was employed, but doses of 150, 200, 250, 300, 350, 400, 450, 600 and 800 etc., cercariae were also used as the experimental considerations warranted. During more recent work a standard dose of 200 cercariae has been used. The infection was allowed to run its course for the required period (usually 100 days, but considerably longer in later experiments) before the animal was killed and the worms recovered. Any animals dying during the course of the experiment were examined and any recoverable worms removed.
In experimental infections of laboratory animals S. haematobium is found in the veins draining the intestine rather than the bladder as is the case in human infections and consequently eggs are generally found in the intestinal tissue and liver and not the bladder; they are also passed out in the faeces rather than the urine. Infected animals were examined periodically for the presence of viable eggs in the faeces by confining them

overnight in an all wire-mesh cage over a dish of saline solution and attempting to hatch any eggs present in the collected faeces in the usual way. The presence or absence of hatched miracidia was determined with the aid of a Point-O-Lite lamp as described by Standen (1949). Any animals found to be passing reasonable numbers of viable eggs (e.g. up to 30 per examination) by this technique were usually retained as a source of material and were not killed for post mortem examination. It was found that animals, especially hamsters, passing more than 30 to 40 eggs in one night had a hyper-infection and were liable to die suddenly although apparently healthy. Hamsters were again found to be the most suitable hosts for this work.

Perfusion of the abdominal venous blood vessels with a suitable anticoagulant is the best method for recovery of the adult schistosomes. Although the technique may appear to be too delicate and protracted to be practicable the only alternative is the manual recovery of individual worms from cut blood vessels. Damage to the worms during recovery is almost inevitable by this method, worm recovery is not always complete, thus wasting material, and can be just as time consuming. With practice a post mortem

examination can be completed within 20 to 30 minutes with a high percentage of worm recovery, using the perfusion technique. Digestion of tissues likely to contain schistosome eggs firstly by trypsin in alkaline solution (pH 8) followed by pepsin in acid solution (pH1.8) is also of advantage as it provides a considerable number of eggs additional to those contained within the female worms recovered by perfusion. A successful digestion may take 8 to 9 hours, but the yield in clean eggs is worth the time taken.

A method combining the perfusion of the abdominal blood vessels and liver and a digestion technique were used routinely for the recovery of undamaged live adult schistosomes and their eggs. The perfusion technique follows in broad outline that of Yolles, Moore et al. (1947). The dorsal aorta and the blood vessels supplying the liver are ligatured and the liver removed and perfused separately, worms being recovered from the cut hepatic portal vein. Worms from the mesenteric veins are similarly recovered from the hepatic portal vein draining the intestine. More complex techniques such as those of Pellegrino and Siquiera (1956) and the Perf-o-Suction methods of Radke, Berrios-Duran and Moran (1957)

were not considered essential, as they involve the use of more expensive apparatus and reagents, are more complex to set up and carry out, and give little or no improvement in percentage yield of worms. The perfusion apparatus itself is essentially similar to that described by these authors (which in turn is based on a method due to Faust and Meleney) (Yolles et. al. 1947) A cannula, consisting of a 2" length of hypodermic needle tubing mounted in a ribbed holder, and an ordinary hypodermic needle were fitted to rubber tubing provided with "roller" clips. Twelve and fourteen gauge cannulae and twelve, fourteen or sixteen gauge hypodermic needles were used, according to the size of the blood vessels being perfused. Citrated saline solution (0.75% sodium citrate plus 0.85% sodium chloride) was used as perfusate as by Yolles & Moore (1947). After perfusion of the liver and gut, eggs were isolated by the technique due to Smithers (1960) and incubation of the digest was carried out in a water bath at 37° C. It was found that careful removal of all fat from the intestine and thorough cleaning of the intestinal mucosa was of importance to obtain a clean digest. Generally, only the posterior region of the intestine (the colon) was affected by the presence of worms, but nevertheless the whole of the intestine was digested. The second (pepsin) digestion

was carried out in conical, stoppered centrifuge tubes, to avoid the additional transference of digest and consequent loss of eggs prior to the final centrifuge. At first eggs were also isolated by merely homogenising the liver or intestine without subsequent digestion, but the produot was not at all satisfactory, eggs generally being surrounded by fibrous host-reaction cells. After recovery of worms at a post-mortem examination of an infeoted animal they were transferred from the perfusate to Ringer's solution as quickly as possible. Worms were left in Ringer at room temperature for several hours and the males and females were separated with the aid of fine paint brushes before being fixed on a glass plate. Cold formol acetic fixative (8 parts water, one part 40% formaldehyde and one part glacial acetic acid) was found to be most satisfactory for whole mounts of all the fixatives tried. Worms were "painted" on to the glass with the minimum amount of Ringer, and fixative was dropped on to them with a pipette. Towards the end of the work a more rapid method for the fixation of larger numbers of worms was used, involving the rapid horizontal shaking of the worms in cold formol-acetic fixative in a stoppered test tube. Both methods gave good results, relaxation

being quite satisfactory. Worms were kept in fixative for 24 hours before being transferred direct to and stored in glycerine alcohol made up in the proportion of one part of glycerol to one part of 10% alcohol. This preservative was the only one found which cleared the worms satisfactorily while not distorting the eggs. Worms were stored in polystyrene tubes fitted with polythene stoppers, as worms stored in ordinary glass specimen tubes fitted with corks were found to turn black after a short while. Eggs digested from tissue were stored in 0.9% saline solution in stoppered centrifuge tubes for as short a while as possible, until they could be measured. In the latest work in this connexion, penicillin at a concentration of 125 i.u. per ml. of 0.9% saline solution was used as a final wash during centrifugation and was used to store the eggs, in order to reduce the amount of bacterial contamination.

c) Histological techniques.

Worms were recovered from freshly killed hosts as described above, the males and females separated and fixed in cold alcoholic Bouin, Susa, or formal saline fixatives. Material was embedded in paraffin wax either by the simple method or by Peterfi's double embedding technique as described by

Carleton and Drury (1957). Longitudinal and transverse serial sections were cut at 6 µ and were stained with Masson's triple stain or Ehrlich's haematoxylin. All drawings were made with the aid of a camera lucida or a squared eye-piece.

d) Statistical methods.

As well as a preliminary morphological study of the adult and larval stages, egg shape was chosen as the best subject for intensive study, as the egg is the only hard, formed structure occurring in either the male or female schistosome, and is therefore less liable to distortion.

It is usually impossible or impracticable to make measurements of all the members of each population under study, which is itself part of an infinite population, and a sampling technique is frequently used. During subsequent treatment of data derived from such samples, it must be remembered that the original data are taken from a sample of the original infinite population and that it is the population which one is trying to compare. Various techniques are available for the treatment of sample data to estimate their nearness to the corresponding population data, but such techniques are still only estimates of the ideal (population) figure.

Preliminary tests using length-frequency curves and "t" tests on one dimension of the eggs at a time indicated a probable difference between at least some of the samples. However, these methods do not take into account the shape of the material. The comparison of shape is more complex mathematically; however, the comparison of "mean shapes" as well as/for each sample is being attempted in this study.
size

The standard "t" test is generally applied to one character mean at a time from two samples (e.g. length of the egg - 1). It is assumed by the null hypothesis that the two samples are drawn from the same population, the variance of which is estimated by pooling the two sample variances. If the difference between the means in terms of its standard error is greater than the expected value of "t" for a given probability, the null hypothesis is rejected and the two samples are in fact taken to have been drawn from two different populations. If the populations cannot be distinguished unequivocally by the use of a single character, the simultaneous use of two or more characters may make this possible, in which case the calculations involved in the comparison of the means are much more complex. In the present work the four dimensions of the egg: overall maximum length minus the spine, maximum breadth

distance of point of maximum breadth from the non-spinose end, and the spine length alone, (Fig. 5, page 65) have been used in an attempt to define the shape of the egg more accurately, and the four dimensions of each egg sample have been examined by application of the technique of multi-variate analysis. Cooley and Lohnes (1962) give a good introductory account (pp. 116-123).

Treatment of data on egg measurements.

Eggs were measured using a crossed micrometer eyepiece fitted to a conventional binocular microscope or using a crossed sliding scale fitted to a Reichert "Visopan" projection microscope. The second method was found to be the easier to use and was also quicker and less tiring than the use of a micrometer eyepiece. Eggs were measured either in utero in adult females in temporary mounts of glycerin alcohol or as suspensions of digested eggs in penicillin-saline. It was necessary to focus accurately on each egg to determine whether it was obscured or distorted by adjacent eggs or whether it was not lying horizontally. Such eggs were always rejected for measurement purposes, but were included in the totals of counted eggs. A large proportion of available eggs had to be rejected for measuring purposes for these reasons, particularly eggs in utero. Magnifications of 125 and 500 diameters were

Figure 5. Diagram showing the four dimensions used in the measurement of the eggs.

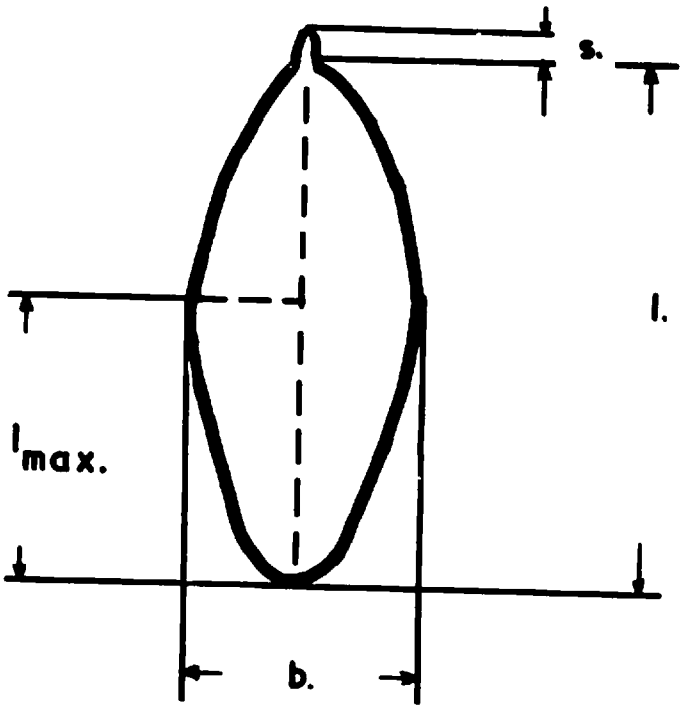


FIGURE 5

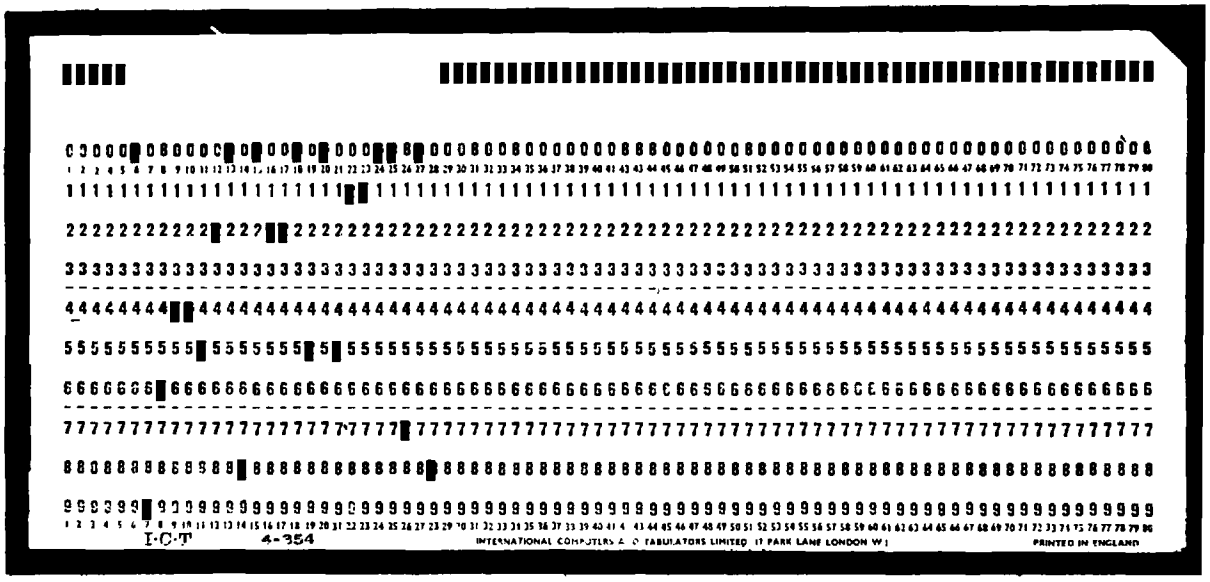
found most useful locating and measuring the eggs respectively. The four dimensions employed as standard are shown in Fig. 5. page 65. The data were punched on to 80 column Hollerith cards, together with the coded reference data, using one card for each egg Fig. 5A, page 66B. The use of punched cards, although more troublesome in the beginning, it more flexible than the use of punched tape. The extra time involved in the preparation of the cards is more than repaid by the increased flexibility in handling them.

After the cards had been prepared, they were sorted mechanically into the desired groups, and the calculations repeated. In this way the same data were used to obtain information on a number of effects without having to re-punch the relevant information. Thus information can be obtained on variation in eggs

1) from a single uterus,
2) from one site within one host animal.
3) due to the site of origin within one individual host animal,
4) from several individuals of one species of host animal,
5) from different species of host animal,
6) due to differences in worm burden,
7) due to duration of infection,

Figure 5a. Photograph of a Hollerith card and interpretation of coded data.

Code		Interpretation	
Column No.	Reading.	Category	Value
6, 7 and 8	096	length	096 μ
9 and 10	44	breadth	44 μ
11 and 12	52	l_{max}	52 μ
13 and 14	08	spine	08 μ
15	0	host species	hamster
16	2	parasite strain	Shambat
17	2	passage number	2
18 and 19	05	infection rate	40-49 individual worms
20 and 21	05	infection length (days)	90-99 days
22	1	material recovered from liver, gut, urine or faeces	liver
23	1	eggs from urine or faeces, tissue, or <u>in utero</u>.	tissue
24, 25 and 26	007	dosage rate (cercariae)	500-549 cercariae
27 and 28	08	reference number	08


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8) due to the dosage rate in the host animal,

and finally,

9) from different geographic strains of the same species of parasite.

The analysis of results by the multivariate method involves a more intricate series of calculations than univariate methods and the use of a desk calculator with considerable carrying facilities is essential; moreover, the analysis of more than four or five variates makes the use of electronic calculating equipment essential as the labour of handling the amount of material involved is very considerable.

Two distinct approaches are available using the multiveriate technique.

These are:

a) The calculation of generalised distances between group means in an n-dimensional space (four dimensions in this case), a projection of which into a three-dimensional model is usually practicable (see illustrations in Lysenko and Sneath, 1959). It is a feature of this method that the axes or vectors are not usually mutually at right angles.

b) The calculation of canonical vectors, mutually at right angles in their own proper space.

The methods employed in the evaluation of the data using these two approaches were as follows:

a) Generalised distance method.

1) For each subsample or group, the four measurements as in Fig.5, page 65, are set down in four columns, x_1, x_2, x_3 and x_4, corresponding to length, breadth, l_{max} and spine respectively.

2) The items of each column are multiplied successively by x_1, x_2, x_3 and x_4, giving further columns of squares and cross-products. All columns are then summed.

3) The sums of squares and products are in fact moments about zero, and in order to relate them to their means each sum of squares is reduced by a correction factor of, e.g. $n.\bar{x}^2$, and each sum of products by, e.g., $n.\bar{x}_1\bar{x}_2$.

4) The results are pooled by adding similar values from all subsamples with due regard for signs, to form the information matrix. This is set out as shown below in lines a - d.

	Left Hand Side				Right Hand Side			
a)	x_{11}	x_{12}	x_{13}	x_{14}	1	0	0	0
b)	x_{21}	x_{22}	x_{23}	x_{24}	0	1	0	0
c)	x_{31}	x_{32}	x_{33}	x_{34}	0	0	1	0
d)	x_{41}	x_{42}	x_{43}	x_{44}	0	0	0	1

5) The information matrix, as above, is inverted following the method described by Goulden (1952), pp. 147-148. This leads to the elements of the inverse matrix (or c-multipliers) as shown below, the information matrix x_{ij} becoming the inverse matrix c_{ij}.

$$\begin{matrix} c_{11} & c_{12} & c_{13} & c_{14} \\ c_{21} & c_{22} & c_{23} & c_{24} \\ c_{31} & c_{32} & c_{33} & c_{34} \\ c_{41} & c_{42} & c_{43} & c_{44} \end{matrix}$$

6) To compare the shape of two groups of eggs, the means of each of the four sets of measurements (x_1, x_2 etc.) of the first group are substracted from the corresponding means of the second group, to give the differences d_1, d_2, d_3 and d_4, due regard being paid to signs.

7) The square of the generalised distance D between the group means is given by

$$D^2 = \beta_1 d_1 + \beta_2 d_2 + \beta_3 d_3 + \beta_4 d_4$$

where

$$\beta_1 = (d_1 c_{11} + d_2 c_{12} + d_3 c_{13} + d_4 c_{14})$$

$$\beta_2 = (d_1 c_{21} + d_2 c_{22} + d_3 c_{23} + d_4 c_{24})$$

$$\beta_3 = (d_1 c_{31} + d_2 c_{32} + d_3 c_{33} + d_4 c_{34})$$

$$\beta_4 = (d_1 c_{41} + d_2 c_{42} + d_3 c_{43} + d_4 c_{44})$$

D^2 is thus a measure of the similarity of the two groups.

8) The angle θ between two comparable vectors L (with α-coefficients) and M (with β-coefficients) is given by

$$\text{Cos } \theta = \frac{\Sigma \alpha_i \cdot \beta_i}{\sqrt{\Sigma^1 \alpha_i^2 \cdot \Sigma \beta_i^2}}$$

Knowing the generalised distances and the angles between the vectors, an n-dimensional model can be envisaged, and this can often be meaningfully represented by a projection into a three-dimensional model. In the present work two such models were constructed of rubber balls separated by glass rods (see Fig. 10, p. 138), the lengths of which (the generalised distances) directed the shape of the model rather than any calculation of angles, and in these circumstances the calculation of angles was not carried out as it was considered unnecessary.

b. Canonical vector method.

The information matrix A, consisting of the variances and co-variances pooled within each group of organisms, is supplemented by a corresponding matrix B, computed between the groups. The roots of the determinantal equation $|B - \lambda A| = 0$ afford an indication of the number, and relative importance, of the contrasts of form shown by the organisms (Table 31, page 133A).

The canonical vectors associated with these roots (Table 31A, p. 133) can be considered as the axes of a graph, within the framework of which the groups may be mutually oriented. Once set within this framework the groups will be separated by a distance D (the

generalised distance already calculated in section a (7) above). The position of each group within the framework of reference can be found by multiplying the means for the group by the corresponding weights in each of the canonical vectors. If these weights are γ_1, γ_2, etc. for any one vector, then the position (or "score") along this vector is $\Sigma\ \gamma_i.\bar{x}_i$. The graph may then be plotted.

The distribution of the points obtained by both the generalised distance method (a) above) and the canonical vector method (b) above) will be basically the same. The distances separating the points (the generalised distances) should theoretically be exactly the same; however, this is unlikely to be the case in practice due to the accumulation of different rounding-off errors in the calculations during each method.

The two techniques are thus two different methods for calculating the same relationships between the various groups. In the one case (generalized distance technique) the points are computed and the axes or framework of reference inserted by inspection, whereas in the other case (canonical variates technqiue) the axes are computed and the points plotted relative to them. Utilising the same data, the same results should be achieved by both methods, the distribution of points and the distances between them being identical in theory. However, as pointed out above this is in fact not the case in practice due to the accumulation of different rounding-off errors.

Both these methods have been used in the present work; the graphical method is perhaps the more difficult owing to the complexity of the graphs and the difficulty both of drawing and interpreting them.

e) Observations on living worms.

The object of these experiments was to ascertain the sequence of events preceding and during the formation of the eggshell and its subsequent shaping within the ootype in order to obtain a better understanding of the structures involved and their functional relationships.

In the initial work, a mixture of equal parts of sterile horse serum (Wellcome No. 5) and Tyrode solution was used as medium for maintaining live excised worms for short periods. Penicillin (crystalline sodium penicillin G, "Crystapen" Glaxo) and streptomycin (streptomycin sulphate Glaxo, for intrathecal injection) were added to the basic medium as antibiotics such that the final concentrations were 1,250 i.u. and 2.500 i.u. respectively per ml. of medium. The latter was

sterilised by Seitz filtration and stored overnight at 37° C. in a waterbath as a test for sterility. Approximately 5 ml. of sterile medium were transferred asceptically to sterile Carrel flasks. A host animal was then killed, and worms recovered manually from the mesenteric veins utilising all the usual precautions to ensure sterility. After a brief wash in sterile Tyrode-antibiotic solution, 3 pairs of undamaged worms were transferred to each Carrel flask. The flasks were closed with sterile serum vial seals, sealed with wax, incubated at 37° C. in the water-bath and examined daily.

It is usual in in vitro culture experiments to rock or rotate the vessels and to change the medium frequently (or use a constant flow device) in order to keep the body of the worm in contact with fresh medium, thus ensuring the removal of waste products and a good supply of nutrients. However, in the present work the apparatus necessary for this was not available.

More recently, individual females have been studied using temporary mounts on well slides and a warm stage. Normal saline solution, Tyrode's solution and medium 199 (Glaxo) previously warmed to 37° C. were used as mounting media. By these

methods microscopic observation was possible at a much higher magnification (480 and 600 diameters) than was possible using the Carrel flasks.

SECTION 4 - RESULTS.

