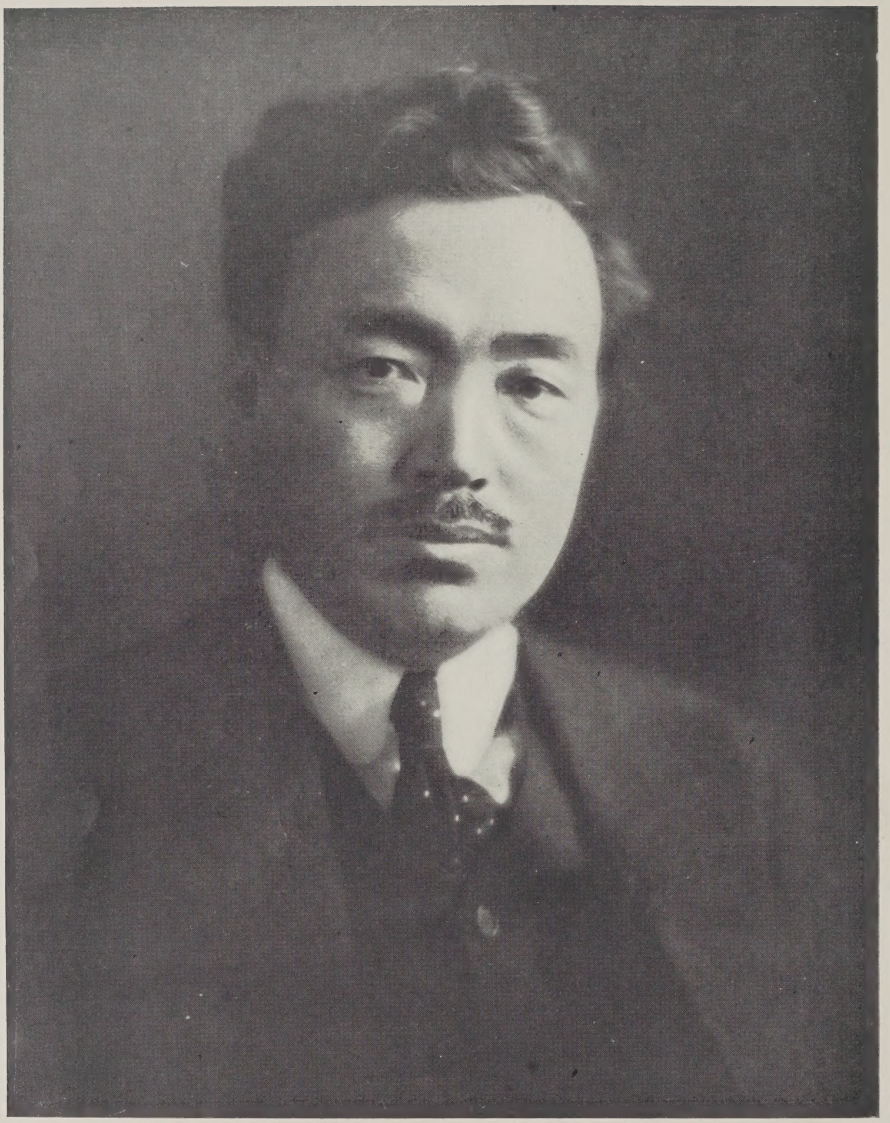
Annals of Tropical Medicine and Parasitology



Hideyo Noguchi

THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

PROFESSOR J. W. W. STEPHENS, M.D.Cantab., F.R.S.

PROFESSOR R. NEWSTEAD, M.Sc., J.P., F.R.S., A.L.S., F.E.S., Hon. F.R.H.S.

PROFESSOR WARRINGTON YORKE, M.D.

PROFESSOR B. BLACKLOCK, M.D.

VOLUME XIX

(March 31, 1925, to December 16, 1925)

With Frontispiece, eight plates, and ninety-six figures in text

LIVERPOOL:
THE UNIVERSITY PRESS OF LIVERPOOL LIMITED

LONDON:
HODDER AND STOUGHTON LIMITED

C. Tinling & Co., Ltd.
Victoria Street
Liverpool

CONTENTS

No. 1. March 31, 1925

CONTENTS

No. 2. July 16, 1925

CONTENTS

No. 3. September 30, 1925

CONTENTS

No. 4. December 16, 1925

SOME CESTODA DESCRIBED BY BEDDARD, 1911-1920

BY

JEAN G. BAER, D.Sc.

(*Zoological Institute, University of Neuchâtel, Switzerland*)

(*Received for publication* 5 *December*, 1924)

Between the years 1911 and 1920, Professor Beddard published in the *Proceedings of the Zoological Society of London* a series of papers dealing with new and known species of Cestoda collected from the animals dying in the Society's Gardens.

A few years ago, Professor Fuhrmann obtained the loan of some of Professor Beddard's types and co-types with the intention of re-examining and eventually redescribing them. However, more important matters having delayed this work, Professor Fuhrmann asked me to undertake it. I tender my sincerest thanks to Professor Fuhrmann for this material, as well as for his kindly advice.

The Cestoda of which we have been able to examine the types or co-types are the following. The names printed in small italics must fall and be considered as synonyms.

1. *ANOPLOTAENIA DASYURI*, Beddard, 1911.
2. *DASYUROTAENIA ROBUSTUS*, Beddard, 1912.
3. *Hyracotaenia hyracis*, Beddard, 1912.
4. *Hyracotaenia procaviae*, Beddard, 1912.
5. *Inermicapsifer capensis*, Beddard, 1912.
6. *Monoecocestus erethizontis*, Beddard, 1914.
7. *Otidiotaenia eupoditis*, Beddard, 1912.
8. *Thysanotaenia gambianum* (Beddard, 1911).
9. *THYSANOTAENIA LEMURIS*, Beddard, 1911.

We have ourselves already re-examined Nos. 3, 4, 5, and 8, and have published our results in a preliminary report (1924). Our conclusions were as follows: *Hyracotaenia hyracis* = *Inermicapsifer capensis* = *Inermicapsifer hyracis* (Rudolphi, 1810), *Hyracotaenia procaviae* = *Inermicapsifer pagenstecheri* (Setti, 1897), and *Thysanotaenia gambianum* = *Inermicapsifer guineensis* (Graham, 1908). No. 7 has

been re-examined by Skrjabin (1914) who finds *Otidiotaenia eupoditis* to be a synonym of *Schistometra conoides* (Bloch, 1782).

We will now consider the remaining species.

ANOPLOTAENIA DASYURI, Beddard, 1911

Synonym :—

Oochoristica dasyuri (Beddard, 1911), Meggitt, 1924.

Host :—*Sarcophilus satanicus*, Thomas. Locality :—Tasmania (Lond. Zoo.).

Of this worm we were able to examine two entire specimens and a few fragments. The length of the largest specimen is 23 mm., and the greatest width is 1 mm. There are altogether about thirty-one segments ; these are at first broader than long ; they then become square, and finally longer than broad, the last segments measuring 2·7 mm. in length, and 1·4 mm. in width.

The *scolex* is very typical, and measures about 0·86 mm. in diameter ; it is provided with four very large suckers, oval in shape

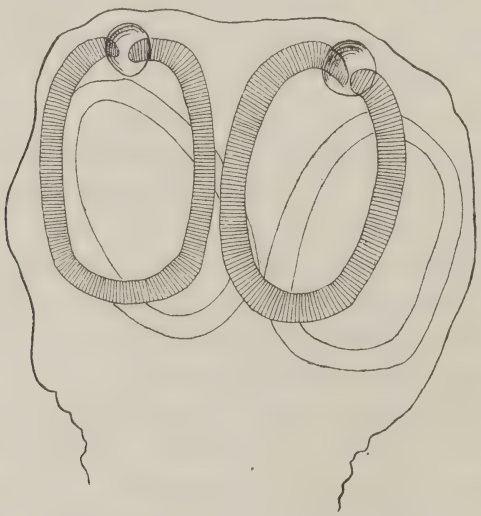


Fig. 1. The scolex of *A. dasyuri*, Beddard.

(fig. 1) measuring 0·48 : 0·29 mm. Neither in whole mounts nor in sections is there any trace of a rostellum. On page 1005, Beddard (1911b) states that ' there is in the same way a kind of hint of a

commencing pseudo-scolex.' What Beddard saw, and interpreted in the above manner, is nothing less than the folds arising from the contraction of the first segments of the strobila.

The cuticle is 3·8μ thick; there are no calcareous corpuscles. The musculature is fairly well-developed. The longitudinal musculature consists of two layers, one outer layer of stout fibres irregularly dispersed throughout the cortical parenchyma, and reaching almost as far as the cuticula (fig. 2), and one inner layer

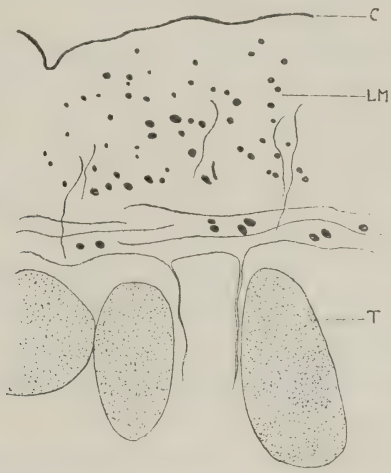


Fig. 2. *A. dasyuri.* A portion of a transverse section. C.—cuticula; L.M.—longitudinal musculature; T.—testes.

of very stout fibres, usually found in pairs, but not forming bundles as described and figured by Beddard (*loc. cit.*, p. 1005, fig. 209). We have, however, occasionally found the latter disposition in the last segments of the strobila. The transverse muscles are but feebly developed; they roughly form two layers separating the inner longitudinal muscle layer from the outer layer on one side, and from the medullary parenchyma on the other. Dorso-ventral fibres are fairly numerous throughout the strobila.