RESULTS.

a) Anatomy and Histology of the female genital apparatus.

In the schistosomes, the ootype is the site of actual shell formation and shaping of the egg and a knowledge of its structure and the relationships of the ducts and glands associated with it is of obvious importance in an investigation of egg shape (Fig. 6a, 77). The ducts consist of the oviduct, along which ova are passed from the ovary towards the ootype, and the vitelline duct which conveys the vitelline material from the vitellaria in the posterior region of the body towards the ootype. These two ducts unite just posterior to the ootype and enter it posteriorly as a common duct. The glands associated with the ootype consist of Mehlis' gland surrounding the oo-vitelline duct and the glandular cells lining the muscular ootype walls.

The most detailed and comprehensive account of the anatomy of the schistosomes parasitising man has been given by Looss (1895). Subsequently Gonnert (1955a and 1955b) has extended and amplified Looss's monograph with special reference to the anatomy of the female genital system of S. mansoni and the relationships of the various organs to the formation of the eggs characteristic of the species. Stephenson (1947)

Figure 6a. - Semi-diagrammatic representation of an adult female S. haematobium.

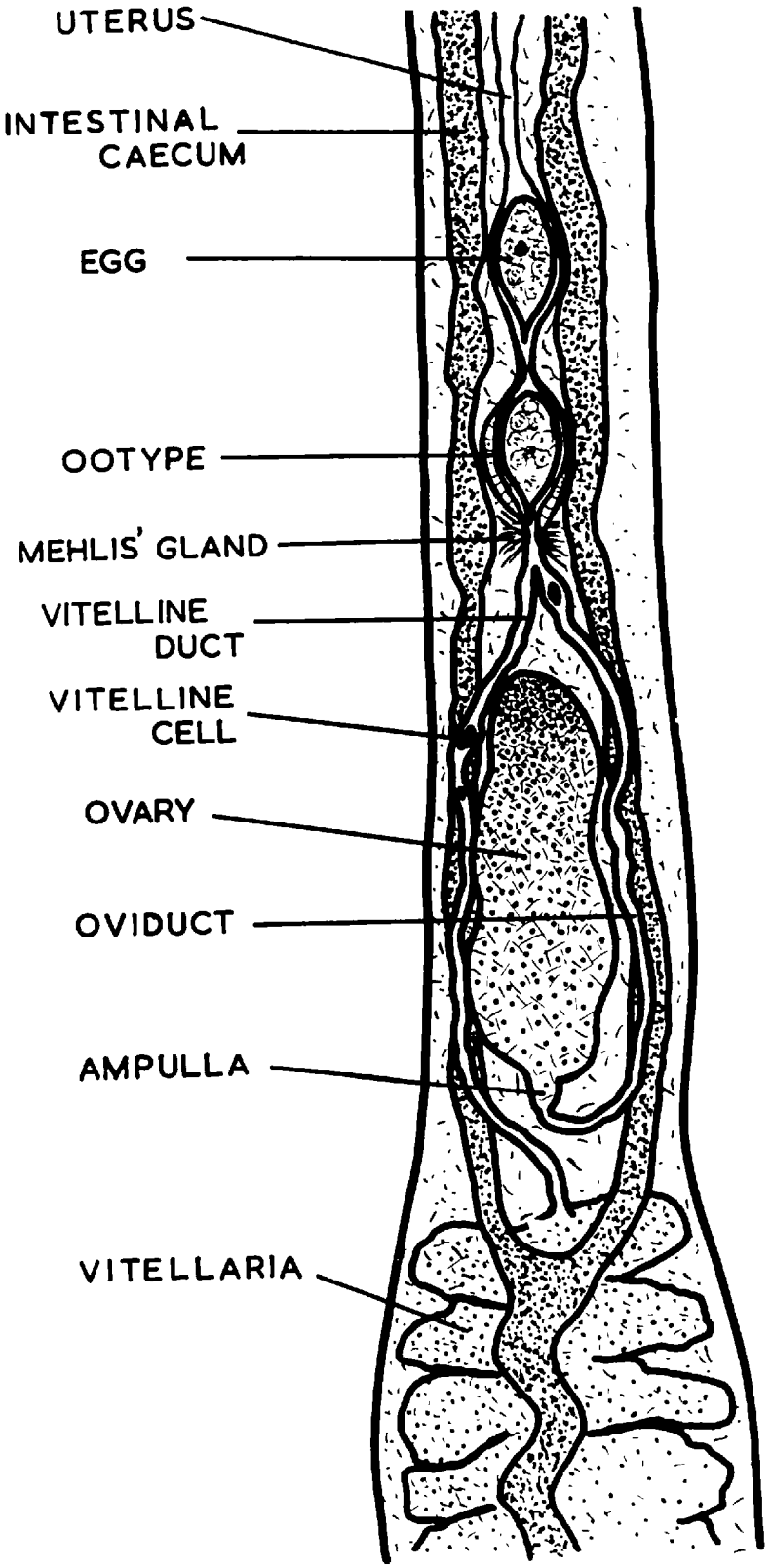

UTERUS
INTESTINAL CAECUM
EGG
OOTYPE
MEHLIS' GLAND
VITELLINE DUCT
VITELLINE CELL
OVARY
OVIDUCT
AMPULLA
VITELLARIA

FIG. 6A

has described the anatomy and especially a series of histo-chemical tests on structures connected with egg formation in Fasciola hepatica.

Ovary.

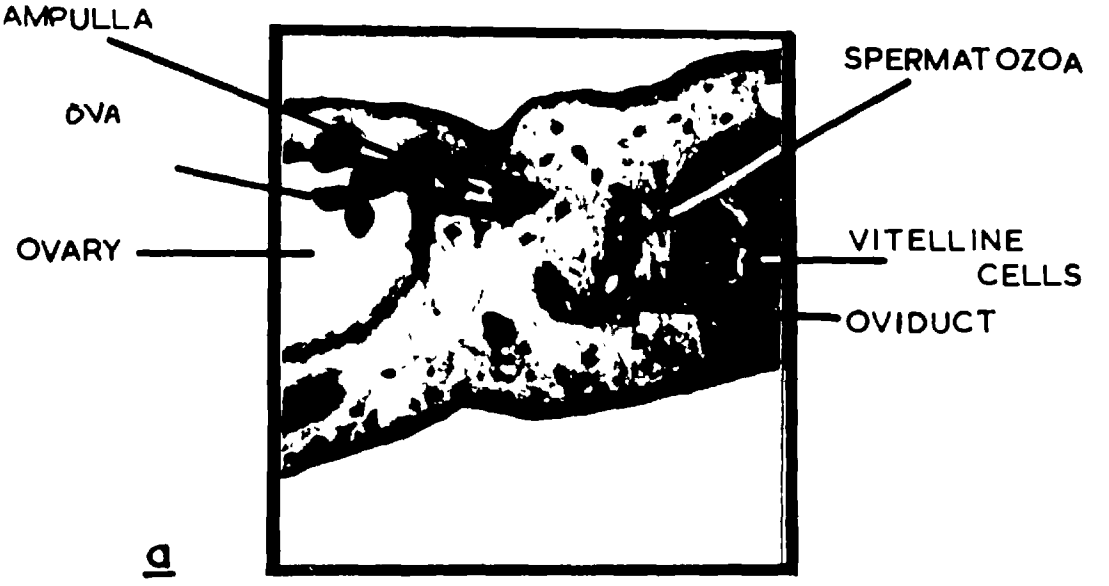
The ovary of S. haematobium is comparatively large, measuring about 450 - 500 μ long by 130 μ wide, and is circular in transverse section (Plates 1a, 2a, and 2b, pages 80 & 82). It lies immediately anterior to the junction of the lateral gut caeca, at about the end of the anterior two thirds of the body length, i.e. it is more posterior in position than in S. mansoni. The ovary lies dorsally with the gut caeca on either side in a ventro-lateral position. The ovary is slightly spirally wound, and is of more or less uniform diameter throughout its length. It appears to be surrounded by a small but discrete layer of circular muscle fibres. Ova are budded off from the germinal epithelium lining the ovary, especially at the anterior end, and gradually pass down the central "lumen" towards the posterior, where the oviduct originates. The ova measure about 26 μ long.

Oviduct

There is a single duct leading from the ovary, ~~leading~~ lying

PLATE 1. a. Longitudinal section of a female *S. haematobium* through the oviduct and ovary. Bouin and Masson, X 570.

b. Longitudinal section of a female *S. haematobium* through the ootype, oviduct and oc-vitelline duct. Bouin and Masson, X 420.


AMPULLA
OVA
OVARY
SPERMATOZOA
VITELLINE CELLS
OVIDUCT
a

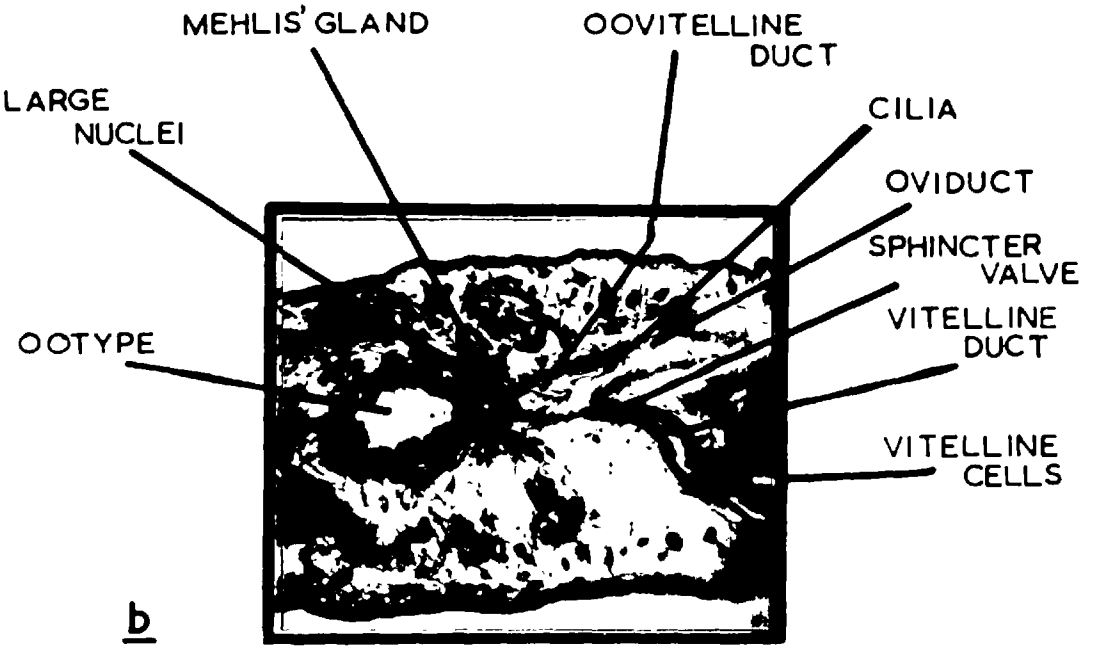

MEHLIS' GLAND
OOVITELLINE DUCT
LARGE NUCLEI
CILIA
OVIDUCT
SPHINCTER VALVE
VITELLINE DUCT
OOTYPE
VITELLINE CELLS
b

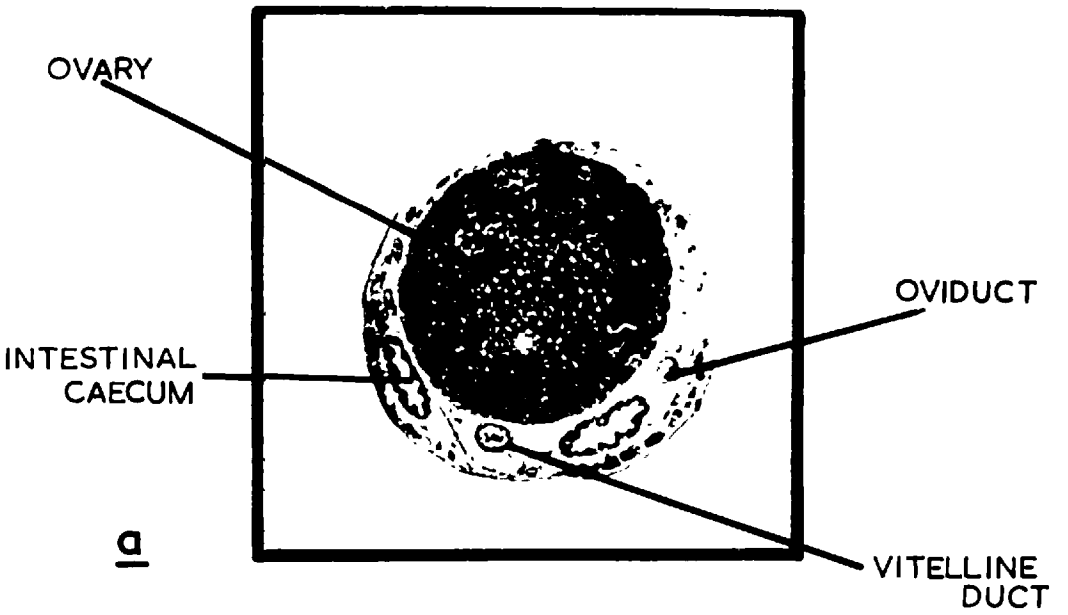
PLATE I

Plate 2. a. Transverse section through the ovary of a female S. haematobium.

Bouin and haematoxylin, X 270.

b. Transverse section through the ovary of a female S. haematobium.

Bouin and Masson, X 310.


OVARY
OVIDUCT
INTESTINAL CAECUM
VITELLINE DUCT
a

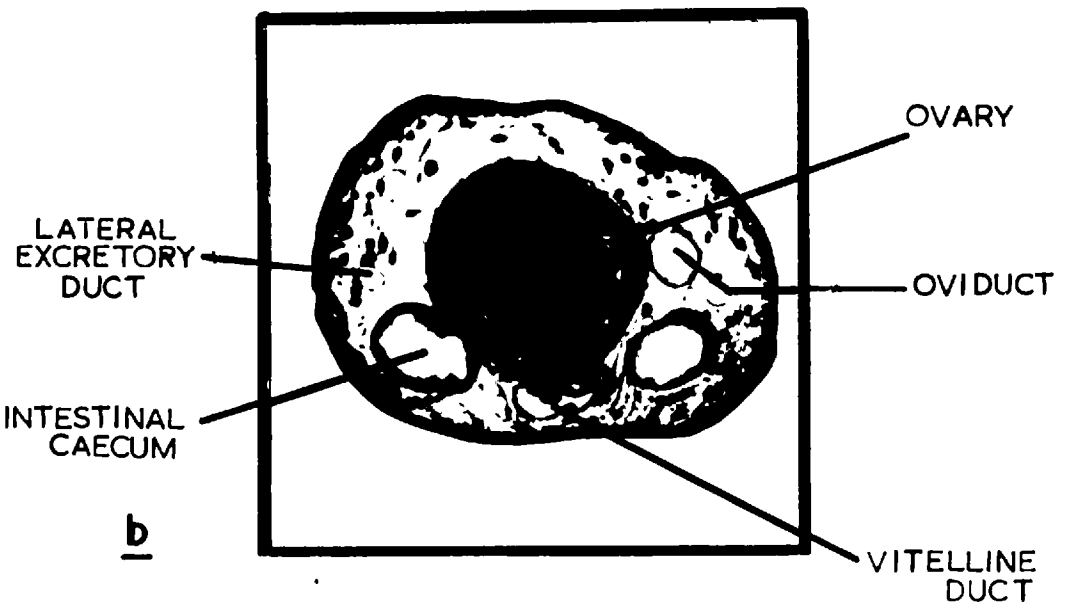

OVARY
LATERAL EXCRETORY DUCT
OVIDUCT
INTESTINAL CAECUM
VITELLINE DUCT
b

PLATE 2

at the posterior end of the anterior two thirds of the body to the exterior, immediately posterior to the ventral sucker. This duct acts in turn as oviduct, receptaculum seminis, ootype, vagina and uterus. It receives secretions from various glands and also the contents of the vitelline duct, which runs from the vitellaria in the posterior third of the body and joins the main duct immediately posterior to the ootype. The uterine portion of the main duct is more or less straight and is not convoluted as in many trematodes.

The oviduct is a ciliated tube about 20 - 25 μ wide, and leads from the ovary in at first a posterior and then an anterior direction. The passage of ova from the ovary into the duct is controlled by a sphincter muscle or ampulla (Plate 1, page 80). Shortly after the oviduct leaves the ovary it dilates somewhat, functioning as a receptaculum seminis (there being no distinct structure), (Plate 1a, page 80). This region contains numerous spermatozoa, lying with their rather spindle-shaped heads against the wall of the receptabulum. As already stated, the oviduct consists of thin, almost membranous ciliated cells. The purpose of these cilia remains obscure; it would appear that ova are fertilized in the receptaculum and are passed down the ovidnct by peristaltic contractions in the latter. The (so-called) cilia may act as a type of

valve, allowing the spermatozoa to pass along the oviduct towards the ovary but prohibiting their return towards the ootype. As Gonnert (1955a) points out, the cilia always appear to be directed towards the ovary; he suggests that a change in the direction of the beat of the cilia may occur, at one moment passing eggs towards the ootype and at another beating in the opposite direction, passing spermatozoa to the receptaculum. However, as described on Page 152 oocytes and spermatozoa are moved back and forth too rapidly for cilia alone. It seems more likely that they act as a type of valve or baffle to the spermatozoa. The oviduct runs laterally to the ovary towards the anterior, and joins the vitelline duct anterior to the ovary.

Vitellaria and vitelline duct.

In S. haematobium the vitellaria are more posterior in position than is the case with S. mansoni, lying in the posterior third of the body. This region is generally somewhat broader than the anterior (uterine) portion. The vitelline duct runs anteriorly and lies at first ventral to the posterior gut appendix and then ventrally to the ovary. The vitelline duct runs along a rather winding course and joins the oviduct just posterior to the ootype. The vitelline cells are passed by peristalsis in groups along the vitelline duct. They contain

a conspicuous nucleus and numerous, darkly staining discrete granules, the shell granules or globules. Immediately prior to joining the oviduct, the lumen of the vitelline duct becomes much narrower and the duct wall much thicker, and appears to control the release of vitelline cells, (Plate 1b, Page 80).

Ovitelline duct and ootype.

The oviduct and vitelline duct join in the centre of the female body, immediately posterior to the ootype itself and directly in line with it, rather than posterio-ventrally as in S. mansoni (Plage 1b, page 80). This difference is of considerable importance as it affects the position and shape of the spine of the egg, as will be shown later. The ovitelline duct is short as in S. mansoni, and its point of entry into the ootype is guarded by two cells with very conspicuous nuclei, protruding into the lumen of the duct (Plate 1b, Page 80). These cells are thus able to seal the ootype off from the rest of the ovitelline duct. The ducts of Mehlis's gland discharge into the lumen of the ovitelline duct between the nuclear valve and ootype proper (the central chamber of Stephenson); Mehlis' gland itself surrounds this region (Plage 1b, Page 80).

In the schistosomes, the ootype is the site of actual

shell formation and shaping, in contrast to Stephenson's (1947) conclusions for Fasciola, which Gonnert (1955b) believes to be incorrect. However, Hanumantha Rao (1959a) is in agreement with Stephenson's conclusion. As Gonnert states (loc. cit.), the ootype has important functions in both the formation and the shaping of the eggshell in the schistosomes.

The wall of the ootype consists of cubical to columnar epithelial cells with large, darkly staining nuclei. The actual size and shape of the cells, as well as their detailed structure, vary according to the stage of formation of the eggshell within the ootype. There appear to be protoplasmic connections between these cells and the surrounding parenchyma of the body. The ootype epithelial cells were shown to be arranged in 3 fields by Gonnert (1955a), and to have a glandular function with a definite rhythm. The various stages in this rhythm are described by Gonnert (loc. cit.) and have all been observed in the present work. Oocytes and vitelline cells were present in several of the ootypes examined, and in one case colourless or lightly staining globules were visible between the early eggshell and the ootype cells. These appear to contain the discharged secretions of the apocrine cells of the ootype; the cells themselves approximated to the "extrusion phase" as

described by Gonnert (1955a). Although the present material has not been investigated with specific reference to the nature of the ootype epithelial cells, all the appearances of the sections agree with Gonnert's theories for S. mansoni.

The ootype discharges the newly formed egg into the uterus, whence it passes to the exterior via the genital pore, posterior to the ventral sucker. The connection between the ootype and uterus is again controlled by a sphincter muscle and the passage of vitelline cells and oocytes into the uterus is prevented by its closure (Plate 3a and b, page 89). However, aggregations of vitelline cells without any conspicuous eggshell have occasionally been observed in the uterus. These are discussed in greater detail subsequently.

The only structure observed in the present material and not mentioned by Gonnert (1955a) is a distinct, quite large granular body, situated dorsally to the oo-vitelline duct and between the ootype and Mehlis' gland (Plate 4a & b, and 5, pages 91 & 93). This is presumably involved in the formation of the egg proper, but its detailed structure and function are obscure.

The origin of the shape of the egg.

The ootype has been shown above to be the site of

Plate 3. a. Longitudinal section of a female *S. haematobium* through the ootype and uterus.

Susa and Masson, X 390.

b. Longitudinal section of a female *S. haematobium* through the ootype and uterus.

Susa and Masson, X 370.

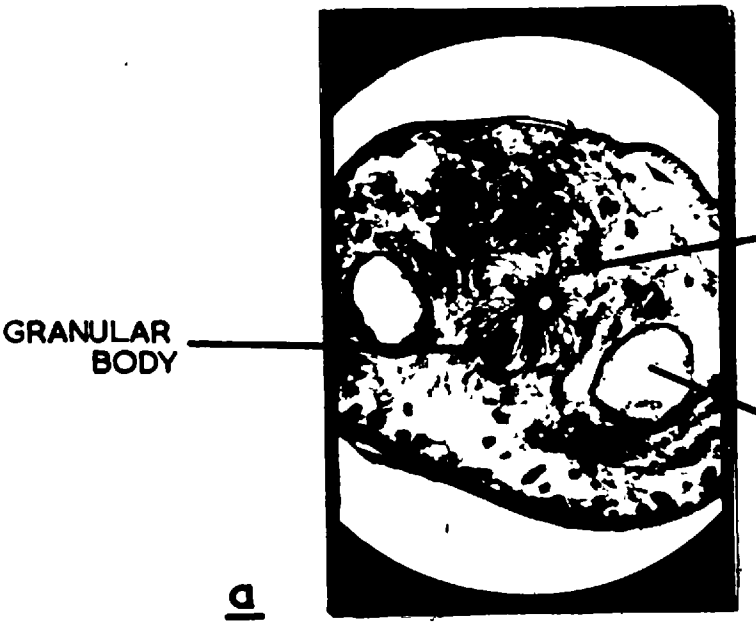

INTESTINAL CAECUM
OO-VIT ELLINE DUCT
EGG
OOTYPE
UTERUS
SPHINCTER VALVE
a


INTESTINAL CAECUM
EGG
OOTYPE
UTERUS
SPHINCTER VALVE
b

PLATE 3

Plate 4. a. Transverse section of a female *S. haematobium* through Mehlis' gland. Bouin and Masson, X 400.

b. Enlargement of the central area of the above section, X 700.


MEHLIS' GLAND
GRANULAR BODY
INTESTINAL CAECUM
a


OO-VITELLINE DUCT
MEHLIS' GLAND
GRANULAR BODY
INTESTINAL CAECUM
b

PLATE 4

Plate 5. Longitudinal section of the ootype and Mehlis' gland of a femals S. haematobium.



PLATE 5

formation and shaping of the egg. All eggs produced by one female S. haematobium (and hence produced in one ootype) are similar, within limits. Although not a hard inflexible structure, the ootype must obviously have the capacity of returning to its basic shape after the passage of a fully formed egg into the uterus. A number of females producing slightly abnormally shaped eggs have been observed, and all the eggs in the uterus have shown the same abnormality (e.g. a curvature of the aspinose end). Within limits, the dimensions (length, breadth, etc.) of the eggs of any one female are similar, but do in fact vary by as much as 15% in some cases (see Figs. 6, 7, & 8, pp. 96, 98 & 100).

The spine is formed in the junction of the oo-vitelline duct with the ootype, which is also lined by an epithelium similar to that in the ootype.

This variation from egg to egg is probably due solely to the slightly plastic nature of the ootype.

Production of malformed eggs.

Of three hamsters killed after 3rd passage (Nos. 23, 26 and 32), all appeared to have an abnormal infection of S.

Figure 6. - Variation in eggs from a single worm.

A - eggs from hamster 7 - liver.

B - eggs from hamster 42 - intestine.

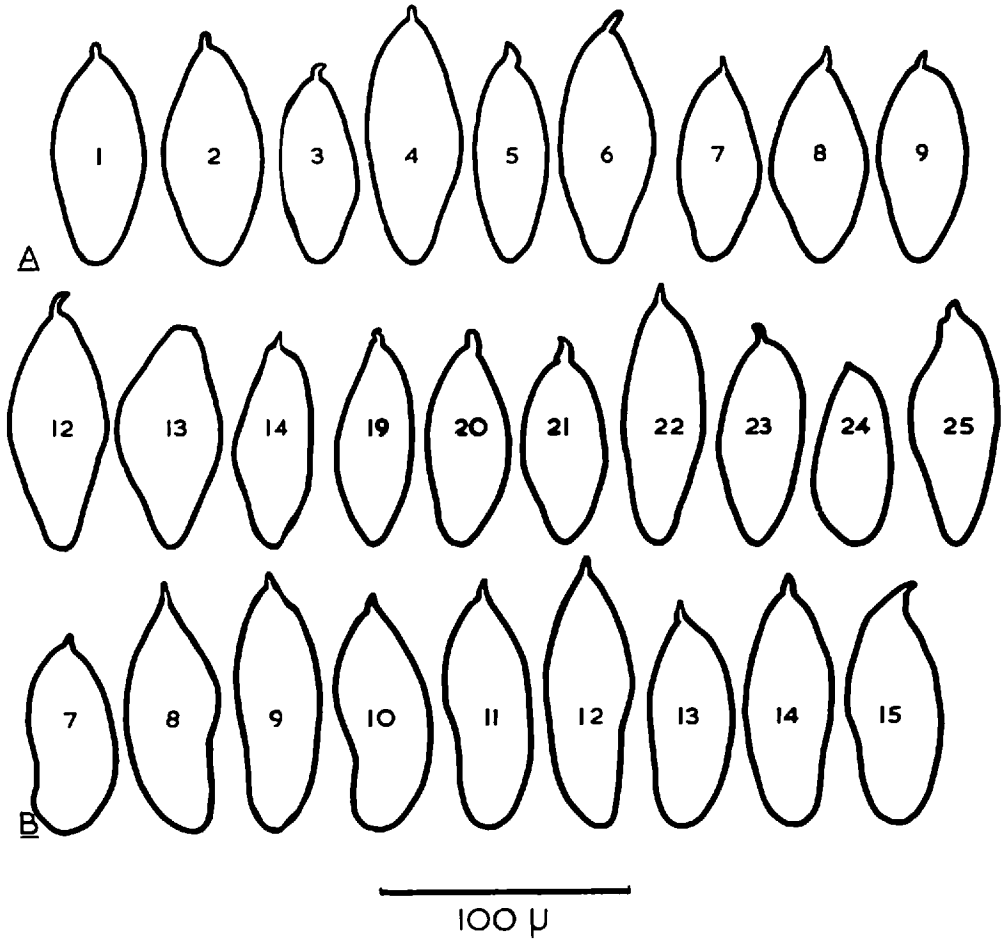

A
1
2
3
4
5
6
7
8
9
12
13
14
19
20
21
22
23
24
25
B
7
8
9
10
11
12
13
14
15
100 μ

FIGURE 6

Figure 7. - Variation in eggs from a single host
(hamster)

A - from the liver.

B - from the intestine.

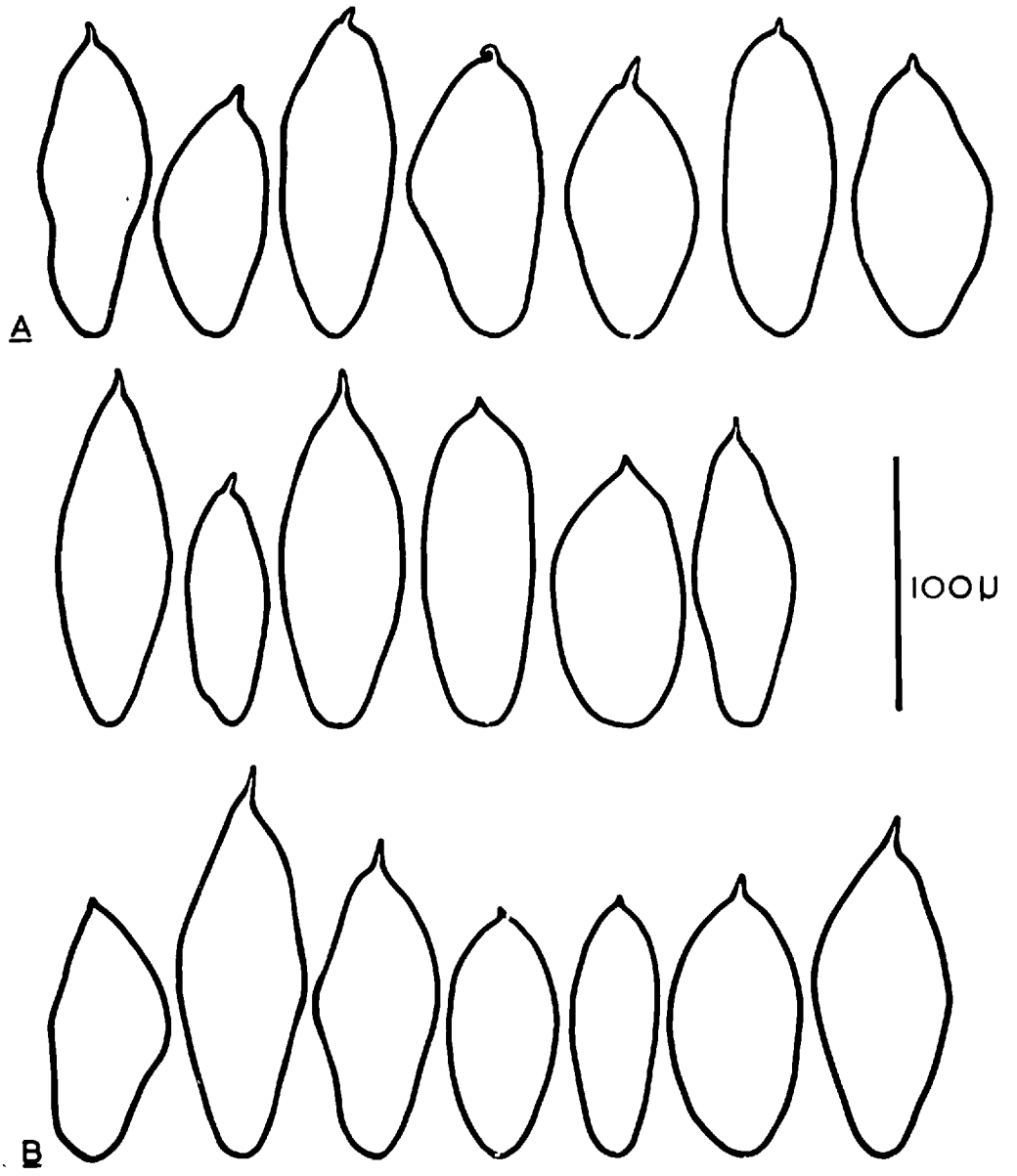


FIGURE 7

Figure 8. - Variation in egg shape.

A - Hamster 12, liver eggs.

B - Hamster 12, intestinal eggs.

C. Hamster 58, intestinal worms.

D. Mouse 19, liver eggs.

E. Mouse 21, liver eggs.

F. Mouse 19, intestinal eggs.

G. Mouse 27, intestinal worms.

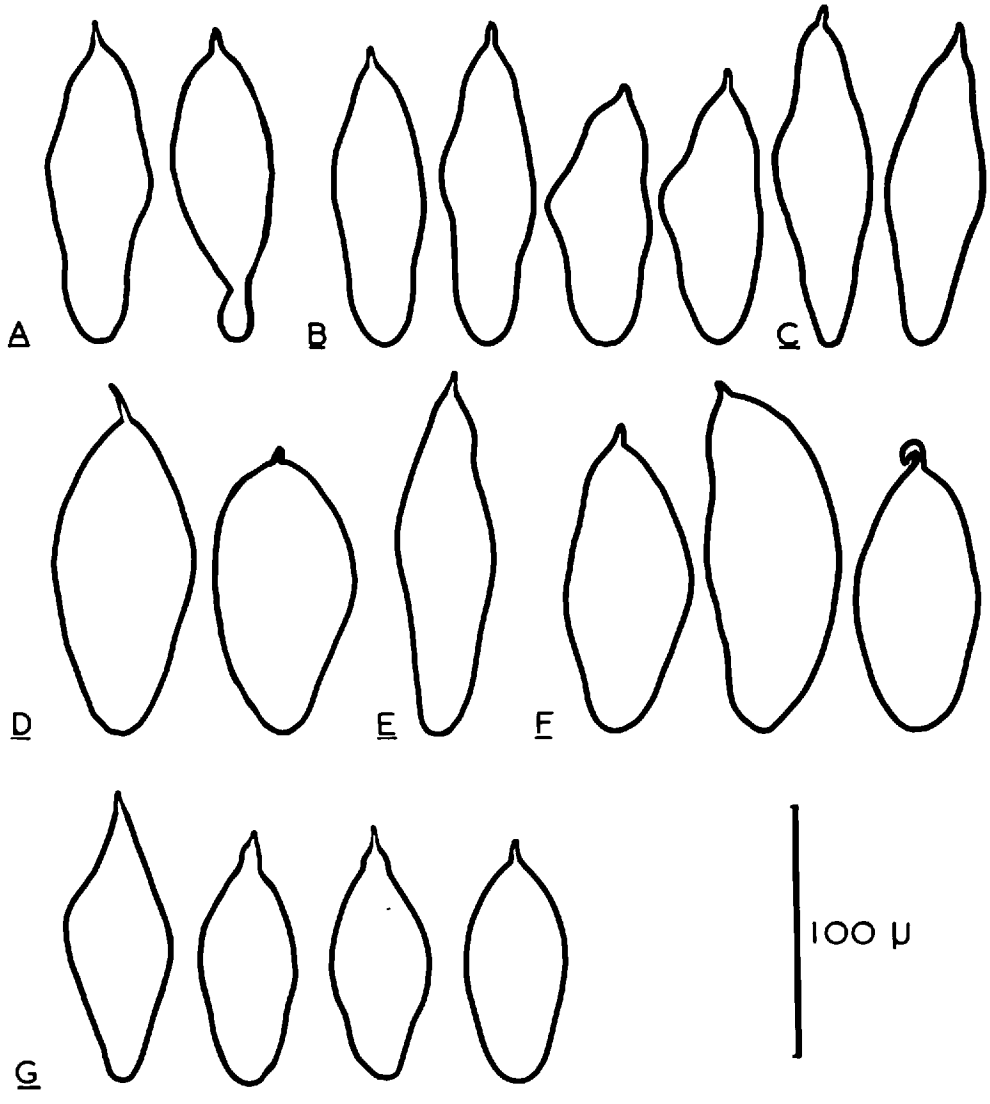


FIGURE 8

haematobium. In one case (No. 32) the infection, though relatively heavy (some 43 worms were recovered after an initial dose of 300 cercariae, i.e. a recovery of 14.3%) consisted entirely of male worms, and all of these were recovered from the liver, as is usual in such cases with S. mansoni at least. The males all appeared to be much more active than usual, and some were still quite active in saline some 6 hours after the liver had been removed from the host.

Of the two mixed infections, the remarkable feature was the great abnormality of the eggs in otherwise apparently normal females. The eggs were much smaller, of very irregular size, and not of a "typical" shape (Fig. 9. page 103). The shell appeared to be more granular and much thinner than usual - there was a considerable amount of distortion of eggshells under conditions (such as the mounting medium) which have not previously been found to cause any distortion at all. The egg contents were of a different appearance also - there did not appear to be any oocyte present, and there was generally no internal organization at all, especially with more badly malformed eggs. The contents appeared to be exclusively granular, and fairly dark in colour. There was

Figure 9. - Malformed eggs in liver worms from Hamster 26.

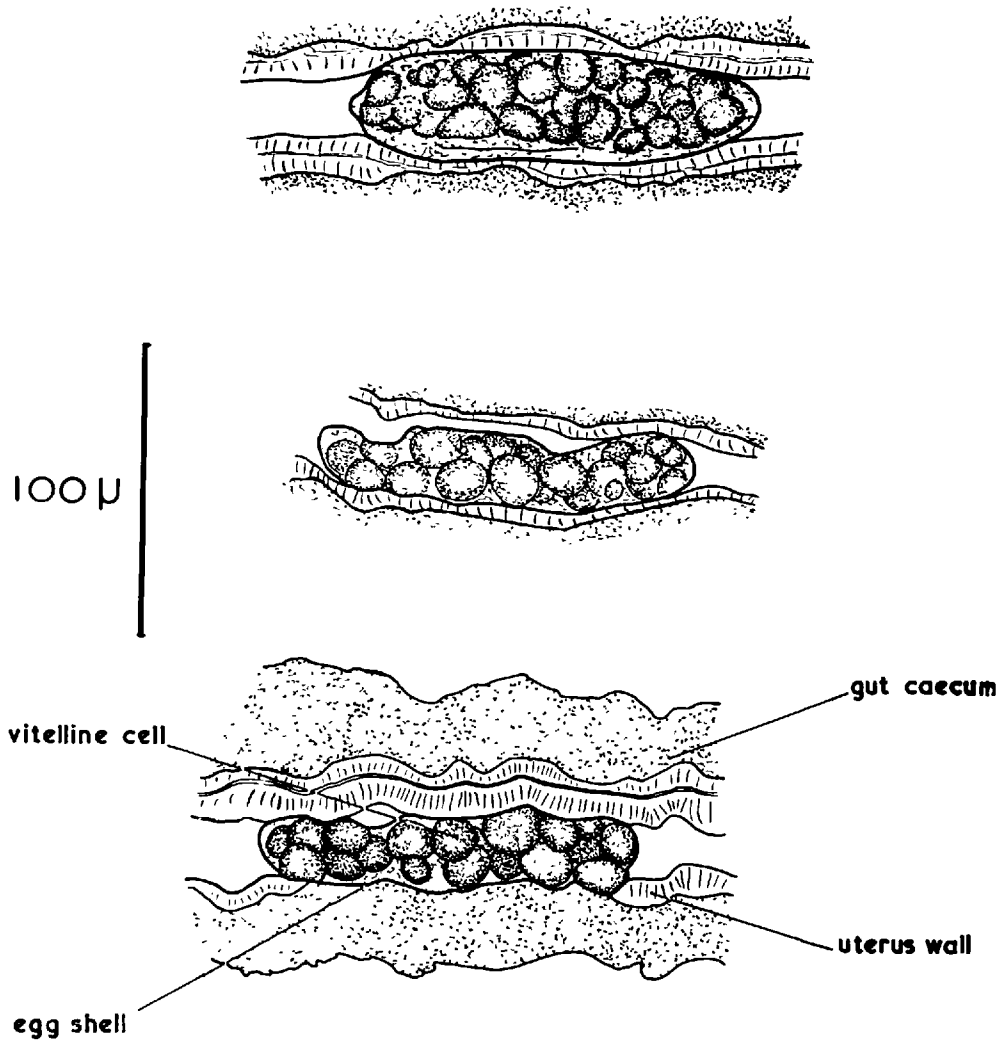


FIGURE 9

often a considerable amount of this granular material lying free in the uteri of the worms, a feature which has not been encountered before; the eggs themselves were variable in size and shape, though most were relatively longer then usual and of a more uniform diameter, and all were small, some being only 60 μ long by 20 μ wide. Some appeared to be almost as wide as long, and almost, if not all, had a spine in the usual terminal position, and of approximately the usual dimensions. These malformed eggs were often common in these two infections, but perhaps did not occur in greater numbers than the round eggs. Other worms were completely eggless.

b) Statistics.

As no apparent differences between the strains could be discerned by straightforward visual observations of the eggs and adult stages of the parasite, a detailed examination of the eggs was made using statistical methods. A summary of the egg measurements is given in Table 4, pages 105. - 109

The calculation of mean squares and products of a sub-sample of 236 eggs was carried out by Mr. M. J. R. Healy at Rothamsted, and the results are given in Table 5, page 110. The sub-sample comprised at least two eggs from each individual sample. These mean squares and products were then used by the

Table 4 - summary of egg measurements.

Strain	Sample No.	Liver, gut urine or faeces	tissue or uterus	No. in sample	Σl	$\bar{l}$	Σb	$\bar{b}$	$\Sigma l_{max.}$	$\bar{l}_{max.}$	Σs	$\bar{s}$
Ghana	1	faeces	-	4	529	132.25	243	60.75	281	70.25	28	7.0
	2	liver	tissue	20	2304	115.20	906	45.30	1357	67.85	154	7.7
	2	gut	tissue	19	2089	109.95	823	43.31	1200	63.16	151	7.95
	2	gut	uterus	72	7387	102.60	2719	37.76	4166	57.86	575	7.99
	3	faeces	-	1	126	126.00	47	47.00	65	65.00	10	10.00
				116	12435	107.20	4738	40.84	7069	60.94	918	7.91
Nigeria	4	faeces	-	14	1393	99.50	621	43.36	722	51.57	84	6.00
	5	faeces	-	2	222	111.00	99	49.50	116	58.00	7	3.50
				16	1615	100.94	720	45.00	838	52.37	91	5.69
Shambat	6	liver & gut	uterus	84	7316	87.09	2854	33.98	3938	46.88	596	7.09
	7	"	uterus	16	1496	93.50	660	41.25	772	48.25	96	6.00
	8	liver	tissue	151	16070	106.42	6692	44.32	8916	59.05	1070	7.09
	8	liver	uterus	81	7356	90.81	2774	34.25	3960	48.89	358	4.42
	8	gut	tissue	208	21868	105.13	9016	43.35	12171	58.51	1529	7.35
	8	gut	uterus	97	8602	88.68	3149	32.46	4625	47.68	449	4.63
	9	liver	tissue	199	20638	103.71	8118	40.79	11810	59.35	1058	5.32
	9	liver	uterus	33	3164	95.88	1142	34.61	1838	55.70	134	4.06
	9	gut	tissue	100	10896	108.96	4490	44.90	6250	62.50	656	6.56
	10	liver	tissue	99	10744	108.52	4242	42.85	6024	60.85	618	6.24
	10	liver	uterus	44	4104	93.27	1518	34.50	2249	51.11	258	5.86
	10	gut	tissue	13	1464	112.61	588	45.23	826	63.54	76	5.85
	11	liver	tissue	99	10861	109.71	4215	42.57	5816	58.75	625	6.31
	11	liver	uterus	106	10098	95.26	3648	34.41	5555	52.40	667	6.29
	11	gut	tissue	100	10208	102.08	4016	40.16	5470	54.70	595	5.95
	12	liver	tissue	100	10676	106.76	4398	43.98	6088	60.88	632	6.32
	12	liver	uterus	5	459	91.80	163	32.60	235	47.00	24	4.80
	12	gut	tissue	100	10916	109.16	4296	42.96	6092	60.92	638	6.38
	13	liver	tissue	100	10836	108.36	4192	41.92	5860	58.60	595	5.95
	13	gut	tissue	100	10674	106.74	4150	41.50	5752	57.52	586	5.86
	13	gut	uterus	346	34296	99.12	12158	35.14	18485	53.42	1626	4.70
	14	liver	uterus	15	1782	118.80	582	38.80	954	63.60	153	10.20
	15	liver	tissue	38	4462	117.42	1765	46.45	2450	64.47	203	5.34
	15	gut	tissue	4	503	125.75	212	53.00	274	68.50	15	3.75
				2238	229489	102.54	89038	39.78	126410	56.48	13257	5.92

l, b, l_{max} and s are the total measurements, and $\bar{l}$, $\bar{b}$, etc. their mean values.