The two longitudinal *nerves* are very much compressed laterally, and measure 15·5μ, the greatest diameter being dorso-ventrally.

The *excretory system* is well developed, and consists of the usual four longitudinal vessels. The ventral vessels are about 0·03 mm. in diameter on transverse sections, and present a very typical aspect in the end segments. At the point where the transverse vessel branches

off, the ventral vessel suddenly swells out, forming a kind of reservoir. This disposition is fairly common among Cestodes, and is also found in *Hemiparonia cacatuae* (Maplestone, 1922), described below. The dorsal excretory vessels are not more than 0·009 mm. in diameter, and are situated dorsally and slightly internal to the ventral vessels. The genital ducts pass between the dorsal and ventral excretory vessels. We have been unable to determine with certainty the position of the longitudinal nerve stem with regard to the genital ducts ; it seems, however, to lie ventral to the latter. The genital pores are irregularly alternate.

Genitalia. The *testes* are very numerous, about 300 or more, and lie in two to three dorso-ventral layers anterior to the cirrus pouch and to the coils of the vas deferens (fig. 3, B). There are no testes to be found dorsal to the cirrus pouch, to the coils of the vas deferens, and to the female gonads ; the latter are, however, surrounded by testes, there being a single row posterior to the vitelline gland (fig. 5). The testes are usually close together, and are ovoid in shape, the greatest diameter being dorso-ventrally.

The *vas deferens* takes up an extraordinary amount of room, pushing aside the testes and the uterus, and occupying most of the available dorso-ventral space.

After forming an intricate mass of coils the vas deferens penetrates into the cirrus pouch. The latter has been described at much length by Beddard, who has, however, failed to interpret this organ correctly, and has caused much confusion by trying to distinguish within the cirrus pouch a vas deferens, a cirrus and a penis. The cirrus pouch is almost spherical in shape, usually broader than long. It measures 0·19 mm. in length, and 0·21 mm. in diameter. Its walls are fairly muscular, and are 5μ thick, being chiefly constituted of longitudinal fibres. Within the pouch the vas deferens forms several loose coils, which Beddard (*loc. cit.*, p. 1015) has interpreted as the cirrus. The *cirrus* is 0·21 mm. long and 0·03 mm. in diameter ; it is unarmed and somewhat swollen towards its extremity, and possesses a *terminal* pore. Beddard's drawing and description of a lateral pore are, of course, due to oblique sections. The cirrus is covered with a fairly thick cuticle, the latter being usually thicker towards the base of the cirrus. Within the cirrus pouch are to be found numerous muscle fibres acting, no doubt, as *retractores*

cirri. There are also numerous small cells with large nuclei, which we believe to be the myoblasts of the above muscles. The cirrus pouch opens into a genital atrium, which is extremely characteristic, reminding one in some ways of a similar

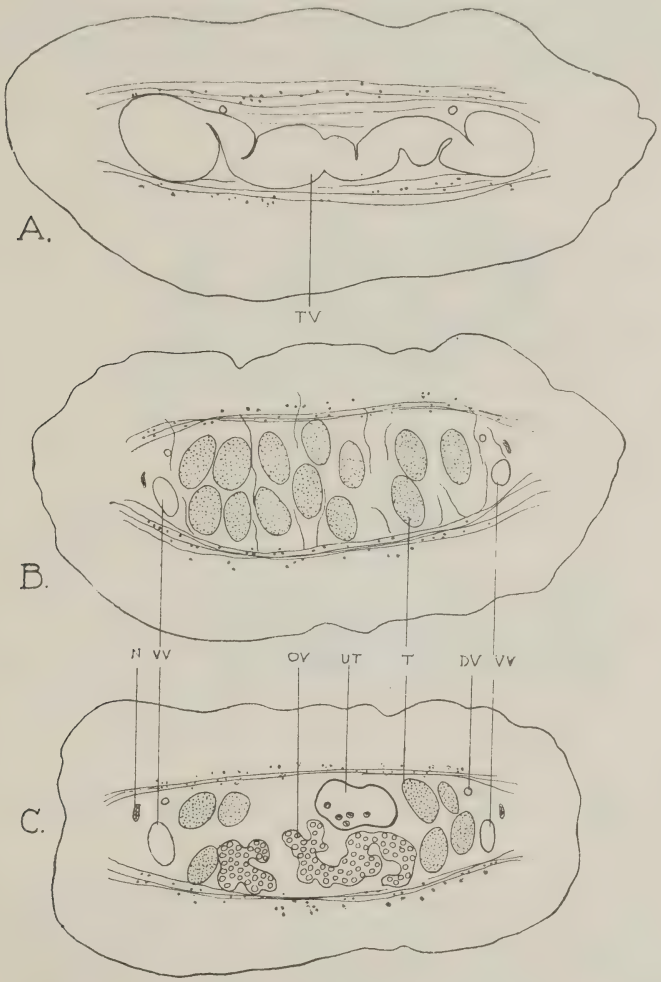


FIG. 3. *A. dasyuri*. Transverse sections: A, between two segments; B, anterior to the cirrus pouch; C, posterior to the cirrus pouch. D.V.—Dorsal excretory vessel; N.—nerve; OV.—ovary; T.—testes; T.V.—transverse vessel; V.V.—ventral excretory vessel; UT.—uterus.

structure found in *Tetrabothrius* spp. The genital atrium may be divided into two regions and not into three or four, as Beddard describes. Immediately next to the cirrus pouch we find a tremendous

sphincter muscle 0·12 mm. in diameter, and 0·1 mm. thick on transverse sections. Curiously enough, this sphincter is pierced laterally to permit the vagina to open into the atrium. Beyond the sphincter we find a second region or atrium proper, the walls of which are provided with numerous radiating muscle fibres, the latter belonging partly to the system of transverse muscles (fig. 4, A and B). It would seem that this complicated genital atrium would

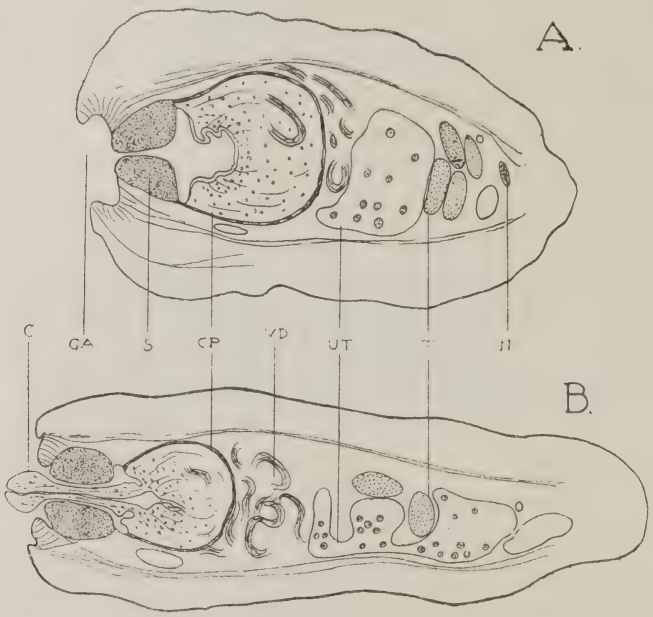


FIG. 4. *A. dasyuri.* A and B, transverse sections passing through the cirrus pouch. C.—cirrus; C.P.—cirrus pouch; G.A.—genital atrium; N.—nerve; S.—sphincter; T.—testes; UT.—uterus; V.D.—Vas deferens.

function as follows. When the cirrus is to be protruded, the transverse muscles contract, thus opening the anterior chamber of the atrium, and at the same time pressing on the walls of the cirrus pouch, causing the cirrus to evaginate; cross-fertilisation is thus made possible. When, however, the sphincter is closed, then self-fertilization is rendered possible, the seminal fluid being expelled through the contractions of the muscular walls of the cirrus pouch. The *vagina*, as we have already mentioned, perforates the sphincter laterally, and passes posterior to the cirrus pouch after forming

a sudden curve, almost at right angles (fig. 5). In its distal portion the vagina forms a distinct and fairly large receptaculum seminis. The *ovary* consists of two wings, of which the poral one is slightly smaller than the aporal one. These wings are made up of fairly numerous and somewhat compressed lobes and remind one strongly of the ovaries of *Taenia* spp. The *vitelline gland* is fairly compact, situated posterior to the ovary, and not extending laterally beyond the latter. The shell gland is well developed. The *uterus* appears very soon and as in *Taenia* spp., consists of a median stem, the

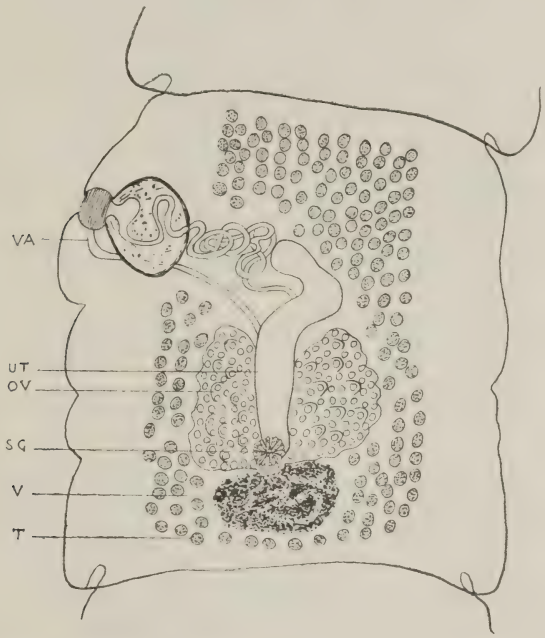


Fig. 5. *A. dasyuri.* A mature proglottid. ov.—ovary; s.g.—shell gland; t.—testes; ut.—uterus; v.—vitelline gland; va.—vagina.