All measurements are in microns.

Table 4 - summary of egg measurements (Contd)

HAMSTER.

Strain	Sample No.	Liver, gut urine or faeces	tissue or uterus	No. in sample	Σl	$\bar{l}$	Σb	$\bar{b}$	$\Sigma l_{max.}$	$l_{max.}$	Σs	$\bar{s}$
Cairo	16	liver	tissue	44	5156	117.18	2046	46.50	2773	63.02	288	6.54
	16	liver	uterus	3	262	87.33	102	34.00	136	45.33	22	7.33
	16	gut	tissue	50	5866	117.32	2364	47.28	3015	60.30	345	6.90
	16	gut	uterus	336	32333	96.23	11851	35.27	16852	50.15	1863	5.54
	17	gut	tissue	47	5304	112.85	2099	44.66	2827	60.15	300	6.38
	17	gut	uterus	208	19592	94.19	7309	35.14	10190	48.99	999	4.80
	18	liver	uterus	1	94	94.00	32	32.00	49	49.00	5	5.00
	18	gut	uterus	19	1747	91.94	621	32.68	886	46.63	95	5.00
	19	liver	uterus	65	6298	96.89	2129	32.75	3374	51.91	464	7.14
	19	gut	uterus	39	3836	98.36	1271	32.59	2022	51.85	277	7.10
				812	80488	99.12	29824	36.73	42124	51.88	4658	5.74
Kimumu	20	gut	uterus	19	1748	92.00	738	38.84	946	49.79	87	4.58
	21	Liver & gut	uterus	18	1474	81.89	661	36.72	762	42.33	63	3.50
	22	"	uterus	13	1245	95.77	491	37.77	657	50.54	59	4.54
	23	gut	uterus	26	2546	97.92	917	35.27	1307	50.27	95	3.65
				76	7013	92.28	2807	36.93	3672	48.31	304	4.00
Baghdad	24	liver	tissue	16	2020	126.25	805	50.31	1181	73.81	125	7.81
	24	liver	uterus	40	4125	103.12	1675	41.87	2372	59.30	232	5.80
	24	gut	uterus	44	4623	105.07	1741	39.57	2669	60.56	290	6.59
	25	liver	tissue	5	606	121.20	250	50.00	344	68.80	42	8.40
	25	liver	uterus	2	228	114.00	74	37.00	138	69.00	9	4.50
	25	gut	uterus	21	2184	104.00	814	38.76	1299	61.86	94	4.48
	26	liver	uterus	49	5137	104.84	1879	38.35	2941	60.02	339	6.92
	26	gut	tissue	50	6357	127.14	2476	49.52	3524	70.48	338	6.76
	26	gut	uterus	4	397	99.25	142	35.50	182	45.50	19	4.75
	27	liver	tissue	20	2443	122.15	972	48.60	1397	69.85	139	6.95
	27	gut	tissue	50	6234	124.68	2488	49.76	3542	70.84	405	8.10
	27	liver & gut	uterus	11	1140	103.64	428	38.90	643	58.45	86	7.82
	28	faeces	-	9	1195	132.78	467	51.89	688	76.44	75	8.33
				321	36689	114.29	14211	44.27	20920	65.17	2193	6.83
Hamster totals	58			3,579	367,639	102.72	141,338	39.49	201,033	56.17	21,421	5.98

Table 4 - summary of egg measurements (Contd.)

Strain	Sample No.	Liver, gut urine or faeces	Uterus or tissue	No. in sample.	Σl	$\bar{l}$	Σb	$\bar{b}$	$\Sigma l_{max.}$	$\bar{l}_{max.}$	N_3	$\bar{a}$
Shambat	29	gut	uterus	14	1235	88.21	476	34.00	646	46.14	63	4.50
				14	1235	88.21	476	34.00	646	46.14	63	4.50
Cabemba	30	liver	uterus	11	1040	94.54	373	33.91	561	51.00	54	4.91
	31	liver	uterus	58	5181	89.33	1980	34.14	2779	47.91	262	4.52
	31	gut	uterus	31	2833	91.39	1080	34.84	1497	48.29	133	4.29
	32	liver	uterus	6	562	93.67	219	36.50	299	49.83	25	4.17
	32	gut	uterus	16	1471	91.94	614	38.37	776	48.50	69	4.31
	33	liver	tissue	100	10857	108.57	4498	44.98	5714	57.14	643	6.43
	33	gut	tissue	15	1712	114.13	703	56.13	878	58.53	71	4.73
	34	liver	tissue	3	293	98.93	90	30.00	169	56.33	25	8.33
	34	gut	uterus	30	2968	97.67	1269	42.27	1557	51.90	188	6.27
				270	26917	99.69	10825	40.09	14230	52.70	1470	5.44
Cairo	35	liver	tissue	6	610	101.66	261	43.50	324	54.00	42	7.00
	35	gut	tissue	1	85	85.00	35	35.00	45	45.00	9	9.00
	35	gut	uterus	3	256	85.33	106	35.33	132	44.00	14	4.67
	36	liver	tissue	4	401	100.25	175	43.75	219	54.75	24	6.00
	36	gut	uterus	49	4504	91.92	1785	36.43	2262	46.18	317	6.47
	37	liver	tissue	4	407	101.75	156	39.00	205	51.25	27	6.75
	37	liver	uterus	8	792	99.00	288	36.00	396	49.50	66	8.25
	37	gut	tissue	1	101	101.00	38	38.00	52	52.00	11	11.00
	37	gut	uterus	139	13244	90.82	5078	35.00	6888	47.34	1055	7.29
	38	liver	tissue	11	1074	97.64	452	41.09	567	51.54	81	7.36
	38	gut	tissue	1	90	90.00	42	42.00	52	52.00	6	6.00
	38	gut	uterus	152	13696	90.68	5475	34.65	7469	47.27	1160	7.39
	39	liver	tissue	50	5422	108.44	2152	42.04	2914	58.20	231	4.62
	39	gut	tissue	49	4959	101.20	1912	39.02	2628	53.63	235	4.79
				484	45641	94.30	17955	37.10	24154	49.90	3286	6.79
Totals	24			768	73,793	96.08	29,256	38.09	39,030	50.82	4819	6.27

Table 4 - summary of egg measurement (Contd.)

RATS.

Strain	Sample No.	Liver, gut urine or faeces.	Tissue or uterus	No. in sample	Σ_1	$\bar{l}$	Σb	$\bar{b}$	$\Sigma l_{max.}$	$l_{max.}$	Σs	$\bar{s}$
Cairo	40	liver	tissue	1	105	105.00	35	35.00	55	55.00	6	6.00
	40	gut	uterus	79	7437	94.14	2861	36.21	3754	47.52	470	5.95
	41	liver	uterus	91	7865	86.43	3115	34.23	4180	45.93	492	5.41
	42	liver	uterus	59	5095	86.35	2189	37.10	2613	44.29	287	4.86
	42	gut	uterus	51	4706	92.27	1653	32.41	2547	49.94	236	4.63
	43	gut	uterus	49	4626	94.41	1763	35.98	2389	48.75	286	5.84
	44	liver	uterus	2	190	95.00	78	39.00	97	48.50	38	19.00
	44	gut	tissue	20	2234	111.70	886	44.30	1115	55.75	158	9.00
Totals	8			352	32,258	91.64	12,580	35.74	16,750	47.58	1,973	5.60

MERIONES.

Shambat	45	liver & gut	uterus	20	1692	84.60	664	33.20	862	43.10	113	5.65
	46	"	uterus	12	982	81.83	418	34.83	518	43.17	62	5.17
	47	liver	uterus	37	3258	88.05	1228	33.19	1660	44.86	178	4.81
	47	gut	uterus	13	1182	90.92	420	32.31	578	44.46	52	4.00
	48	liver	tissue	8	735	91.87	300	37.50	410	51.25	40	5.00
	48	gut	tissue	7	696	99.43	274	39.14	366	52.28	37	5.28
	48	gut	uterus	96	7844	81.71	3211	33.45	4135	43.07	462	4.81
	49	liver	uterus	1	72	72.00	30	30.00	39	39.00	0	0.00
	49	gut	uterus	13	1160	89.23	460	35.38	619	47.61	68	5.23
Totals	9			207	17,621	85.12	7,005	33.84	9,187	44.38	1,012	4.89

MASTOMYS

Cairo	50	liver	tissue	2	185	92.50	86	43.00	100	50.00	7	3.50
	50	gut	uterus	3	259	86.33	107	35.67	145	48.33	15	5.00
	51	liver	tissue	1	96	96.00	41	41.00	46	46.00	5	5.00
	52	liver	tissue	6	551	91.83	227	37.83	265	44.17	36	6.00
	52	gut	tissue	3	291	97.00	110	36.67	162	54.00	23	7.67
	52	gut	uterus	43	3663	85.19	1508	35.07	1839	42.77	272	6.32
Totals	6			56	5,045	86.98	2,079	35.84	2,557	44.09	358	6.17

TABLE NO. 4. - summary of egg measurements. (Contd.)

.MOI MAN.

Strain	Sample No.	Liver, gut urine or faeces tissue or uterus	No. in sample	Σl	$\bar{l}$	Σb	$\bar{b}$	$\Sigma l_{max.}$	$\bar{l}_{max}$	Σc	$\bar{c}$
Ghana	53	urine -	46	6237	135.59	2718	59.09	3346	72.74	313	6.80
Total			46	6237	135.59	2718	59.09	3346	72.74	313	6.80
Nigeria	54	urine -	50	6751	135.02	2918	58.36	3660	73.20	346	6.92
Total			50	6751	135.02	2918	58.36	3660	73.20	346	6.92
Cairo	55	urine -	25	3312	132.48	1338	53.52	1745	69.80	164	6.56
	56	urine -	25	3400	136.00	1370	54.80	1699	67.96	172	6.88
	57	urine -	25	3359	134.36	1384	55.36	1756	70.24	184	7.36
	58	urine -	20	2691	134.55	1097	54.85	1375	68.75	152	7.60
Total			95	12,762	134.34	5189	54.62	6575	69.21	672	7.07
Dirgag	59	urine -	8	1156	144.50	464	58.00	571	71.37	53	6.62
	60	urine -	17	2331	137.12	953	56.06	1218	71.65	110	6.47
	61	urine -	3	438	146.00	188	62.67	226	75.33	24	8.00
	62	urine -	25	3450	138.00	1535	61.40	1803	72.12	186	7.44
	63	urine -	25	3357	134.28	1417	56.68	1706	68.24	182	7.28
	64	urine -	1	143	143.00	52	52.00	76	76.00	5	5.00
Total			79	10,875	137.66	4609	58.34	5600	70.89	560	7.09
Said	65	urine -	25	3027	121.08	1349	53.96	1541	61.64	181	7.24
	66	urine -	25	3158	126.32	1417	56.68	1579	63.16	178	7.12
	67	urine -	12	1421	118.42	642	53.50	737	61.42	77	6.42
	68	urine -	1	139	139.00	54	54.00	75	75.00	4	4.00
Total.			63	7745	122.98	3462	54.95	3932	62.41	440	6.98
Kuwait	69	urine -	13	1536	118.15	587	45.15	819	63.00	95	7.31
Total			13	1536	118.15	587	45.15	819	63.00	95	7.31
Baghdad	70	urine -	34	4603	135.38	2034	59.82	2389	70.26	259	7.62
Total			34	4603	135.38	2034	59.82	2389	70.26	259	7.62
Totals	18		380	50,509	132.92	21,517	56.62	26,321	69.26	2,685	7.06

TABLE 5. Mean Squares and Products: Sub-sample of 236 eggs.

	Length	Breadth	L_{max}	Spine
Length	7416.40	3517.00	3715.20	197.40
	476.85	163.15	339.54	13.08
	226.55	67.96	127.29	12.37
	252.85	106.00	164.11	2.77
	88.12	15.80	52.15	0.72
	332.74	130.11	187.54	8.58
Breadth		1694.20	1721.60	91.00
		84.77	105.69	7.00
		54.92	35.29	5.90
		76.00	73.92	1.40
		14.16	9.75	0.34
		76.60	71.50	4.08
L_{max}			1994.60	96.80
			274.00	8.08
			97.02	4.12
			122.58	3.38
			40.96	1.37
			125.99	4.82
Spine				24.00
				8.15
				14.59
				8.30
				3.84
				7.85

The order in each column is:

a) Between host species.

b) Between parasite strains within host species.

c) Between individual hosts within parasite strains.

d) Between sites within individual hosts.

e) Within sites, and

f) Total variation.

author to carry out analyses of variance and to compare the host species and the strains of parasites used.

The general symbols used and the methods adopted are as follows:-

General symbols:

	Totals	Means
Host species	H	$\bar{h}$
Parasite strains	L	$\bar{l}$
Samples (animals)	A	$\bar{a}$
Sites	S	$\bar{s}$
Grand total	X	$\bar{x}$

Source	Sum of Squares	Degrees of Freedom	Mean Squares	F	P
a	$\Sigma(H\bar{h}) - (X\bar{x})$	5	7416.40	a/b	
b	$\Sigma(L\bar{l}) - \Sigma(H\bar{h})$	13	476.85	b/c	
c	$\Sigma(A\bar{a}) - \Sigma(L\bar{l})$	51	226.55	c/d	
d	$\Sigma(S\bar{s}) - \Sigma(A\bar{a})$	53	252.85	d/e	
e	$\Sigma(x^2) - \Sigma(S\bar{s})$	113	88.12	-	
f	$\Sigma(x^2) - (X\bar{x})$	235	232.74		

Comparison of host species.

Variance of mean ($\bar{h}$) of n_1 values.

$$= \frac{\text{Error variance}}{n_1}$$

In the present circumstances, where the components of the above analysis of variance are not of equal status but each can be regarded as subordinate to the one above it, and where (moreover) many of the unit cells of the body of data are unoccupied (e.g. no mice from Ghana), it would be unfair to use line e (variance within sites) as a measure of random variation, since this would ignore the large effects of samples and strains. For each line in the analysis it would be fairer to use as a measure of random variation the mean square in the line immediately below it. Thus the variation between host (line a) will be judged by that within strains (line b), with 13 degrees of freedom. The variance of the difference between two host means will be taken as the sum of their variances, so that:

$$t = (\bar{h}_1 - \bar{h}_2) \Big/ \sqrt{s^2/n_1 + s^2/n_2}$$

Comparison of parasite strains.

The scatter of means about the general mean can be obtained (in the sum of squares column of table 5, page 110) by successive addition:

	S. of S.	N.	D.F.	M.S.
Host species	$\Sigma(H\bar{h})-(X\bar{x})=37082.00$	6	5	7416.40
Parasite strains with in host species	$\Sigma(L\bar{l})-(X\bar{x})=43281.05$	19	9	4809.00
Animals within strains	$\Sigma(A\bar{a})-(X\bar{x})=54385.10$	70		
Sites within animals	$\Sigma(S\bar{s})-(X\bar{x})=68236.15$	123		

From this information, the parasite strains could be compared as for the host species. However, because of the irregularities in the data already referred to, this becomes impracticable, especially in view of the fact that any significant differences might be attributable to hosts rather than strains. The difficulty can be illustrated by turning forward to Table 17, page 120. Here it would be misleading to compare the Shambat mean (based on 2517 eggs from assorted non-human mammals) with the Kuwait mean (based on only 13 eggs of human origin). If there were a significant difference it would in fact be ascribable to hosts rather than strains. Thus, the only satisfactory procedure is to follow the analysis of variance and compare strains <u>within hosts</u>. Thus, (Table 17) it would be fair to compare Shambat mouse and Cairo mouse. The relevant measure of random variation would be the third line of the analysis: "between samples within strains" with 51 degrees of freedom.

The results of the analysis of variance are given in Tables 6 to 9, pages 115 and 116, and are summarised in Table 10, page116. The results of the comparison of host species for the three most promising dimensions determined by the analysis of variance (length, breadth and $l_{max.}$) are given in Tables 11 to 16, 117 - 119. The results of the comparison of parasite strains within the same host species (man, hamster and mouse) are given in Tables 17 to 29, pages 120 - 132 for the dimensions length, breadth and $l_{max.}$.

Although the comparison of host species and parasite strains using one dimension of the egg at a time revealed significant differences in a considerable proportion of the cases, it was felt that the simultaneous use of all four dimensions would make the relationships of the groups even clearer. Accordingly, the means of each of the groups under study were calculated (Tables 20A, 20B, and 30, pages 123 and 133). The canonical vectors (Tables 31 to 35, pages 133-137) were then used to calculate the generalised distances separating successive pairs of groups. Models were constructed illustrating the relationships of these groups (Fig. 10, page 139) and from these it was evident that there was only one basic dimension of variation.

ANALYSES OF VARIANCE : Subsample of 236 eggs.

Table 6: length

Source	Sum of Squares	D.F.	Mean Squares	F.	P.
a	37082.00	5	7416.40	15.55	< 0.1%
b	6199.05	13	476.85	2.10	< 5%
c	11554.05	51	226.55	0.89	-
d	13401.05	53	252.85	2.87	< 0.1%
e	9957.56	113	88.12	-	
f	78193.90	235	332.74		

Table 7: Breadth

Source	Sum of Squares	D.F.	Mean Squares	F.	P.
a	8471.00	5	1694.20	19.98	< 0.1%
b	1102.01	13	84.77	1.54	-
c	2800.92	51	54.92	0.72	-
d	4028.00	53	76.00	5.37	< 0.1%
e	1600.08	113	14.16	-	
f	18001.00	235	76.60		

Table 8: L_{max}.

Source	Sum of Squares	D.F.	Mean Squares	F.	P.
a	9973.00	5	1994.60	7.28	< 1%
b	3562.00	13	274.00	2.82	< 1%
c	4948.02	51	97.02	0.79	-
d	6496.48	53	122.58	2.99	< 0.1%
e	4628.48	113	40.96	-	
f	29607.65	235	125.99		

Table 9 : Spine

Source	Sum of Squares	D.F.	Mean Squares	F.	P.
a	120.00	5	24.00	2.94	< 10%
b	105.95	13	8.15	0.56	-
c	744.09	51	14.59	1.76	< 5%
d	439.90	53	8.30	2.16	< 0.1%
e	433.92	113	3.84	-	
f	1844.75	235	7.85		

Table 10 : Summary of probabilities of components of the analysis being due to chance.

Source	Category	Length	Breadth	L_{max}	Spine
a	Host species	< 0.1%	< 0.1%	< 1%	< 10%
b	Parasite strains within host species	< 5%	-	< 1%	-
c	Samples (animals) within strains	-	-	-	< 5%
d	Sites within samples	< 0.1%	< 0.1%	< 0.1%	< 0.1%

N.B. The lower the value of P., the higher the probability of distinguishing between the categories.

COMPARISON OF HOST SPECIES.

Table 11 : Host means - length.

Error variance = 476.85 D.F. = 13.

Host species	n	$\Sigma' l$	mean (μ)	Var. of mean.	S.E.
Hamster	3579	367639	102.72	0.13	0.36
Mouse	768	73793	96.08	0.62	0.79
Arvicanthis	352	32258	91.64	1.35	1.16
Meriones	207	17621	85.12	2.30	1.52
Mastomys	58	5045	86.98	8.2	2.86
Man	380	50509	132.92	1.25	1.12

Table 12 : "t" table - length*

	Hamster	Mouse	Arvicanthis	Meriones	Mastomys	Man
Hamster	-	7.65<0.1%	9.08<0.1%	11.28<0.1%	5.45<0.1%	25.81<0.1%
Mouse		-	3.16<1%	6.41<0.1%	3.06<1%	26.89<0.1%
Arvicanthis			-	3.72<1%	1.51 -	25.64<0.1%
Meriones				-	0.57 -	25.42<0.1%
Mastomys					-	14.91<0.1%
Man						-

* $t = \frac{\text{difference of means}}{\text{S.E. of difference}}$

Table 13 : Host means - breadth

Error variance = 84.77 D.F. = 13.

Host species	n_i	Σb	mean (μ)	var. of mean	S.E.
Hamster	3579	141338	39.49	0.024	0.15
Mouse	768	29256	38.09	0.11	0.33
Arvicanthis	352	12580	35.74	0.24	0.49
Meriones	207	7005	33.84	0.41	0.64
Mastomys	58	2079	35.84	1.46	1.21
Man	380	21517	56.62	0.22	0.47

Table 14 : "t" table - breadth

	Hamster	Mouse	Arvicanthis	Meriones	Mastomys	Man
Hamster	-	3.784<1%	7.294<0.1%	8.574<0.1%	2.994<2%	11.424<0.1%
Mouse		-	3.984<1%	5.904<0.1%	1.80 -	12.114<0.1%
Arvicanthis			-	2.374<5%	0.08 -	13.304<0.1%
Meriones				-	1.46 -	14.064<0.1%
Mastomys					-	10.824<0.1%
Man						-

Table 15 : Host means - l_{max}.

Error variance = 274.00 D.F. = 13

Host species	n_1	l_{max}	mean(μ)	var. of mean	S.E.
Hamster	3579	201033	56.17	0.076	0.87
Mouse	768	39030	50.82	0.36	0.60
Arvicanthis	352	16750	47.38	0.78	0.88
Meriones	207	9187	44.38	1.32	1.15
Mastomys	58	2557	44.09	4.72	2.17
Man	380	26321	69.26	0.72	0.85

Table 16 : "t" table - l_{max}.

	Hamster	Mouse	Arvicanthis	Meriones	Mastomys	Man
Hamster	-	8.11<0.1%	9.45<0.1%	9.99<0.1%	5.51<0.1%	14.17<0.1%
Mouse		-	3.21<1%	4.99<0.1%	3.00<2%	17.73<0.1%
Arvicanthis			-	2.07 -	1.40 -	17.93<0.1%
Meriones				-	0.12 -	17.40<0.1%
Mastomys					-	10.80<0.1%
Man						-

Table 17 : length

Locality	Hamster	Mouse	Arvicanthis	Meriones	Mastomys	Man
Ghana	12435 (116)					6237 (46)
Nigeria	1615 (16)					6751 (50)
Shambat	229489 (2238)	1235 (14)		17621 (207)		
Cairo	80488 (812)	45641 (484)	32258 (352)		5045 (58)	12762 (95)
Kisumu	7013 (76)					
Baghdad	36689 (321)					4603 (34)
Cabemba		26917 (270)				
Dirgag						10875 (79)
Said						7745 (63)
Kuwait						1536 (13)
D.F. between strains within hosts	5	2				6
					Total d.f. =	13

The total lengths are in microns, and the figures in brackets refer to the sample size.

COMPARISON OF PARASITE STRAINS.

Table 18 : breadth.

Locality	Hamster	Mouse	Arvicanthis	Meriones	Mastomys	Man.
Ghana	4738 (116)					2718 (46)
Nigeria	720 (16)					2918 (50)
Shambat	89038 (2238)	476 (14)		7005 (207)		
Cairo	29824 (812)	17955 (484)	12580 (352)		2079 (58)	5189 (95)
Kisumu	2807 (76)					
Baghdad	14211 (321)					2034 (34)
Cabemba		10825 (270)				
Girgag						5600 (79)
Said						3462 (63)
Kuwait						587 (13)
D.F. between strains within hosts	5	2				6
					Total d.f. =	13

The total lengths are in microns, and the figures in brackets refer to the sample size.

COMPARISON OF PARASITE STRAINS.

Table 19 : 1 max.

Locality	Hamster	Mouse	Arvicanthis	Meriones	Mastomys	Man
Ghana	7069 (116)					3346 (46)
Nigeria	838 (16)					3660 (50)
Shambat	126410 (2238)	646 (14)		9187 (207)		
Cairo	42124 (812)	24154 (484)	16750 (352)		2557 (58)	6575 (95)
Kisumu	3672 (76)					
Baghdad	20920 (321)					2389 (34)
Cabemba		14230 (270)				
Dirgag						5600 (79)
Said						3932 (63)
Kuwait						819 (13)
D.F. between strains within hosts	5	2				6
				Total d.f. =		13

The total lengths are in microns, and the figures in brackets refer to the sample size

Table 20 : Mean dimensions. (microns)

A. Man

Strain	Length	Breadth	$L_{max.}$	Spine
1. Ghana	135.59	59.09	72.74	6.80
2. Nigeria	135.02	58.36	73.20	6.92
3. Cairo	134.34	54.62	69.21	7.07
4. Baghdad	135.38	59.82	70.26	7.62
5. Dirgag	137.66	58.34	70.89	7.09
6. Said	122.94	54.95	62.41	6.98
7. Kuwait	118.15	45.15	63.00	7.31

B. Hamsters

Strain	Length	Breadth	$L_{max.}$	Spine
1. Ghana	107.20	40.84	60.94	7.91
2. Nigeria	100.94	45.00	52.37	5.69
3. Shambat	102.54	39.78	56.48	5.92
4. Cairo	99.12	36.73	51.88	5.74
5. Kisumu	92.28	36.93	48.31	4.00
6. Baghdad	114.29	44.27	65.17	6.83

C. Mice

Strain	Length	Breadth	$L_{max.}$	Spine
1. Shambat	88.21	34.00	46.14	4.50
2. Cairo	94.30	37.10	49.90	6.79
3. Cabemba	99.69	40.09	52.70	5.44

Table 21

Strain means - man - length

Error variance = 226.55 D.F. = 51

Strain	n_1	Σ_1	mean (μ)	var. of mean	S.E.
Ghana	46	6237	135.59	4.92	2.22
Nigeria	50	6751	135.02	4.53	2.13
Cairo	95	12762	134.34	2.38	1.54
Baghdad	34	4603	135.38	6.66	2.58
Dirgag	79	10875	137.66	2.87	1.69
Said	63	7745	122.94	3.59	1.89
Kuwait	13	1536	118.15	7.43	2.72

"t" table

	Ghana	Nigeria	Cairo	Baghdad	Dirgag	Said	Kuwait
Ghana	-	0.18	0.46	0.06	0.74	4.33 $<0.1\%$	4.97 $<0.1\%$
Nigeria		-	0.26	0.11	0.97	4.24 $<0.1\%$	4.89 $<0.1\%$
Cairo			-	0.34	1.45	4.67 $<0.1\%$	5.17 $<0.1\%$
Baghdad				-	0.74	3.89 $<0.1\%$	4.59 $<0.1\%$
Dirgag					-	5.79 $<0.1\%$	6.08 $<0.1\%$
Said						-	1.44 -
Kuwait							-

Table 22

Strain means - man - breadth

Error variance = 54.92 D.F. = 51

Strain	n_1	b	mean (μ)	var. of mean	S.E.
Ghana	46	2718	59.09	1.19	1.09
Nigeria	50	2918	58.36	1.10	1.05
Cairo	95	5189	54.62	0.58	0.76
Baghdad	34	2034	59.82	1.61	1.27
Dirgag	79	4609	58.34	0.69	0.83
Said	63	3462	54.95	0.87	0.93
Kuwait	13	587	45.15	4.22	2.05

"t" table

	Ghana	Nigeria	Cairo	Baghdad	Dirgag	Said	Kuwait
Ghana	-	0.48	3.36<1%	0.44	0.55	2.89<1%	6.01<0.1%
Nigeria		-	2.28<1%	0.88	0.01	2.42<5%	5.72<0.1%
Cairo			-	3.51<0.1%	3.29<1%	0.27	4.32<0.1%
Baghdad				-	0.97	3.10<1%	6.09<0.1%
Dirgag					-	2.71<1%	5.97<0.1%
Said						-	4.34<0.1%
Kuwait							-

Table 23

Strain means - man - $l_{max.}$

Error variance = 97.02 (max.) D.F.= 51

Strain	n,	$\Sigma l_{max.}$	mean (μ)	var. of mean	S.E.
Ghana	46	3346	72.74	2.11	1.45
Nigeria	50	3660	73.20	1.94	1.39
Cairo	95	6575	69.21	1.02	1.01
Baghdad	34	2389	70.26	2.85	1.69
Dirgag	79	5600	70.89	1.23	1.11
Said	63	3932	62.41	1.54	1.24
Kuwait	13	819	63.00	7.46	2.73

"t" table

	Ghana	Nigeria	Cairo	Baghdad	Dirgag	Said	Kuwait
Ghana	-	0.22	1.99	1.11	1.01	5.40 < 0.1%	3.152 < 1%
Nigeria		-	2.32 < 5%	1.34	1.23	5.80 < 0.1%	3.33 < 1%
Cairo			-	0.53	1.12	4.25 < 0.1%	2.13 < 5%
Baghdad				-	0.31	3.75 < 0.1%	2.26 < 5%
Dirgag					-	5.10 < 0.1%	2.67 < 2%
Said						-	0.19
Kuwait							-

Table 24.

Strain means - hamsters - length

Error variance = 226.55 D.F. = 51

Strain	n_i	l	mean (μ)	var. of mean	S.E.
Ghana	116	12436	107.20	1.95	1.39
Nigeria	16	1615	100.94	14.16	3.76
Shambat	2238	229469	102.54	0.10	0.32
Cairo	812	80488	99.12	0.28	0.53
Kisumu	76	7013	92.28	2.98	1.73
Baghdad	321	36689	114.29	0.70	0.84

"t" table

	Ghana	Nigeria	Shambat	Cairo	Kisumu	Baghdad
Ghana	-	1.56	3.26<1%	5.43<0.1%	6.72<0.1%	4.34<0.1%
Nigeria		-	0.42	- 0.48	- 2.09	<5% 3.47<1%
Shambat			-	5.61<0.1%	5.86<0.1%	13.20<0.1%
Cairo				-	3.80<0.1%	15.32<0.1%
Kisumu					-	11.46<0.1%
Baghdad						-

Table 25

Strain means - hamsters - breadth

Error variance = 54.92 D.F. = 51

Strain	n,	b	mean (μ)	var. of mean	S.E.
Ghana	116	4738	40.84	0.47	0.68
Nigeria	16	720	45.00	3.43	1.85
Shambat	2238	89038	39.78	0.02	0.14
Cairo	812	29824	36.73	0.68	0.82
Kisumu	76	2807	36.93	0.72	0.85
Baghdad	321	14211	44.27	0.17	0.41

"t" table.

	Ghana	Nigeria	Shambat	Cairo	Kisumu	Baghdad
Ghana	-	2.11<5%	1.51	-	3.84<0.1%	3.59<0.1%	4.29<0.1%
Nigeria		-	2.81<1%	4.07<0.1%	3.95<0.1%	0.39	-
Shambat			-	3.63<0.1%	3.31<1%	10.44<0.1%
Cairo				-	0.17	-	8.19<0.1%
Kisumu					-	7.81<0.1%
Baghdad						-

Table 26

Strain means - hamster - l_{max}.

Error variance = 97.02 D.F. = 51

Strain	n_i	l_{max}	mean (μ)	var. of mean	S.E.
Ghana	116	7069	60.94	0.84	0.92
Nigeria	16	838	52.37	6.06	2.46
Shambat	2238	126410	56.48	0.04	0.20
Cairo	812	42124	51.88	0.12	0.35
Kisumu	76	3672	48.31	1.28	1.13
Baghdad	321	20920	65.17	0.30	0.55

"t" table

	Ghana	Nigeria	Shambat	Cairo	Kisumu	Baghdad
Ghana	-	3.26<1%	4.74<0.1%	9.24<0.1%	8.65<0.1%	3.95<0.1%
Nigeria		-	1.66	0.19	1.50	5.08<0.1%
Shambat			-	2.15<5%	7.10<0.1%	14.98<0.1%
Cairo				-	3.02<1%	20.45<0.1%
Kisumu					-	13.38<0.1%
Baghdad						-

Table 27

Strain means - mice - length

Error variance = 226.55 D.F. = 51

Strain	n,	l	mean (μ)	var. of mean	S.E.
Shambat	14	1235	88.21	16.18	4.02
Cairo	484	45641	94.30	0.47	0.68
Cabemba	270	26917	99.69	0.84	0.92

"t" table

	Shambat	Cairo	Cabemba
Shambat	-	1.49	2.77<1%
Cairo		-	4.73<0.1%
Cabemba			-

Table 28

Strain means - mice - breadth

Error variance = 54.92 D.F. = 51

Strain	n,	breadth	mean (μ)	var. of mean	S.E.
Shambat	14	476	34.00	3.92	1.97
Cairo	484	17955	37.10	0.11	0.33
Cabemba	270	10825	40.09	0.20	0.45

"t" table

	Shambat	Cairo	Cabemba
Shambat	-	1.54	3.00<1%
Cairo		-	5.34<0.1%
Cabemba			-

Table 29

Strain means - mice - l_{max}.

Error variance = 97.02 D.E. = 51

Strain	n,	max.	mean (μ)	var of mean	S.E.
Shambat	14	646	46.14	6.93	2.63
Cairo	484	24154	49.90	0.20	0.45
Cabemba	270	14230	52.70	0.36	0.60

"t" table

	Shambat	Cairo	Cabemba
Shambat	-	1.41 -	2.43 < 2%
Cairo		-	3.73 < 0.1%
Cabemba			-

Table 30 : Host means (microns)

	Length	Breadth	$L_{max.}$	Spine
Hamsters	102.72	39.49	56.17	5.98
Mice	96.08	38.09	50.82	6.27
Arvicanthis	91.64	35.74	47.58	5.60
Meriones	85.12	33.84	44.38	4.89
Mastomys	86.98	35.84	44.09	6.17
Man	132.92	56.62	69.26	7.06

Table 31.

Roots of determinantal equation $|B - \lambda A| = 0$, based on 180 eggs from Hamsters and Meriones.

Root	% of total information contributed by each dimension
200.5557	38.08
161.8283	30.73
111.5067	21.17
52.7140	10.01

From the above table, it is evident that length is the most important dimension, contributing approximately 38% of the total information. The canonical variates do not correspond simply to the four original measurements. Each variate is generated by one of the four roots, i.e. the vector X (Table 31A, page 133B) is generated by the root 200.5557, vector Y is generated by root 161.8283 etc.

Table 31A.

Values of canonical vectors derived from the roots of the determinantal equation $|B - \lambda A| = 0$ (Table 31, page 133A), and based on 180 eggs from Hamsters and Meriones.

	Vector			
Relative weights of	X	Y	Z	Q
Length	+1.0000	+0.0351	+0.0082	-0.5103
Breadth	+0.5022	-0.8137	+0.5474	+0.1289
L_{max}	-0.0270	+0.2307	-0.0188	+1.0000
Spine	-0.2358	+1.0000	+1.0000	-0.6120

The vectors are orthogonal in the space defined by the pooled information matrix, but are unlikely to be orthogonal in Euclidean space. Thus the sum of products is not necessarily zero in Euclidean space, and the failure of such a cross-product test for orthogonality in Euclidean space does not necessarily indicate non-orthogonality in the proper space occupied by the vectors. A cross-product test is not available for operation on vectors expressed in Euclidean space (as above) to test whether or not they are orthogonal in their own proper space.

The vectors in the above table are evaluated in Tables 37 to 39 inclusive and the results expressed graphically on pages 145, 147 and 149.

Table 32 : Generalised distances.

Hosts.

Mouse	-	Hamster	6.80
Arvicanthis	-	Hamster	12.65
Meriones	-	Hamster	20.03
Mastomys	-	Hamster	17.10
Man	-	Hamster	33.73
Man	-	Mastomys	51.77
Man	-	Meriones	54.20
Man	-	Arvicanthis	46.80
Man	-	Mouse	41.73
Arvicanthis	-	Mouse	5.47
Arvicanthis	-	Meriones	7.25
Meriones	-	Mouse	12.71

Table 33 Generalised distances between strains.

Man.

Ghana	Nigeria	0.63
Ghana	Cairo	- 1.67
Nigeria	Cairo	- 0.33
Ghana	Baghdad	- 2.38
Nigeria	Baghdad	- 2.87
Baghdad	Cairo	- 3.70
Baghdad	Dirgag	1.34
Cairo	Dirgag	3.52
Ghana	Dirgag	1.82
Baghdad	Said	14.21
Cairo	Said	11.20
Dirgag	Said	15.93
Kuwait	Nigeria	18.89
Kuwait	Baghdad	18.51
Kuwait	Cairo	17.66

Table 34 Generalised distances between strains.

Hamsters.

Baghdad	-	Cairo	18.47
Ghana	-	Nigeria	1.52
Baghdad	-	Nigeria	13.23
Cairo	-	Ghana	11.36
Cairo	-	Shambat	4.66
Baghdad	-	Shambat	13.74
Baghdad	-	Ghana	7.55
Shambat	-	Nigeria	6.40
Shambat	-	Kisumu	12.25
Kisumu	-	Cairo	6.79
Kisumu	-	Ghana	18.35
Nigeria	-	Kisumu	10.05
Nigeria	-	Cairo	6.45
Baghdad	-	Kisumu	26.05
Ghana	-	Shambat	6.10

Table 35 Generalised distances between strains.

Man - Hamsters.

Hamsters	Man	
Baghdad	Kuwait	3.76
Baghdad	Dirgag	25.76
Kisumu	Kuwait	29.47
Ghana	Said	14.60
Baghdad	Said	3.00
Shambat	Ghana	37.28

Figure 10. - Photograph of 3-dimensional models showing the relationships of the host species and the parasite strains.

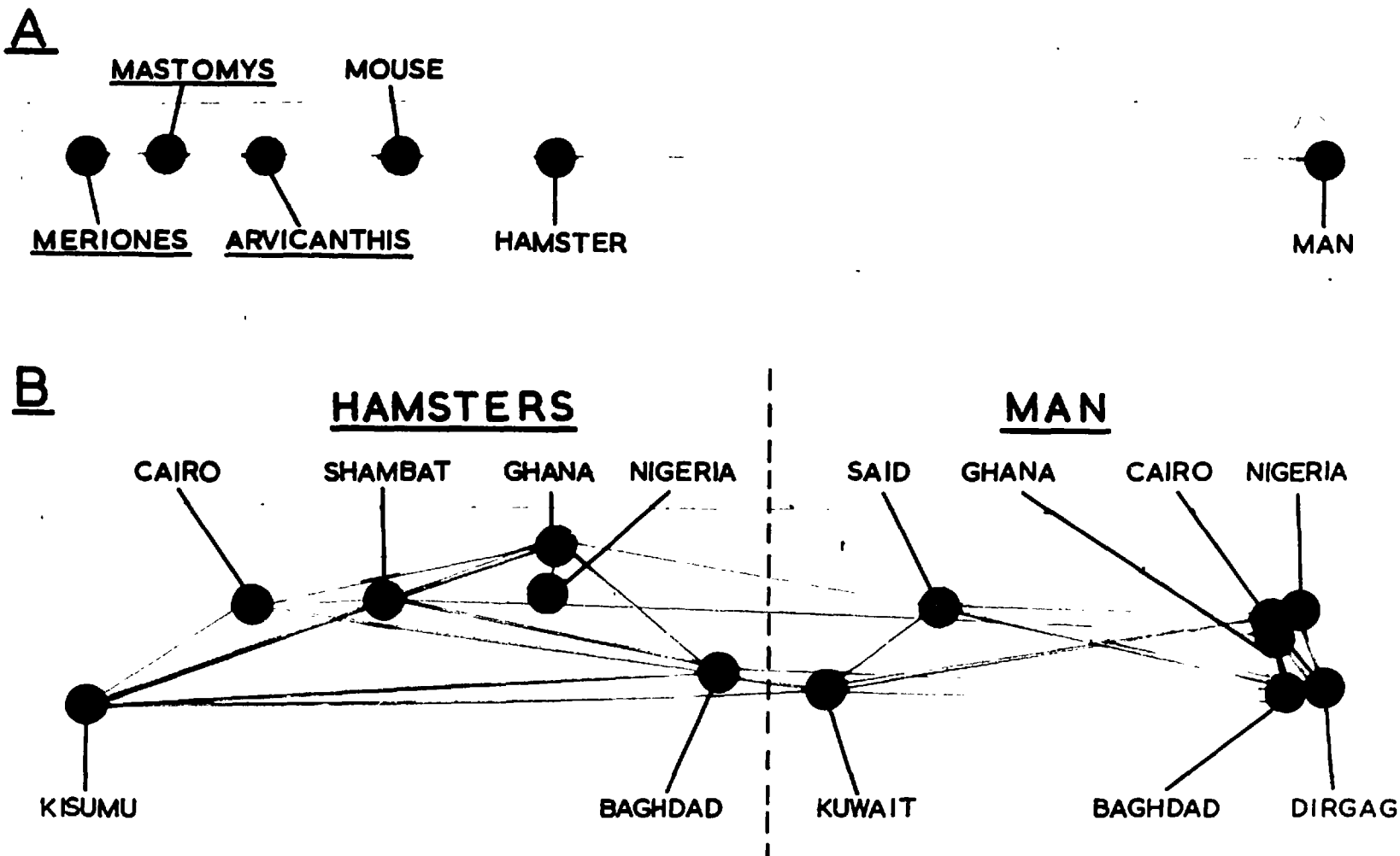

A
MASTOMYS
MOUSE
MERIONES
ARVICANTHIS
HAMSTER
MAN
B
HAMSTERS
MAN
CAIRO
SHAMBAT
GHANA
NIGERIA
SAID
GHANA
CAIRO
NIGERIA
KISUMU
BAGHDAD
KUWAIT
BAGHDAD
DIRGAG

FIG. 10

The X, Y, and Z coordinates were also calculated for eggs from one worm, for eggs from worms from one site within a single host, and for eggs from different sites within different hosts. The results of these calculations are given in Tables 37 to 39, pages 141, 142 and 143, and the graphs on pages 145, 147 and 149.

c) Results of observations on living worms.

The object of these experiments was to ascertain the sequence of events preceding and during the formation of the egg shell and its shaping within the ootype in order to get a better understanding of the structures involved and their functional relationships.

1st experiment.

Culture flasks were set up as described (pages 70 and 71) but only with 100,000 i.u. (instead of 150,000 i.u.) of streptomycin per 30 ml. of medium giving a final concentration of 3,333 i.u. per ml. instead of 5,000 i.u.