anterior portion of which is pushed aside by the coils of the vas deferens, thus forming a very characteristic kink. The uterus soon begins to branch out laterally until it fills almost the entire segment. On no occasion was the uterus observed to be reticular, neither were ova found embedded in the parenchyma, both these observations being due to errors of interpretation. The gravid uterus presents a very characteristic aspect, there being always more diverticula on the aporal than on the poral side (fig. 6). The *ova* are thin-shelled and measure 27 : 19μ.

As can be gathered from the above description, the genus *Anoplotaenia*, Beddard, 1911, is entirely justified, although to our mind its systematic position is not correct.

Beddard, after a somewhat lengthy discussion, places his genus in the sub-family ANOPLOCEPHALINAE because the head is unarmed, and because the host is a Marsupial.

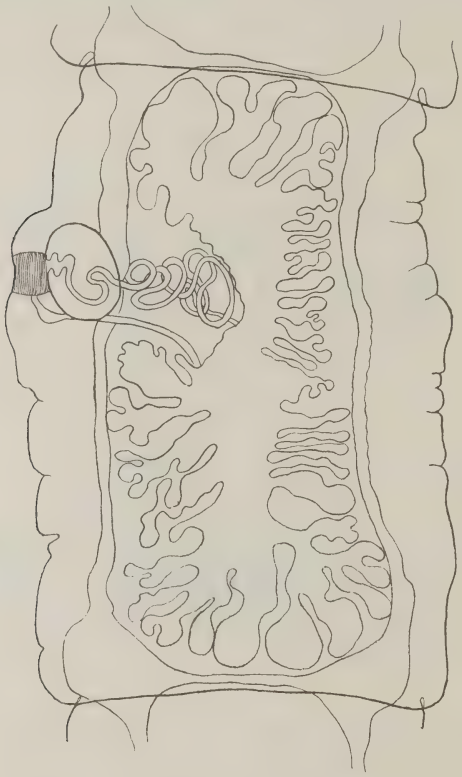


FIG. 6. *A. dasyuri.* A gravid segment showing the structure of the uterus.

Meggitt (1924a) basing his argument on Beddard's error of interpretation, considers the genus *Anoplotaenia* as a synonym of *Oochoristica*, because the uterus dissolves and the ova are scattered in the parenchyma, and he lists it accordingly. To our mind the presence of an unarmed scolex is not necessarily a reason for including this genus in the ANOPLOCEPHALIDAE. Several families of *Cyclophyllidea* contain genera with unarmed scolices, the same genera being also found to possess species with armed scolices; for instance,

Hymenolepis and *Taenia s. str.*, not to mention *Anonchotaenia*, *Rhabdometra*, *Octopetalum*, etc. On the other hand, the uterus of *Anoplotaenia*, as Beddard himself states, is very similar to that of *Taenia s. str.*

If we now take the above characters into consideration, keeping in mind the general anatomy, we can but place the genus *Anoplotaenia* in the family TAENIIDAE, Perrier e. p., and re-define it as follows :—

TAENIIDAE *of small size. Head unarmed, suckers large. Genital pores irregularly alternating. Genital ducts pass between the excretory vessels and dorsal (?) to the nerve. Testes form a single field interrupted dorsal to the coils of the vas deferens and to the ovary and vitelline gland. A single row of testes posterior to the latter. Cirrus pouch spherical, opening into a highly differentiated genital atrium provided with an exceedingly powerful sphincter. The latter is perforated laterally by the vagina. Uterus a median stem with numerous lateral diverticula.*

Adult in *Marsupials*. Type : *Anoplotaenia dasyuri*, Beddard, 1911.

DASYUROTAENIA ROBUSTA, Beddard, 1912

Host :—*Sarcophilus satanicus*, Thomas. Locality :—Tasmania (Lond. Zoo).

This second interesting genus was also obtained by Beddard from a Tasmanian Devil. Unfortunately we have only been able to examine a few fragments of this worm.

The greatest length according to Beddard (1912b) is 31 mm. and the greatest width 9 mm. The *scolex* 3·5 mm. in diameter bears four suckers, each of which measures 0·35 mm. in diameter. The fragments to hand, and also the material examined by Beddard, judging from his drawings, are extraordinarily contracted. This will serve to explain certain of the errors committed by Beddard.