Table 37 - co-ordinates for Graph 1

Variations from egg to egg (1 worm - Mouse 27 - intestine)

X	Y	Z	O
102	- 13	26	7
101	- 10	27	3
100	- 8	26	6
97	- 10	25	9
97	- 8	25	6
99	- 9	25	10
98	- 7	27	8
96	- 7	26	5
99	- 6	26	9
101	- 5	27	5
98	- 7	25	4
96	- 7	26	4
96	- 10	28	6
101	- 7	25	8
101	- 10	25	5
93	- 6	27	2
96	- 6	28	2
94	- 8	25	3
101	- 8	25	2
98	- 4	25	6
99	- 4	29	5
95	- 5	25	5
95	- 8	26	2
101	- 10	27	4
104	- 7	27	5
100	- 8	28	7
98	- 9	24	7
101	- 7	24	7
94	- 6	24	7
93	- 7	25	8
98	- 6	26	6
105	- 4	26	10
102	- 6	25	10

Table 38 - co-ordinates for Graph 2.

Variations from worm to worm (Hamster 42 - gut)

X	Y	Z	O
106	- 3	23	3
118	- 7	26	1
101	- 7	21	5
106	- 10	23	5
108	- 7	24	.10
111	- 6	26	.05
111	- 8	25	1
110	- 10	25	.009
119	- 2	29	- .54
118	- 5	28	1
103	- 10	23	1
118	- 9	24	1
111	- 5	27	10
119	- 5	25	5
114	- 7	24	4
109	- 5	25	- .352
112	- 9	24	2
113	- 7	24	6
121	- 5	26	5
102	- 11	22	2
111	- 7	24	.439
113	- 9	27	1
110	- 8	23	2
109	- 6	25	2
109	- 14	26	2
117	- 8	22	4
107	- 10	23	.403
110	- 11	25	- .383
102	- 9	23	2
118	- 6	25	1
109	- 9	25	1
108	- 9	25	1
107	- 10	23	6
114	- 10	27	2
98	- 14	23	3
108	- 4	26	2
112	- 7	25	.015
109	- 10	24	1
111	- 4	27	- 2
107	- 7	24	5
117	- 8	24	2
116	- 3	31	.576
113	- 4	24	4
112	- 8	29	3
109	- 8	23	.47
111	- 8	24	3
110	- 8	23	4

Table 39 - co-ordinates for Graph 3

Variation in eggs (Hamsters - liver worms)

X	Y	Z	O
106	- 9	23	4
111	- 7	23	8
108	- 7	25	4
110	- 6	25	4
106	- 7	23	1
134	- 3	31	2
110	- 4	25	2
102	- 7	26	0.278
107	- 6	22	2

Variation in eggs (Hamsters - intestinal worms)

112	- 4	25	1
111	- 8	25	2
109	- 9	24	2
106	- 7	23	1
109	- 12	26	5
103	- 7	23	4
114	- 8	24	4

Variation in eggs (Mice - liver worms)

114	- 6	23	- 1
109	- 7	24	4
104	- 9	23	4
109	- 10	24	4

Variation in eggs (Mice - intestinal worms)

105	- 7	26	1
101	- 7	26	3
101	- 10	24	2
103	- 9	23	3
107	- 9	24	3
109	- 12	26	3
110	- .025	24	6
107	- 9	26	0.168

Table 39 (Contd) - co-ordinates for Graph 3

Variation in eggs (Mastomys - liver worms)

X	Y	Z	0
81	- 14	23	1

Variation in eggs (Mastomys - intestinal worms)

101	- 9	25	6
100	- 9	25	.138

Variation in eggs (Arvicanthis - liver worms)

101	- 8	24	3
102	-12	25	2
113	- 9	25	6

Variation in eggs (Arvicanthis - intestinal worms)

110	- 9	26	.390
106	- 7	23	4
110	- 8	26	1
109	- 8	26	.061

Variation in eggs (Meriones - liver worms)

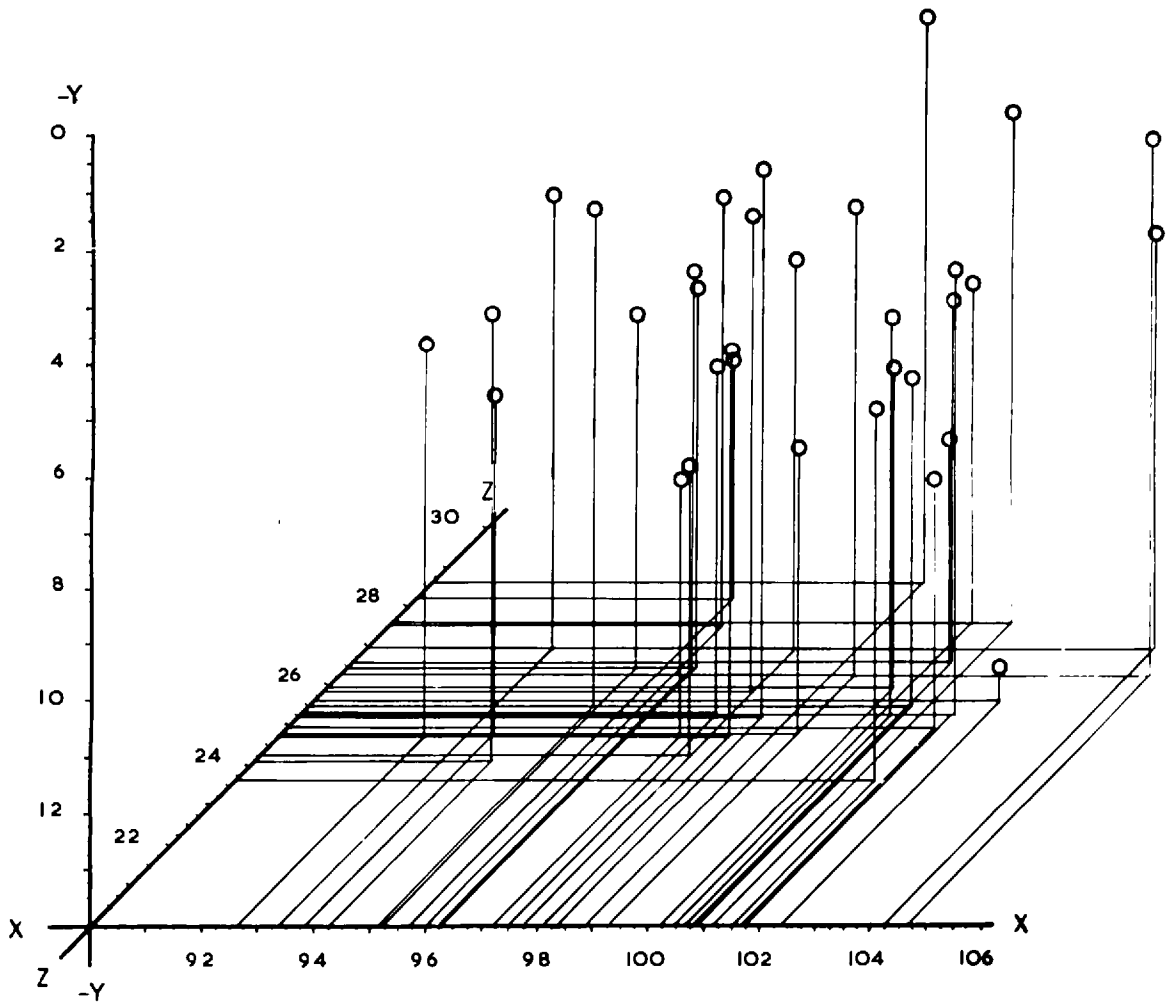
86	-13	16	6

Variation in eggs (Meriones - intestinal worms)

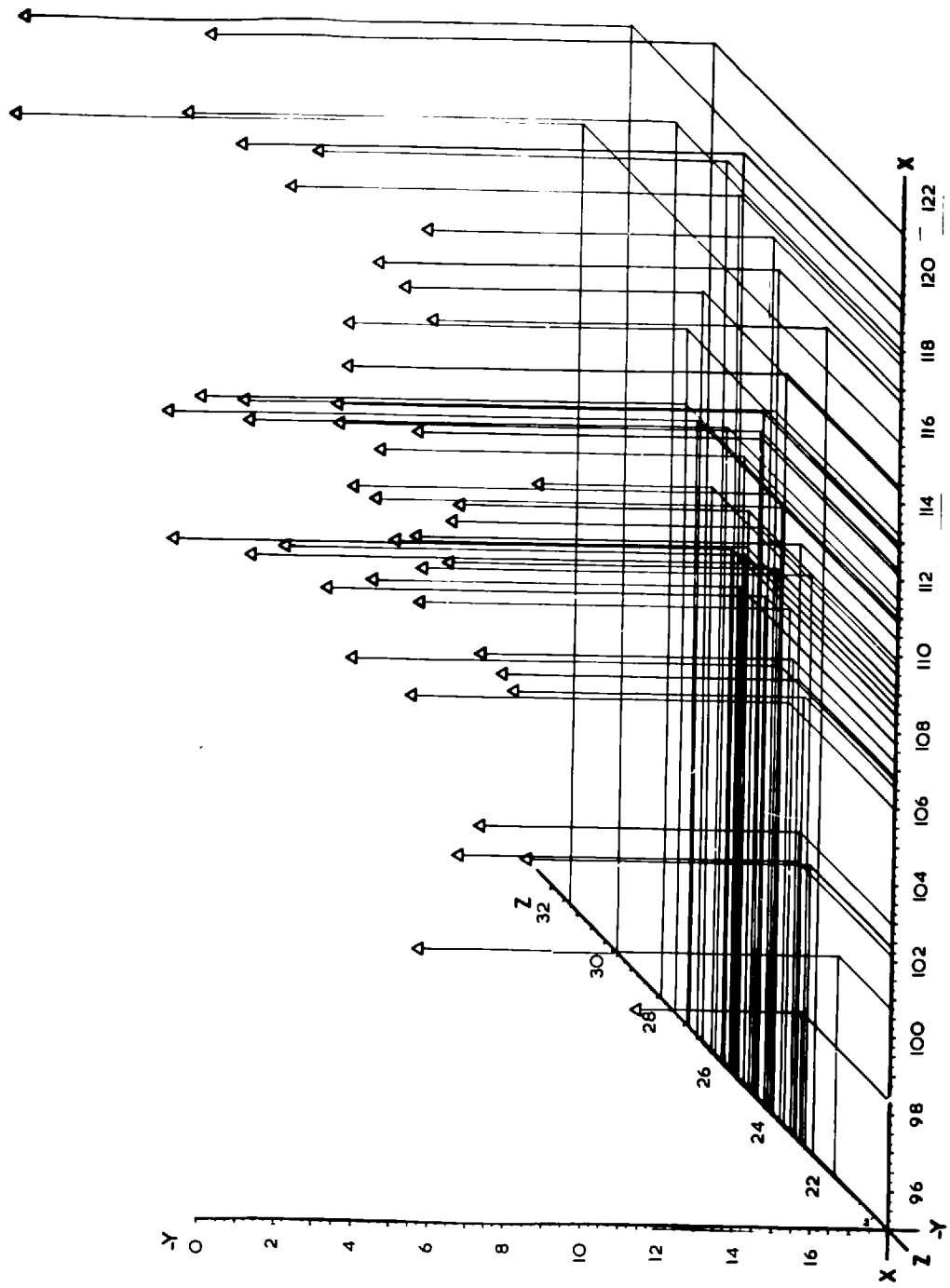
96	- 9	23	3
104	- 9	25	3

Graph 1. - Variation in eggs from one worm -

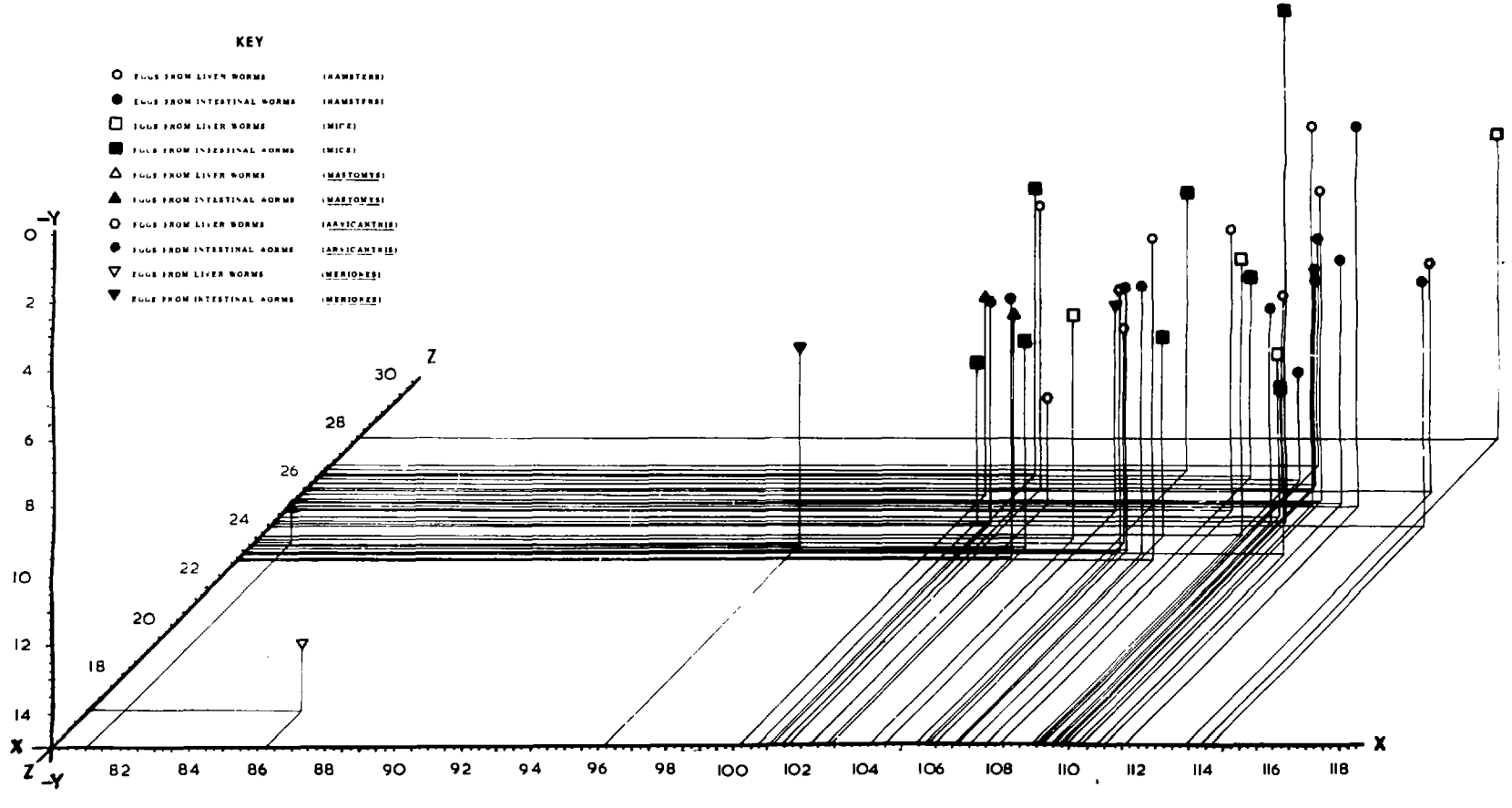
(Mouse 27, intestinal worm).


-Y
0
2
4
6
8
10
12
Z
30
28
26
24
22
X
Z
-Y
92
94
96
98
100
102
104
106
X

Graph 2. - Variation in eggs from worm to worm -
(Hamster 42, intestinal worms).


-Y
0
2
4
6
8
10
12
14
16
Z
22
24
26
28
30
32
X
96
98
100
102
104
106
108
110
112
114
116
118
120
122
X
-Y
Z

Graph No. 3. - Variation in eggs from different hosts and different sites within a host.


KEY
EGGS FROM LIVER WORMS (HAMSTERS)
EGGS FROM INTESTINAL WORMS (HAMSTERS)
EGGS FROM LIVER WORMS (MICE)
EGGS FROM INTESTINAL WORMS (MICE)
EGGS FROM LIVER WORMS (MASTOMYS)
EGGS FROM INTESTINAL WORMS (MASTOMYS)
EGGS FROM LIVER WORMS (AAVICANTHIS)
EGGS FROM INTESTINAL WORMS (ARVICANTHIS)
EGGS FROM LIVER WORMS (MERIONES)
EGGS FROM INTESTINAL WORMS (MERIONES)
Y
0
2
4
6
8
10
12
14
Z
30
28
26
24
22
20
18
X
82
84
86
88
90
92
94
96
98
100
102
104
106
108
110
112
114
116
118
X
Z
-Y

The following day, all the worms appeared healthy and were quite active. The females particularly were apparently feeding actively as seen by movements of the pharynx and oral sucker, although most pairs of worms had separated. Egg laying was seen to occur in several females, the eggs being moved forwards along the uterus by peristaltic contractions, followed by the return of the eggs to almost the same point in the uterus by a reflex wave or reverse peristalsis. Eggs were laid in groups of six or so, followed after a short interval by further waves of peristaltic contractions in the uterus. Muscular contractions and movements of the gut contents were also frequently observed, and it was noticed that the contents of the anterior of one gut caecum would often be passed into the other in a flowing motion.

Five days after the flasks were set up, the worms were observed to have changed to a brownish colour and were very sluggish and inactive. The worms appeared to be somewhat shrunken in size, with numerous blister-like protuberances along the cuticle. There were few or no paristaltic movements observable in the body, only occasional very sluggish general body movement. Regions of apparent muscle tetanus were apparent, especially in the females. Contamination of the cultures was apparent, fungal growths and probably bacteria being evident. The experiment

was terminated at this stage; at no time was egg formation observed, possibly because of the excessive contamination of the cultures with metabolic products, plus fungi and bacteria.

This experiment was repeated several times with essentially the same results. It did not appear possible to maintain the schistosomes in the serum/Tyrode solution for longer than about four or five days using these methods.

It was not possible to examine the cultures for long periods without the use of a warm stage to maintain the incubation temperature, nor to observe the actual formation of the eggs, because the depth of the Carrel flasks prohibited the use of the high magnifications essential for this, a magnification of some 80 diameters being the limit.

In an attempt to overcome these difficulties using a warm stage at 37° C. individual females were examined in well slides under high power (480 diameters), using normal saline solution, Tyrode's solution and some out-dated medium 199 of Morgan and Parker, marketed by Messrs. Glaxo. These media again did not circulate in the "culture". Worms did not survive for more than 15 - 30 minutes using these methods, possibly due

to the intense light necessary for high power observation, but observation of the female reproductive system was much more successful.

Although the actual formation of an egg and its shell was not seen, these methods allowed certain conclusions to be reached about the activity within the ootype and the ducts leading to it.

Groups of spermatozoa were observed in the oviduct, posterior to the ootype. These groups were shot backwards and forwards along the oviduct (probably by peristalsis, although this is not certain), and were separated by sausage-shaped clear areas which appeared to have a definite membranous boundary, within the duct. The overall movement of the spermatozoa was towards the ovary. An undulating membrane or similar ciliary action was observed in the oviduct, immediately posterior to the ootype, again with the effective beat directed towards the ovary. Vitelline cells were similarly passed backwards and forwards along the vitelline duct, but the overall direction was towards the ootype. Ova were not seen leaving the ovary. The ootype was more active than was anticipated, and had an apparently continuous, slow massaging action rather than periods of short activity separated by longer quiescent periods during

the formation of the shell. The egg became more organized while being observed, although the shell was still soft and pliable when the egg entered the uterus. The shell itself became gradually more conspicuous over a period of about 10 minutes' observation in one instance, and was not formed as quickly as had been expected.

SECTION 5 - DISCUSSION.

Discussion

Anatomy and Histology

The results obtained in the present work confirm those of Looss (1895) for S. haematobium, and of Gonnert (1955a and 1955 b) for S. mansoni (with the exception of differences which have been accorded specific taxonomic importance in the latter case).

The only structure observed in the present material and not apparently mentioned by these authors is the granular body situated between the ootype and Mehlis' gland, (page 87 and Plates 4, a and b, and 5, pages 89 & 91).

Mechanism of egg-formation.

Further experiments to amplify the preliminary observations on the mechanics of egg formation will be carried out along the same lines when sufficient material is available. In the meantime the use of complex culture media and apparatus appears to be unnecessary. Normal saline was reasonably effective in the series of experiments with well slides although the worms became moribund within about 30 minutes as was the case with the other media used in this series. Fresh medium 199 may well maintain the worms in an active condition for longer periods however. Blisters were seen to form on the

cuticle very shortly after the insertion of the worm into each of the media in the well slide series. These gradually expanded and burst after about 20/30 minutes, by which time the worm had gradually become opaque and moribund, much as was observed in the experiments with serum/Tyrode medium and Carrel flasks after several days. This may be due to the osmotic pressure but this is doubtful as the same thing happened using 0.85% solution and Tyrode solution alone. Also, 0.85% saline solution had no obvious effect on the worms when a series of saline solutions of different strengths was tested previously, and no blisters were formed. Standen (1962) has described very similar phenomena occurring in S. mansoni obtained from mice, treated with a schistosomicidal drug and subsequently maintained in vitro. The male worms became much shortened 48 hours after drug treatment and between 48 and 96 hours after treatment a precipitate was formed over the body surface. This deposit was of flocculent nature and was interspersed with small, clear globules similar to those described above. Standen does not report the presence of globules in female worms although a flocculent oral deposit was observed. Survival times for female worms were also longer. The blister-like appearance may possibly be due to a chemically unsuitable medium. The experiments with well-slides described above will have to be repeated with media freshly and

accurately made up in the light of Standen's report, if a longer survival time is to be obtained. The use of a constant flow device or some other means of changing the medium in contact with the worms would also be beneficial.