The *cuticle* is exceedingly thick, and measures as much as 11·4μ. There are no calcareous corpuscles to be found.

The *musculature* is extremely well developed (fig. 7). Immediately beneath the cuticula we find a layer of irregularly disposed muscle fibres ; these are very stout and show a tendency to form bundles of about four fibres each. Beneath this layer of longitudinal muscles are to be found several fibres of transverse muscles. Beneath

these again we find a second layer of longitudinal muscles now definitely grouped in bundles containing about fifteen fibres. We next find a second layer of transverse muscles, beneath which lies a third layer of longitudinal muscles forming bundles containing about fifty fibres each. This layer is separated from the next by a third layer of transverse fibres. The fourth layer of longitudinal muscles consists of bundles containing about thirty fibres each. We then find a fourth layer of transverse fibres, beneath which lies a fifth layer of longitudinal muscle bundles containing about twenty fibres each. Finally we have a fifth layer of transverse fibres.

FIG. 7. *D. robusta.* A portion of a transverse section. C.—cuticula; L1-L5.—Longitudinal musculature; T1-T5.—transverse musculature.

Dorso-ventral fibres are very numerous. It is interesting to note the very numerous and exceedingly distinct myoblasts, the latter being found in all three of the muscular systems. This exceedingly powerful musculature reminds one of that of *Cotungia* spp. and also to a certain extent of that of the ACOLEIDAE.

The *excretory* system also presents a very interesting disposition, and seems to have given Beddard much trouble. The most striking character of this system is the truly extraordinary development of the two ventral excretory vessels. The latter are about 0·69 mm. in diameter, and form two exceedingly large coils in the lateral fields of the proglottides. Owing to extreme contraction of the worm, we find these coils touching one another, with the result that on

sections we find what Beddard describes as membranes and valvules, and which are caused by the sections passing somewhat obliquely through two consecutive coils. In the same way the genital ducts do not *pierce* the ventral vessel, but pass between two coils. We have endeavoured to figure this diagrammatically in fig. 8. The latter

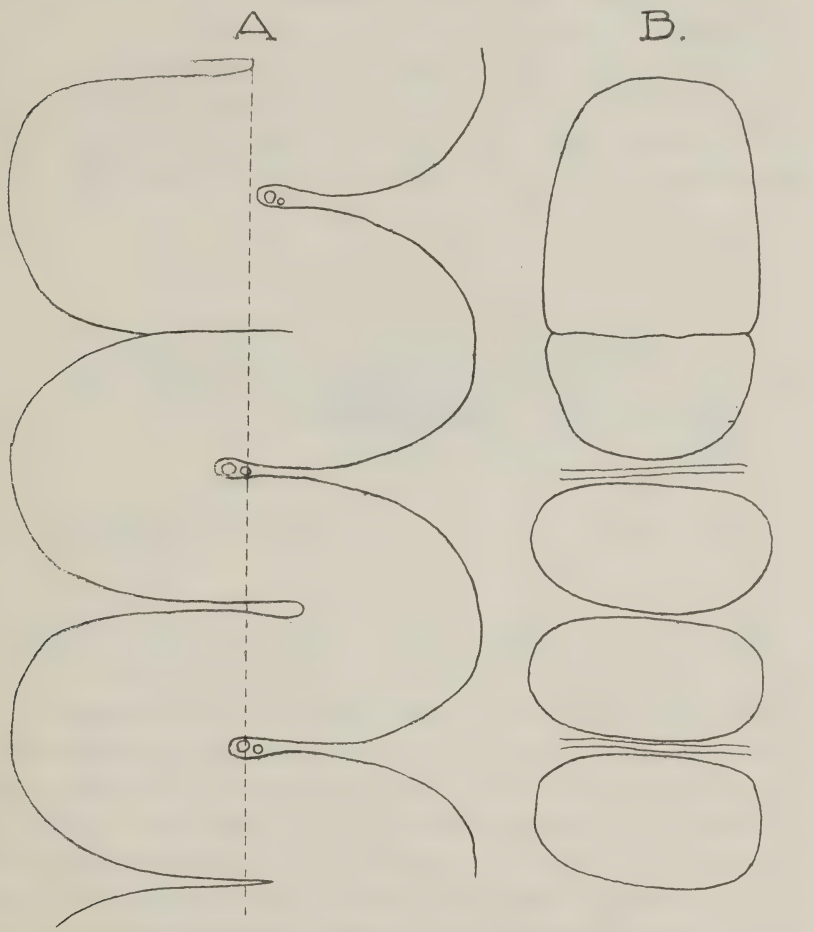


FIG. 8. *D. robusta.* Diagram of the ventral excretory vessel: A, sagittal view; B, section along the dotted line viewed horizontally.

represents a ventral vessel coiled dorso-ventrally; in reality the coils are spiral, and next to it is a section passing along the dotted line. As Beddard rightly remarks, there are no transverse vessels to be seen, although perhaps in less contracted specimens such

a structure might be seen, and might have been obliterated owing to the extraordinary contraction of the worm. The dorsal vessels are about 0·007 mm. in diameter, i.e., about a hundred times smaller than the ventral vessels. They are also very much coiled and are exceedingly difficult to make out.