Stephenson (1947) carried out a comprehensive series of tests on the chemical nature and physical properties of the eggshell of Fasciola. He has demonstrated that the eggshell of Fasciola is derived from globules contained in the vitelline cells, and that these globules contain protein and an orthodihydroxyphenol. He has demonstrated that the globules are liberated in the more proximal uterine coils in Fasciola after passage through Mehlis' gland, as described by Kouri and Nauss (1938), and he believes that mechanical forces due to the churning action of the uterus are responsible for freeing most of the granules. However, Smyth and Clegg (1959) are doubtful of Stephenson's observation. Stephenson (loc. cit.) states that this liberation can be correlated with the presence of spermatozoa in the proximal part of the uterus beyond Mehlis' gland, and he suggests that the latter are responsible for the aggregation of the vitelline cells around the ovum and for the formation of the eggshell round them from fused vitelline cell globules. He also suggests that Mehlis' gland has a lubricating or more

probably a spermatozoan-activating function and plays no direct part in the formation of the eggshell. The polyphenol of the newly formed eggshell is oxidised to a quinone with a consequent shrinkage, and becomes characteristically hard, brittle and selectively impermeable.

Function of Mehlis' gland.

Smyth and Clegg (1959) have produced evidence that Mehlis' gland secretion does not become part of the eggshell, and according to Hanumantha-Rao (1959a) the secretion is probably concerned with the release of shell formative substances in Fasciola hepatica through the stimulation of the vitelline cells. The same author (Hanumantha-Rao, 1959b) has described a series of histological tests on the Mehlis' gland of Fasciola hepatica and its relation to eggshell formation. He states that at no stage did the eggshell or its precursors give any positive reactions for phospholipids, whereas Mehlis' gland was found to be positive to all the tests made. He also states that there was no indication that Mehlis' gland secretion participated in the characteristic forming of the eggshell. He concludes that the secretion is connected with the mechanism effecting the release of the eggshell precursors from the vitelline cells, but does not itself form part of the eggshell. Gonnert (1955b)

put forward similar ideas in an earlier paper, although he suggested that the secretions of the ootype epithelium may be responsible for the actual confluence of the shell drops to form the eggshell.

Although the anatomy (among other features) of the female genital system of the schistosomes differs in detail from that of Fasciola, Gonnert (1955b) agrees with Stephenson (1947) that the vitelline cells of schistosomes contain globules which are liberated and fuse to form the eggshell. However, he disagrees with Stephenson (1947) as to the site of liberation of these globules, and states that they are released within the ootype in S. mansoni. The present author confirms this with respect to S. haematobium. However, Gonnert (1955b) agrees that mechanical forces probably play a part in their release. He suggests that the passage of the vitelline cells through the narrow lumen of the terminal portion of the vitellin duct (analogous with Stephenson's "uterine valve") causes the breakdown of the mechanism isolating the globules within the vitelline cell and facilitates their exit. Gonnert (1955b) goes on to suggest that the secretion of the ootype epithelium is responsible for the fusion of the granules to form the eggshell, the Mehlis' gland secretion possibly having a lubricating or spermatozoan-stimulating function, or

more probably as suggested by Stephenson (1947), effecting the release of the eggshell granules in the vitelline cells. The results of the present work support Gonnert's theories rather than those of Stephenson where they differ. Gonnert is in agreement with Stephenson's results concerning the physical and chemical nature of the eggshell, namely that the eggshell is formed from the granules contained within the vitelline cells and consists of a quinone - tanned protein (ortho-dihydroxyphenol).

For the schistosomes (or S. mansoni and S. haematobium at least) the site of release of the shell globules and of the formation of the eggshell and its shaping is undoubtedly the ootype. The vitelline cells provide at least the major part of the eggshell material in the form of orthodihydroxyphenol and protein, contained in the vitelline granules, according to Gonnert (1955a). The fusion of the secreted granules is aided by secretions from the ootype epithelium, and not by those from Mehlis' gland in all probability. However, these latter may be important in the release of the shell granules from the vitelline cells. The eggshell is formed and shaped and tanning of the orthodi-hydroxyphenol to quinone commences almost immediately in the ootype as demonstrated in the present material by rapid darkening of the discrete, newly-formed shell to a brown colour, and is completed during the passage of the egg along the uterus, although in trematodes which produce

many eggs in a short while (e.g. S. japonicum and Fasciola) tanning takes place largely in the uterus, as stated by Stephenson (1947).

Malformed eggs.

Describing the effect on S. mansoni harboured by guinea pigs maintained on vitamin C deficient diets, Hoffman and Axtymer (1944) reported the formation of abnormal eggs and stated the following:-

> "The genital structures including the vitellaria were apparently normal in all. On the other hand, the uteri of the schistosomes, whose hosts had been on a scorbutic diet (i.e. vitamin C deficiency) plus 5 mgm of ascorbic acid (vitamin C) weekly, occasionally contained a normal well-formed ovum.
>
> Most often in its place there were granular structures forming an ill-shaped structure in which the germinal cell could not be clearly identified; or an imperfect irregular granular shell without clearly defined inner contents. These shells were smaller than the normally formed ones, and at times spent vitelline cells were found close by... Such imperfectly formed ova were raroly found in the parasites derived from guinea pigs on the scorbutic diet plus 9 mgm. ascorbic acid weekly, and not at all from those derived from animals which were fully protected against scurvy."

The observations of these authors on S. mansoni in guinea pigs maintained on a vitamin C. deficient diet agree very closely with those of the present author on S. haematobium in hamsters as described in the section on the production of malformed eggs (Page 101).

The reason for the formation of these abnormal eggs appears to lie in the diet of the host animal, with special reference to vitamin C. According to Sealock and Silberstein (1939) vitamin C is essential in the metabolism of tyrosine, and Painter & Zilva (1947) found that guinea pigs deprived of vitamin C and receiving more than 0.5 mg. of l-tyrosine daily excreted large quantities of hydroxyphenyl compounds. They concluded that this was a result of the incomplete metabolic breakdown of the tyrosine in the absence of Vitamin C, the breakdown product being excreted in the form of hydroxyphenyl compounds. Similarly, Rienits (1950) found that the oxidation of tyrosine in slices of guinea pig liver depended on the l-ascorbic acid retention of the animal. In the case of material from scorbutic animals the oxidation could be restored to values obtained from non-scorbutic animals by the addition of l-ascorbic acid.

Proceeding a stage further, Smyth and Clegg (1959)

described a series of experiments to determine the identity of the precursors of sclerotin in trematode eggshell formation, and concluded that 13 amino acids were present in the hydrolysate of homogenised vitelline gland cells of Fasciola (containing a protein and orthodihydroxyphenol according to Stephenson (1947)) after various treatments. Of these 13 amino acids, three were definitely identified and occurred in considerable quantities. These three amino-acids were phenylalamine, histidine and tyrosine.

It therefore appears that a diet deficient in vitamin C, even though sufficient to maintain the host animal in apparently good health, is insufficient to enable the female schistosome to produce typical eggs by affecting the tyrosine metabolism of the eggshell precursors in the vitelline cells. According to Hovde (1950) hamsters maintained on a vitamin C deficient diet require a daily allowance of 0.3 mg. of vitamin C per 100 g. of body weight for normal growth. In contrast Cooperman, Waisman and Elvejem (1943) concluded that vitamin C was unnecessary for growth of hamsters in their experiments on nutrition; however, their artificial diets under investigation were supplemented with "greens", whole corn and wheat, which can be reasonably expected to contribute the requisite amount of vitamin C. Their conclusions that vitamin C is unnecessary for

growth in hamsters must therefore be open to doubt.

According to the analysis of the modified diet 41B which was supplied by Messrs. Oxoid and on which the present host animals were maintained, there is no vitamin C added in the vitamin supplement. Specific enquiries were made by the author and apparently vitamin C is not added to the diet because of its extremely rapid oxidation and consequent inactivation. At the beginning of the present work, animals were maintained on diet 41B alone, without the supplement of greenstuff given more recently, and it is from this period that females producing abnormal eggs were encountered. It therefore appears that the basal diet of Oxoid 41B alone does not contain sufficient vitamin C to enable typical schistosome eggs to be formed unless additional vitamin is provided (in the form of a supplement of greenstuff in the present case).

It is possible however, that the production of malformed eggs is not a result of a dietary deficiency such as lack of vitamin C, but is the result of a genetical mechanism, in which the abnormal host environment (i.e. laboratory host rather than man) may cause a selection pressure on the schistosome strain, with the result that an attenuated strain results which

may produce malformed eggs and which is incapable of surviving three passages in laboratory animals. If the malformation is due to dietary deficiencies, why are malformed eggs not encountered in earlier passages? These deficiencies may be cumulative if they are of dietary origin and may require more than one passage to become evident but this seems less likely than that a selection of an attenuated strain takes place. On the other hand it seems more likely at first sight that a selection pressure would select a more ~~adequate~~ adapted strain of parasite rather than a less well adapted strain (attenuated strain). It has also been suggested (Smyth, pers. comm.) that malformed eggs may be unfertilised and haploid; Smyth is of the opinion that such eggs would be smaller than normal diploid eggs. It is necessary to examine fresh material and to count the number of chromosomes to determine this. Whatever the reason, the fact that it is difficult or impossible to maintain a laboratory strain of S. haematobium beyond the third passage, despite a successful report by Pitchford (1961), may well be connected with the formation of malformed eggs by whatever method is responsible.

The possibility of producing an attenuated strain of S. haematobium or alternatively of enabling the strain to be maintained beyond the third passage by decreasing or increasing

the vitamin supply, requires further investigation. This, together with the effect on the schistosomes of the addition of ascorbic acid to the basal diet of Oxoid 41B without greenstuff supplement is under further investigation by the author at the present time.

Experimental results so far obtained gave the following results for hamsters:

Treatment	mg. ascorbic acid/ 100 ml. serum *
Control (normal diet + greenstuff)	0.75
46 days without greenstuff (normal diet)	0.48
" " "	0.56
49 " " "	0.46
	0.34
49 with " "	2.25
	0.59

* adapted from Lindlan and Butler, J. biol. Chem. 1937-38, 122 p. 673. (Determinations were made by Mr. G. C. Ross of the British Museum (Natural History) to whom I am grateful.

The removal of greenstuff from the diet of the animal resulted in a reduced ascorbic acid level; it appears that this reduction is progressive but becomes evident only

after the experiment has been in progress for a few days, while the reserves of ascorbic acid are used up. The addition of ascorbic acid tablets to the drinking water at the rate of 200 mg./100 ml. of water has been attempted. Further work is to be carried out to determine the effect of vitamin C on egg formation in hamsters.

Statistics.

In all subsequent discussion, it is essential to remember that all experiments were carried out on strains of the same, well-defined species of parasite, and that all differences exhibited as a result of these experiments and discussed hereafter are in the same, theoretically uniform, species and are largely the results of these experiments. All the material came originally from human subjects of the disease known as urinary bilharzia or urinary schistosomiasis, and therefore a more detailed identification is not considered necessary. The question of whether such material is S. haematobium or S. capense is still open to question and is not entered into in any detail here.

From a consideration of the probabilities of

distinguishing between material from the four host categories (a) host species, (b) parasite strains within a host species, (c) samples (individual animals) within a parasite strain, and (d) sites within a host animal (Table 10, Page 116) by the use of each of the four dimensions (length, breadth, $l_{max.}$ and spine length of the egg), a number of preliminary conclusions can be drawn. Thus it is possible to distinguish between material from each of the host species with considerable certainty using either length, breadth, or $l_{max.}$. Spine length, however, is less satisfactory, but $l_{max.}$ is the only dimension which will distinguish between parasite strains from the same host species with any certainty, and material from different animals of the same species of host infected with the same strain of parasite cannot be distinguished at all. (The spine length gives a reasonable separation but this dimension is the most inaccurate and is therefore not really reliable). Material from different sites (eggs measured within the uterus of the female or from tissue, either digested out or from faeces) can be clearly distinguished by each of the dimensions with a high degree of certainty.

It is also possible to obtain information on the efficiency of each of the four dimensions in distinguishing between the various categories. Thus the dimension $l_{max.}$

appears to be most likely to separate the categories, it being effective at the 1% probability level or better in each case except that involving individual animals within a parasite strain (Table 10). It should perhaps be emphasised that this dimension is a new one as far as the examination of schistosome eggs is concerned and has not in fact been used in other studies on egg shape, although Alves (1949) used a similar dimension fairly successfully in an analysis of egg shape of S. haematobium, S. bovis and S. mattheei. The next most acceptable dimension appears to be the length; consideration of the breadth will distinguish only between host species and between sites within a host animal, although it is reliable in both these cases. However, it offers no improvement on length and although more reliable than $l_{max.}$ at separating material from different host species the improvement is not great and it is not effective in distinguishing between material from different parasite strains within a host species. The spine length is not really a reliable dimension, as it is both difficult to measure and not very effective in distinguishing any of the categories except sites within an individual host, at which other dimensions are equally efficient.

It therefore appears that material from different host species does differ, that material from different parasite

strains from the same species of host animal differs, and that the site within the host animal from which material is obtained is of great importance (material from the tissues or faeces, and also presumably from the urine, are all larger than material measured within the female uterus). Furthermore, these different categories can be distinguished by the use of one dimension, the best being $l_{max.}$, followed by length as the second best.

Considering the probabilities of distinguishing between material from the host species, it is evident from Tables 12, 14, and 16, Pages 117,118 and 119, that material from man can be distinguished with a high degree of certainty from material from all other host species using either length, breadth, or $l_{max.}$. However, material from hamsters can also be distinguished by the use of length, breadth, or $l_{max.}$.

Applying a similar method to attempt to distinguish between geographic strains of parasite (Table 21, Page 124) it is evident by considering length, that material from Kuwait and Said can be distinguished from all the other strains, but that these two strains cannot be distinguished from each other. Using the dimension breadth (Table 22, Page 125) the same is largely true with the addition that Cairo material is distinct from all other material also, with the exception of Said, and

that the latter is now distinguishable from Kuwait material. Consideration of $l_{max.}$ (Table 23, Page 126) reveals a situation mid-way between these two. Material from Kuwait and Said is again significantly distinct from all other material but not from each other, while material from Cairo can be distinguished with reasonable significance from only Nigerian, Said and Kuwait material. Thus material from Said and Kuwait can be distinguished from all other material with a high degree of certainty by each of the three dimensions and from each other by use of breadth alone. Cairo can also be separated, by the use of breadth, and with less certainty using $l_{max.}$. All other strains cannot be separated from each other with any confidence.

Turning to material from hamsters, material from Baghdad can be distinguished from all other material using length (Table 24, Page 127), which was not the case with similar material from man. Kuwait material is likewise distinct from all other material, but was not available from man.

Of the remaining strains, material from Nigeria is not significantly distinct from Ghana, Shambat or Cairo, whereas all others are distinct from each other. Considering breadth (Table 25, Page 128) no strain is distinct from all others; Ghana and Shambat cannot be satisfactorily separated

nor can Kisumu and Cairo, and Nigeria and Baghdad. Turning to $l_{max.}$ (Table 26, Page 129) a similar situation is found as for length. Material from Baghdad is again distinct from all other strains, but material from Nigeria can only be distinguished from Ghana and Baghdad; material from Ghana is now distinguishable from all other strains. Shambat is distinct from all strains other than Nigeria, as was the case with length, and Cairo is distinguishable from all strains other than Nigeria, again as was the case with length. The material from Baghdad (from hamsters) is distinct from all other material and Kisumu material is fairly distinct also, as is Ghana.

Considering the material from mice (Tables 27, 28, and 29, Pages 130 - 132) material from Cabemba can be distinguished from Cairo and Shambat, but the latter two cannot be separated. This is of some interest in that the Cabemba strain has the same origin as that from Shambat but has been passaged through a different sub-species of snail (Bulinus truncatus rohlfsi) from Angola. This has evidently affected its size as it is significantly larger in each of the three dimensions than material of the other strains from mice.

It is not possible to make any really reliable

estimate of the relationships of the various strains from man, hamsters and mice to each other; it is however evident that material of two strains may not be distinguishable when taken from one host species but may in fact become so when passaged through another (e.g. Baghdad material from man and hamsters).

Material from Arvicanthis and Mastomys cannot be distinguished by these methods, nor can material from Mastomys and Meriones. Material from Arvicanthis and Meriones can be distinguished by using length and breadth only, and then not with great certainty.

It is possible to obtain more information on the relationships of the material from different host species and on the relationships of the geographic strains by considering all four dimensions at once, i.e. considering the shape of the egg rather than one dimension at a time.

Material from man is shown to be more obviously distinct to material from hamsters and all the other host species used by this method. Material from each of the non-human hosts can also be distinguished, unlike the use of one dimension alone.

It is evident from Table 31, page 133A, that egg length is the most important of the four dimensions, contributing about 38% of the total information. Similarly, considering Table 31A, page 133B, the vector X is generated by the largest root, Y by the second largest, Z by the third largest and 0 by the residual variation. In the case of vector X, it is evident from a consideration of the relative values of the weights that length (+1.0000) is of greater importance than breadth (+0.5022) and the vector X is therefore tentatively identified as "length". The vector Y, generated by the second largest root, the greatest contrast is between breadth (-0.8137) and spine (+1.0000) and most closely represents the dimension "spine". In the vector Z, length and spine run together. Throughout, the dimension L_{max} is of little value, i.e. the individual eggs are not more or less pear-shaped but are in fact similar in shape, as distinct from size (defined by l and b, contributing the maximum information). This is in contrast to the univariate methods (Table 10, page 116) where L_{max} is apparently the dimension of choice.

In Figure 10A, the long axis of the model represents the overall relative size of the eggs, and not differences in shape. All dimensions collapse onto that represented by size. The eggs showing closest similarity to those from man (to the right of the model) are from hamsters. However, although they lie next to eggs from man in the model, these are less like the latter than they are like eggs from mice, Arvicanthis, Mastomys, and even Meriones, eggs from the latter being most unlike eggs from man. The "average" eggs from

the non-human hosts show a greater similarity to each other than any of them do to those from man. However, it is evident from Figure 10B that some eggs from hamsters (Baghdad) do in fact show some similarity to some eggs from man (Kuwait).

Considering, Figure 10B further, it is evident that all eggs from man lie to the right and all eggs from hamsters to the left of the model, and although some eggs from hamsters (Baghdad) and some eggs from man (Kuwait) lie adjacent to each other, they are still distinct. Eggs from Baghdad lie towards the opposite side of the model from eggs from Cairo, Ghana and Nigeria, in both hamsters and man, and eggs from Ghana and Nigeria lie close together in both cases. The close relationship in the model of these two strains is reasonable when their geographical relationship is considered, although on the other hand they are transmitted by members of different species-groups of snails, the forskali group (Ghana) and the africanus group (Nigeria). On the other hand, the close relationship on the right hand side of the model of material from man from Cairo, Ghana, Nigeria, Baghdad and Dirgag, transmitted by members of the truncatus, forskali and africanus species groups, suggests that the taxonomic status of the intermediary may not be an important factor.

LeRoux's (1958) theory that the strain of S. haematobium carried by members of the africanus group should be regarded as S. capense is not supported by the present results, as the methods adopted here have failed to reveal any major difference between the Ghana strain and the rest of the typical group such as could be expected if such a theory were true. It is interesting to note also that material from Said and Dirgag can also be differentiated by the appearance of the lesions on the intestine of infected hamsters caused by the parasites. The intestine of hamsters infected with the Said strain almost invariably exhibited lesions in the posterior regions of the intestine, particularly in the loop of the colon and the rectum, whereas hamsters infected with the Dirgag strain possessed lesions in a more anterior position, usually in the ileum. In the majority of the infections with other strains, lesions are found along the colon, and in the liver to a lesser extent.

The relationships of the hamster material to each other and to that from man are also difficult to evaluate, partly because not all strains are common to both hosts. All that can be said is that a passage of material through hamsters appears to affect different strains to different degrees. However, the

predominant relationship is still one of overall size, material from man being larger than that from hamsters, although there is also some small difference in shape (as shown by the three dimensional nature of the models).

In both figures, 10A and 10B, the largest eggs lie to the right of the model and the smallest to the left. Evaluation of material from mice, Arvicanthis, etc. would extend Figure 10B further to the left, were sufficient material available. The group centres of the hamster material and the material from man lie the same distance apart as do these points for Figure 10A allowing for rounding-off errors, and it it therefore possible to extrapolate the Figure 10B to the left to obtain an idea of the size of the model if material from mice, Arvicanthis etc. was added.

Computation of the angle between the long axes of the models in Figures 10A and 10B, represented by man-Merionés (Figure 10A) and Kisumu-Baghdad (Figure 10B), using the formula

$$\text{Cos } \Theta = \frac{\Sigma \alpha i . \beta i}{\sqrt{\Sigma \alpha i^2 . \Sigma \beta_i{}^2}}$$

gave an answer of = $0^{\circ}9'$ approximately, i.e. the long axis of each of the two models is the same and that therefore the difference between the groups of eggs is basically a question of size.

Since there are no relevant multivariate tests of significance available at present along predetermined axes in generalized distance charts, it is not possible to determine whether there is a vector at right angles to the long axis of the model (Figure 10B). However, it is possible to carry out a univariate test on the multivariate scores along a discriminant function selected as running in as nearly the right direction as possible (Nigeria-Dirgag in this case)(Blackith, pers comm). The steps adopted were as follows.

1) The discriminant functions β_1, β_2, β_3 and β_4 for Dirgag-Nigeria were calculated and gave $\beta_1 = -2.6523$, $\beta_2 = +0.2539$, $\beta_3 = -0.2241$, and $\beta_4 = +3.7638$.

2) Knowing $\bar{l}$, $\bar{b}$, and $\overline{l_{max}}$ and $\bar{s}$ for eggs from Dirgag and Nigeria, the scores for each group of 25 eggs were calculated from $\Sigma\beta_1\bar{l} + \beta_2\bar{b} + \beta_3\overline{l_{max}} + \beta_4\bar{s}$ for each group. The scores obtained for the average values of eggs from Dirgag and Nigeria along the discriminant function were -339.5042 and -333.6547 respectively.

3) The values for individual eggs from a) Dirgag and b) Nigeria were obtained from $\Sigma\beta_1 l + \beta_2 b + \beta_3 l_{max} + \beta_4 s$ and were found to be -8661.5572 and -8340.6318 respectively.

A standard univariate t-test was carried out on these figures and resulted in a significance level of 60% approximately i.e. that the probability of discriminating between individual eggs along the postulated vector is very low and it seems likely therefore that there is no significant vector present running in that general direction.

The presence of negative generalised distances (Table 33, Page 135) indicates that the two samples concerned are identical, as it is impossible for a real negative distance to exist (two samples cannot be more alike than identical). Such negative numbers arise from sampling variations in the calculation of the canonical vectors. These negative values have been increased by a small amount to make them positive and to clarify the relationships of the samples in the construction of the models. The calculated generalised distances between

the Nigeria strain and the Ghana, Baghdad, Kisumu and Cairo strains from hamsters do not meet at a single point, suggesting that there is a fourth dimension involved in this region of the model. As this is impossible to visualise, the Nigeria strain is given an approximate location relative to Ghana.

In the present work, the average size of eggs from human cases was found to be 140 /u long by 57 /u wide. No comparable adult worms were available, and recourse has been made to Girges (1934). He states that the average length of a sample of eggs from Cairo (i.e. topotype material) measured by him is 146.8 /u and the breadth 58.75 /u (recalculated from data in Girges, 1934), i.e. slightly larger than the present material. Girges (loc. cit.) gives an average length of adult male worms of 11.75 mm (recalculated) and states that the average length of a female worm is "about 20 mm."

Material obtained from hamsters in the present work gave the following results: eggs 109 /u long by 39.5 /u wide, male worms 7.4 mm. long, females 11.15 mm. There is therefore a considerable reduction in size of both adult worms and eggs recovered from hamsters as compared with material from human cases. This amounts to a reduction of 20 to 30% in both length

and breadth for the eggs and 37% and 49% for the length of the male and female worms respectively. The reduction in the length of the eggs from other hosts is even greater and amounts to about 35% for material from Mastomys (producing the smallest eggs) compared with material from man.

Eggs measured within the uterus of the female worm, whether from the liver or intestines, show an almost equally marked reduction in size compared with eggs digested out from liver or intestinal tissue or recovered from faeces, regardless of the species of host or the strain of parasite (Table 4, Page 105). It seems reasonable to suppose that eggs measured within the uterus of female worms recovered from man would also be reduced in size compared with those recovered from the urine, although comparable material is not available at present. There is a reduction of up to 15% in length and 20 to 30% in breadth with similar reductions in the size of the other two dimensions for material from hamsters. Material from mice, Arvicanthis, Meriones and Mastomys also exhibit reductions in size relative to man but to a greater and more variable extent.

Why there should be a different between eggs measured in utero and those recovered from tissues or faeces

is difficult to answer. There is little difference between eggs from either liver tissue, intestinal tissue, or faeces, or between eggs from worms recovered from the liver or intestine. It seems surprising that eggs undergo an increase in size after being laid by the female. Eggs digested out from tissues and stored and measured in saline solution may possibly swell after recovery but preliminary experiments at the start of this work failed to show any detectable increase in size after prolonged storage. The increase in size in eggs laid by the females may be due to the absorption of materials, such as nutrients, from the host tissues, or may be a result of the growth and development of the embryo within the egg. However, this would necessitate the eggshell being sufficiently permeable for nutrients to diffuse through on the one hand, or sufficiently impermeable on the other to prevent waste products from escaping. The latter seems somewhat less likely as it has been suggested that one of the causes of the pathological conditions associated with schistosomiasis is the toxic waste products produced by the developing miracidium within the egg (Hoeppli 1932); also the accumulation of waste products within the egg would presumably be detrimental to the developing embryo.

The reasons underlying the overall reduction in size

of both adult parasites and eggs recovered from experimental hosts compared to those from man are even more difficult to ascribe. There is possibly a reduction in the metabolic rate of the parasite in an abnormal host as a result of the abnormal physiological environment resulting in a smaller adult worm, which is in turn capable of producing only smaller eggs. In this connection, DeWitt (1962) has drawn attention to the effect of host diet on worm morphology and egg production in S. mansoni. The size of the definitive host may also have some effect on the size attained by the parasite, smaller species of host only being able to produce smaller parasites. The reasons behind the host effect are similarly difficult to determine at present. They too are almost certainly connected with the environment presented by the laboratory hosts and are presumably related in some way to the genetical constitutions of the host and the parasite, but the details of the relationship remain to be elucidated. This relationship is affected principally by the species of host, secondly by the geographic strain of the parasite, and thirdly by the site of occurrence of the eggs of the parasite, (i.e. whether they are from the female worm or from the tissues, faeces or urine).

In the light of these results from S. haematobium originally from man, it is perhaps unnecessary to emphasise the

dangers of creating a new species of parasite for material from unusual hosts before searching investigations havebeen made. Passage of the material through other (laboratory) host species would emphasise the amount of variation possible in the same species of parasite under differing conditions. Also, by measuring samples of eggs and by calculating the generalised distances in the way described, from the same set of canonical vectors, it will be possible to build up a comprehensive analysis of the relationships of additional material which will be of considerable value to all who work with the schistosomes. It is suggested that similar techniques could be employed with considerable advantages in other fields of parasitology. An investigation of eggs of S. capense, S. mattheei, S. intercalatum and S. bovis, would also contribute much to the elucidation of their inter-relationships in all probability.

Susceptibilities of experimental hosts to infection.

Martins (1958) summarised the extent of knowledge at that time on the non-human vertebrate hosts of Schistosoma mansoni and S. haematobium. He concluded that S. haematobium has a much more striot host specifity than does S. mansoni. During the present work hamsters, mice, Mastomys natalensis, Meriones shawi and Arvicanthis abisynicus have been infected with S. haematobium, and some Saccostomys from South Africa have also been received for use in experimental work but are

still under investigation.

As a result of these experimental infections, the golden hamster (Mesocricetus auratus) was found to be the best all round host for experimental purposes although only 37% survived the infection. Moore and Meleney (1954) and Kuntz and Malakatis (1955a) also arrived at this conclusion as a result of their more extensive work. Kuntz (in a personal communication) has also stated that the hamster is reasonably satisfactory; he also found that although Arvicanthis was apparently a fair host (Kuntz & Malakatis 1955b) later work demonstrated that the passing of eggs in the faeces was erratic and often light (Kuntz 1961 and personal communication). The present work has confirmed Kuntz's (loc. cit.) findings in this respect. Comparatively few adult schistosomes were recovered at autopsy of Arvicanthis, and these were often largely males. Further, only once could miracidia be induced to hatch from faeces of this animal. As a result of these experiences, Arvicanthis was largely dispensed with for experimental purposes.

The jird, Meriones shawi was examined as a potential host by Kuntz & Malakatis (1955b), who concluded that it was a rather poor host, basing their conclusion on the low recovery of adult schistosomes. Only six Meriones were used in the

present work, all in one passage, as they were found to be unsatisfactory hosts for the experiments in progress, yielding only 3.7% of cercariae as adult worms and no viable eggs in the faeces. Mastomys natalensis was also found to be less satisfactory than hamsters in that a lower percentage of worms was recovered, although Pitchford & Visser (1962) are of the opinion that this species and Saccostomys may in fact prove to be good hosts for experimental work.

Kuntz (1961) has investigated the passing of eggs in the faeces of hosts infected with S. haematobium. He found that egg counts were erratic but generally followed a pattern, building up from a few eggs to a peak before tailing off again. Although no specific attempt has been made in the present work to determine the rate of passing of eggs in infected animals, it was observed that this was often very irregular. Kuntz (personal communication) is of the opinion that both passage of eggs in the faeces and susceptibility to infection by cercariae are "individual" characteristics of the host animal and has experienced essentially similar effects in S. japonicum infections in laboratory animals in Formosa. He has found that some infected individuals of a host species seem to produce reasonable numbers of eggs, in either faeces or tissues, while in another series of

exposuresin the same host species worm production and passing of eggs may be low. Evans and Stirewalt (1951) investigated this phenomenon by a study of variation in infectivity of the same strain of S. mansoni from the same snails to white mice under the same experimental conditions. They found that statistically significant variations in infectivity of the cercariae did in fact occur, and that these variations appeared to be related to the physiological condition of the snail host. Their mice were of an inborn strain, and they considered that in theory the mice offered minimal heritable variation. They did not, however, subject their mice to a preliminary bath before exposure, in order to stimulate the evacuation of urine and faeces which would otherwise pollute the cercarial suspension with deleterious effects on their infectivity. This bath preliminary to exposure was advocated by Watson and Abdel Azim (1949) and has been used as a standard procedure by Standen (1949) and Kuntz (1961) among others, and also in the present work. In the evaluation of their results, Evans and Stirewalt considered that the variation in the infectivity lay with the snails and the cercariae, rather than the cercariae and the mice. However, in the light of Kuntz's views (personal communication) (supported by the present author) that variation in infectivity in schistosome cercariae is almost an individual characteristic, too much weight should not perhaps be

given to Evans and Stirewalt's (1951) views. It seems more likely that this variation is due to a combination of variation in infectivity of the cercariae together with variation in the susceptibility of the vertebrate host, of which the latter may well comprise the more important part. The occasional occurrence during the present work of individual host animals (e.g. Meriones and Arvicanthis) which were evidently totally resistant to infection, lends support to this view. Such animals were found to be uninfected and showed no sign of ever having been infected, although other individuals of the same species were successfully exposed under the same conditions, at the same time, and to a similar number of cercariae from the same snails. The possibility of an essential period of gradual adaptation of the human strains of S. haematobium used in the present work to the somewhat abnormal hosts used does not appear likely in view of the results obtained by Moore and Meleney (1952) during their investigations with Schistosoma mansoni. These authors found that of 96 snails exposed to miracidia (hatched from eggs passed by 10 human cases) 65 shed cercariae; 111 mice were exposed to these cercariae and all these mice passed viable eggs in the faeces 6 to 6½ weeks after exposure (i.e. within the usual range for the appearance of S. mansoni eggs in the faeces of mice).

The effect of population density of adult parasitic helminths in their vertebrate hosts on the pattern and physiology of growth of the parasite has been investigated by Roberts (1961) in Hymenolepis diminuta (Cestoda). Roberts concluded that population density has a direct effect on the germinative region in Hymenolepis and that the gravid proglottids are reduced in number in dense infestations, and that the rate of maturation is essentially independant of the rates of growth in length and weight. The effect of population density on the worms becomes apparent during patency, when a higher population density resulted in a slower growth rate, although the rate of maturation was apparently independent of population size.

Daily egg production per worm in nematodes parasitic in animals may be lower in larger populations than in smaller ones, and Rankin (1957) suggested that crowding may have been responsible for the occurrence of smaller (though sexually mature) trematodes in Salamanders, compared with the same species from lighter infections. However, Roberts (1961) is of the opinion that neither the largest nor the smallest population size may furnish optimal conditions for growth, maturation and egg-laying of an individual

parasite. It seems probable that the mechanism of the crowding effect is very complex and does not necessarily depend entirely on a limited food supply or on the accumulation of toxic metabolic products. The possibility of the production of an "antigen" by one parasite against another of the same species must also be considered in this connexion.

The influence of the host on variation in a given parasite as a concept has received increasing attention in recent years. For some time now, parasitologists have accepted that worms of the same species from different host species may exhibit quite striking differences in both morphology and physiology when the host specificity is not too strict to allow this. However, in 1959, Schiller (1959b) was still able to say that "no satisfactory means of evaluating the extent to which the host may influence intraspecific variation in parasitic flatworms had been developed". Although in previous papers (Read, Schiller and Phifer 1958, and Schiller 1959a) he had been able to demonstrate that both the definitive host and its diet played an important part in the size attained by the dwarf tapeworm Hymenolepis nana in the mouse, he was able to demonstrate conclusively that certain host species had

a marked effect on the frequency with which variant characters were expressed, and that the extent of this effect varied with the different hosts (Schiller 1959b). Schiller found that the hosts which induced the higher increases in the frequency of variation were those in which infections with this parasite had not been previously recorded. On the other hand, he was able to demonstrate that the frequency of occurrence of certain variant characteristics in H. nana was essentially constant within one host species (the mouse) (Schiller, 1959b). Although comparison between cestodes from mice, hamsters and grey squirrels indicated that the growth rate, strobila size and egg productivity varied according to the host, these were grossly affected by both the quantity and quality of the host diet, particularly in respect of carbohydrates. Other morphological characters of taxonomic importance were not apparently affected however.

It seems, therefore, that infection rates, the species of host and the host diet may all have a greater or lesser general effect on the morphology of the parasite, and that the environment presented by an individual host, regardless of species, may have an effect differing from that of another host of the same species under the same conditions.

There does not appear to be any valid reason why the environment provided by the host, composed of many factors due to diet, host species, etc, should be regarded as in any way different in principle from that of a more "conventional" environment. The form adopted by a free-living organism is regarded as the expression of interaction between the genetical make-up of that organism on the one hand with its environment on the other. There seems to be no valid reason why the form adopted by a parasitic organism should be regarded in any way other than as the result of the interaction of the genetical constitution of the parasite and the physiological environment presented by the host. The only basic difference between the environment of the free living organism and that of the parasite is that the latter is often much more complex and is itself a phenotypic character. It is in the complexity of the environment, which is itself a phenotypic expression, within the host (i.e. variability from host to host and even from time to time) that the reason for variation within a parasitic species lies.

Variation in eggs.

Cort (1951) has drawn attention to the variation encountered in eggs in a trematode species. He found that

the variation in egg size in groups of frog lung flukes morphologically of the same species, was greater than the difference between eggs of two distinct species of the same genus.

Buchanan (1937) has pointed out that a reliable diagnosis of disease due to schistosome infections cannot be made on either size or shape of the schistosome eggs, as these show a marked variation even in the same host individual and such differences alone do not justify the "labelling" of a new species. Buchanan points out that the egg illustrated by Blackie (1932) as that of S. mattheei is indistinguishable from S. bovis, and he points out that McHattie and Chadwick (1932) stated that "diseased" S. bovis may produce eggs corresponding in shape and size to those of S. haematobium. McHattie and Chadwick (1932) observed that about 1% of female S. bovis showed many typically shaped S. bovis eggs in utero and in the same uterus one or more typically shaped S. haematobium eggs, having the same measurements as the egg of this parasite in man. They were able to distinguish such females (schistosomes from cattle) from those from human cases by the fact that the vitellaria occupy about half the total length of the female in the former, instead of one-third as

in the material from man. In his original description of S. intercalatum from man Fisher (1934) stated that none of the morphological characteristics of his material, apart from the egg, were sufficient to distinguish it clearly from either S. haematobium or S. bovis, and in the light of the warnings given by Chandler (1923) McHattie and Chadwick (1932), Buchanan (1937) and Stunkard (1957) among others, this species must be regarded as of doubtful validity pending further investigation.

Smithers (1956) has figured, without comment, the variation to be found in S. haematobium eggs in utero which he recovered from mice infected with cercariae from naturally infected snails of the species Bulinus in the Gambia. Pitchford (1961) has also presented illustrations of variation in eggshape in schistosomes of the haematobium-mattheei complex, and Alves (1949) has attempted to differentiate between eggs of S. bovis, S. mattheei, and S. haematobium on statistical grounds. He measured the width of the egg at a point 50 microns from the aspinose end, in addition to the maximum width and length, in an attempt to define the spindle shape of S. bovis. On the basis of his results, he was able to say that normal S. haematobium and S. bovis, should never be confused and wrongly classified, while there is a 3%

chance of wrongly classifying S. bovis as S. mattheei, and 1½% of classifying S. mattheei as S. bovis on the basis of eggshape. He found that while an egg of S. mattheei should not be regarded as that of S. haematobium, there is a half per cent chance of wrongly classifying S. haematobium eggs as S. mattheei. He also noticed that his West African and Sudanese S. haematobium eggs were quite distinct from those of Rhodesian origin. Schwetz (1951) concluded on the basis of egg shape that S. intercalatum could be distinguished from both S. bovis and S. haematobium but not from S. mattheei.

Pitchford & Visser (1960) were able to demonstrate the effect of host diet on S. mansoni var. rodentorum of Schwetz (1953) from various species of wild rodents in the Transvaal which had been infected both naturally and in the laboratory. They concluded that Schwetz's variety was in fact S. mansoni, the differences in morphology of Schwetz's form being due to the abnormal environment in a rodent instead of man. Despite the observations made by Stunkard (1957) that the nutrition of the host probably plays an important part in variation in the parasite, little work has been done on the effect of host diets on parasitic helminths in general and on the schistosomes in particular. The problem is obviously

very complex and will need much careful study before it is fully elucidated. Illustrations of the variation in egg shape encountered in the present work are given in Figs. 6, 7, 8 & 9, Pages 96, 98, 100 and 103.

It was the purpose of the present work to draw attention to the phenotypic variation which it is possible to inflict upon strains of parasitic helminths by various comparatively simple and commonplace treatments, and therefore to draw attention to the need for extreme caution in creating definite, distinct taxonomic status for particular examples of a single variable group of organisms. The methods were not used in the present study to suggest taxonomic relationships between the various groups, as is one of the more normal uses of the multivariate technique, as there are implicit in the origins

of the material, but were used to demonstrate the effects of various treatments in an attempt to create pseudo-taxonomic differences in a similar manner and of a similar order of magnitude as are often encountered in helminthology and accorded definite taxonomic status. Whether in fact any genetic modifications are implicit in such experimental phenotypic modifications is not entered into here and would have to be the subject of a separate study.

The multivariate approach was used to extract the maximum amount of information from the data by comparing the dimensions of one group with those of another group. There is no means of knowing in advance whether such an approach will reveal further information. There is in fact little improvement compared to the information obtained by the univariate approach, but it is not possible to arrive at this conclusion without trying both approaches.

To summarise therefore, examination of material by conventional methods alone - e.g. anatomy or histology - does not necessarily reveal the extent of variation present in a sample of parasitological material, and detailed statistical analysis may often be of value and give a more precise answer. Furthermore, results thus obtained can be of much greater value as they are less likely to be based on subjective interpretations of colour or shape, etc. It is essential when examining material, whether it be from a new or little known host or not, that consideration be given to the effect of the host upon the egg (in the case of the schistosomes at least), and also upon the general anatomy of the parasite. Similarly, it is very important to realise the effect on size (and possibly on shape) of the site of occurrence of the material in the host animal. The geographic strain of the parasite

is also of considerable importance; variation in material from the same site from several animals of the same species infected with the same geographic strain of parasite is the only one which appears to be of relatively minor importance, and is often the only one which is given consideration; variations above this level are all too frequently used for specific or even generic differentiation. The warnings given by Nelson at el. (1962) concerning the diagnosis of species by egg size alone cannot but be repeated here.

It is important that the amount of variation exhibited by the same species of parasite under different conditions should be much more widely realised, and considerable caution must be exercised before material from a new host is described as a distinct species, basing the conclusion on size and shape alone. It has been shown above that it is possible to considerably affect the size of eggs of S. haematobium merely by passaging through another host species. Differences may also be found in other aspects such as enzyme and biochemical systems. The implications of this must be realised before new species are created. This conclusion probably has much wider applications and may be applicable

to all parasites capable of surviving as adults in hosts of different species. More work is needed to amplify and confirm these results for other genera and species of parasites.

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THE RECOVERY AND TRANSPORTATION OF *Schistosoma haematobium* EGGS FROM THE FIELD

SIR,—The recovery of eggs from field cases of urinary schistosomiasis, and more particularly their successful transportation over long distances to the laboratory, has presented considerable difficulty in the past. The following technique has proved very successful in the transportation of viable eggs from Africa and the Middle East to the United Kingdom on numerous occasions and may be of interest to other workers.

Terminal urine samples and those collected after exercise yield the highest concentration of eggs and make the collection of larger quantities (e.g., 24 hour samples) largely unnecessary.

Procedure

Set up the sample in clean urine glasses in a cool place and away from bright light if possible. Allow to sediment for at least an hour. Examine with a microscope for the presence of viable eggs. Pipette the sediment from heavily infected samples to centrifuge tubes, preferably of the conical type. Add normal saline solution, shake to resuspend the sediment and centrifuge at 1500 rpm. for 2 to 3 minutes. Suck off the supernatant liquid, resuspend in saline containing 100 i.u. penicillin per ml. and centrifuge again. Repeat. Finally resuspend the deposit in penicillin-saline or saline alone. The sample should be chilled (*but not frozen*) until required.

For transport to the U.K. a small vacuum flask is used and should be chilled for 2 to 3 hours before use. The washed sediment prepared as above should be transferred to a chilled screw-topped Universal bottle and sealed with wax. The bottles are packed in the flask, together with a little ice to keep the contents cool and sufficient cotton wool to protect the vacuum vessel. They are then sent by air to the U.K. by the quickest route.

Timing of the whole operation is important to eliminate as much travelling and waiting time as possible during transit. The flask and contents should be kept chilled whenever possible, even on arrival in the U.K. Written instructions to this effect should be attached to the outside of the flask. It is important that the pressure in the flask and that within the aircraft are allowed to equilibrate to prevent the flask from shattering.

This method has yielded thousands of miracidia from each urine sample and has enabled laboratory cultures to be successfully established on numerous occasions. It is considerably superior in results to sending newly infected snails by air, when mortality is often high.

I am, etc.,
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2nd January, 1964.

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