The two lateral *nerve stems* are compressed laterally and measure on transverse section 76 by 19μ.

Genitalia. The genital pores are unilateral, and the genital ducts pass between the excretory vessels, and ventral to the nerve.

The *testes* are fairly numerous, about 250, and are inclined to be dorsal (fig. 9). They occupy three to four dorso-ventral layers in the

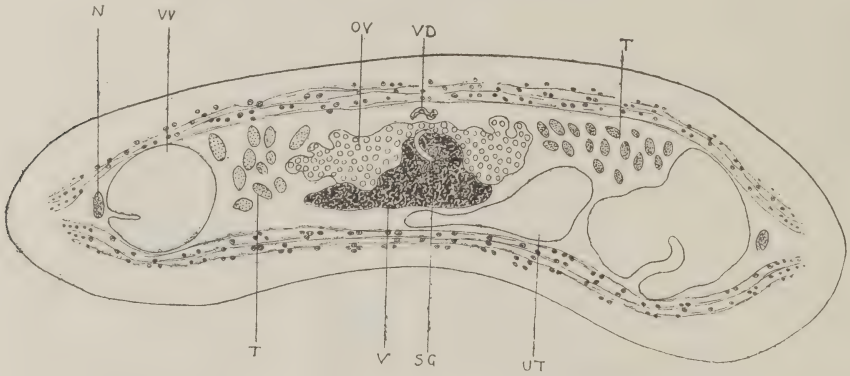


Fig. 9. *D. robusta.* Transverse section. N.—nerve; OV.—ovary; S.G.—shell gland; T.—testes; UT.—uterus; V.—vitelline gland; V.D.—vas deferens; V.V.—ventral excretory vessel.

lateral fields, and only a single dorsal layer in the region of the female genitalia. This layer is, however, soon pushed aside by the growing female organs and especially by the uterus. When examined in horizontal sections (fig. 10), the testes are flattened antero-posteriorly, owing to contraction. Laterally the testes extend as far as the ventral excretory vessels. The *vas deferens* is extremely coiled, but straightens out suddenly when passing between the excretory vessels. We have not noticed the glands surrounding the male duct and described by Beddard as 'interstitial prostatic cells.' The cirrus pouch is an elongate pear-shaped organ 0·34 mm. long and 0·25 mm. at its greatest diameter. The walls are fairly muscular and measure 8μ in thickness. Immediately on entering the cirrus pouch the vas deferens forms several coils much distended with

spermatozoa, and probably replacing functionally an internal vesicula seminis. The *cirrus* is 0·19 mm. long and 0·015 mm. in diameter. Although none of the cirri were evaginated, we have been unable to observe on them any small spines such as Beddard describes on page 693. The *vagina* opens into a small genital atrium posterior to the cirrus pouch ; it is thick-walled in the first part of its course and runs almost in a straight line towards the centre of the segment, where it forms a large receptaculum seminis. We have noticed an interesting and somewhat problematic structure situated on the course of the vagina and just before the latter enters the receptaculum seminis. The vagina suddenly increases in diameter and becomes thick-walled resembling very much an ootype. This structure contains circular muscle fibres, and probably acts as

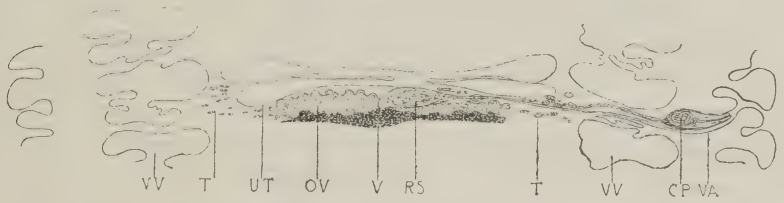


Fig. 10. *D. robusta.* Horizontal section. c.p.—cirrus pouch ; ov.—ovary ; r.s.—receptaculum seminis ; t.—testes ; ut.—uterus ; v.—vitelline gland ; va.—vagina ; v.v.—ventral excretory vessel.

a sphincter to close the receptaculum seminis once the latter is filled with spermatozoa. The *ovary* is only slightly lobed and forms two wings, of which the poral one is slightly smaller than the aporal one. The *vitelline gland* is situated posterior and slightly ventrally to the ovary, extending laterally as far as the latter. A distinct shell-gland is also to be found. The *uterus* is not as Beddard states ' a large cavity extending right across the segment,' but presents the typical structure met with in the genus *Taenia*, i.e., it possesses a median stem with two or more lateral diverticula. These latter are club-shaped when distended with ova. With regard to the ' uterine pore ' described in a later paper (1915), we cannot but interpret this as an artefact due to the sections not being in an horizontal plane.

The ova measure 19 by 15μ, and are provided with a thin shell.

It is obvious that the above description is far from being complete as no young segments, and especially no scolex, have been examined, the description being entirely based on gravid or mature segments.

We have attempted to clear up certain obscure anatomical details, but remain totally in the dark with regard to the scolex.

Beddard's description of a globular scolex with two external and two internal suckers depicts an entirely new arrangement, and suggests that the author has misinterpreted his sections. We cannot, of course, say that the arrangement described by Beddard is impossible without having examined the material ; we may, however, formulate an hypothesis based on the general structure of the worm, and on Beddard's drawings of the head. The hooks figured on page 682, text-fig. 96, are typical hollow hooks as found on the rostellum of many species of Cestodes, and especially in the family TAENIIDAE. The anatomy, also, is that of a member of this family ; we are, therefore, led to conclude that the '*two inner suckers armed with hooks*' represent a rostellum ! The fact is much more apparent if the figure is held upside down. Hence we conclude that the genus *Dasyurotaenia* should be maintained, and placed in the family TAENIIDAE and not in the ANOPLOCEPHALIDAE as Meggitt (1924b) has done, basing his classification on Beddard's description.

We would re-define this genus as follows :

TAENIIDAE *of small size, with an exceedingly well-developed muscular system. Ventral excretory vessels hypertrophied, a transverse vessel being absent* (?) *Scolex provided with four small suckers and a rostellum armed with a double* (?) *crown of hooks. Genital pores unilateral ; genital ducts passing between the excretory vessels and ventral to nerve. Large receptaculum seminis present. Uterus with a short median stem and very long, club-shaped, lateral diverticula.*

Adult in Marsupials. Type : *Dasyurotaenia robusta*, Beddard, 1912.

MONOECOCESTUS ERETHIZONTIS, Beddard, 1914

Host : *Erethizon dorsatum* L. Locality : N. America (Lond. Zoo).

We have been able to examine two entire specimens of this worm ; all our observations being made on total mounts, the latter being remarkably clear, as the material is rather macerated.

A careful examination of our preparations has shown us that the excretory vessels show distinct anastomoses such as are always found in the genus *Schizotaenia*. Beddard describes at much

length (p. 1048) the presence in certain segments of a rudimentary vagina, and based on this character, he places his genus in the ACOLEIDAE! One of the characteristics, however, of the ANOPLOCEPHALINAE, with the exception of *Aporina*, is the possession of a vagina which soon atrophies and disappears as the segments grow older. Beddard has himself described this particularity, but he does not seem to have realised its importance.

The rudimentary vagina of the ACOLEIDAE is of a totally different type, the vaginal *pore* being absent.

We have found the cirri to be covered with small spines, and what is more, we have found that the material examined contains *two* different species! Our observations lead us to conclude that what Beddard has described as *Monoecocestus erethizontis*, gen. et sp.n. is nothing else than *Schizotaenia americana* (Stiles, 1895), and *Schizotaenia variabilis*, Douthitt, 1915. Beddard's paper is dated 1914, and Douthitt's 1915. Although the latter author's description of *S. variabilis* is excellent, we must comply with the rules of priority. This species must, therefore, be named *S. erethizontis* (Beddard, 1914), syn. *S. variabilis*, Douthitt, 1915.

In the following table we have endeavoured to place all the species of the genus *Schizotaenia*, Janicki, 1904, giving the differential characters of each species. It will be noticed that we retain the specific name *americana* for the species described by Stiles (1896). Douthitt (1915) has definitely shown the existence of two species of Tapeworms in the American Porcupine, neither of which can be identified with *T. laticephala*, Leidy, 1855. As the types of the latter have been lost (*vide* Stiles, *loc.cit.*, p. 165), it seems undesirable to maintain this name. We, therefore, propose to consider *T. laticephala*, Leidy, as a *nomen nudum*. We have removed from the genus *Schizotaenia* the species *S. cacatuae*, Maplestone, 1922, as this species is the type of a new genus to be described below. In a previous paper (1923), we removed to the genus *Anoplocephala* the species *S. latissima* (Deiner, 1912) and *S. gigantea* (Peters, 1856). We are almost inclined to include in the genus *Schizotaenia* the species actually known as *Oochoristica didelphydis* (Rudolphi, 1810) from *Marmosa murina*, L. It will be remembered (*vide* Janicki, 1906) that only fragments of this species exist, egg-capsules have not been found, and the scolex is unknown. On the other hand, the

vagina lies anterior to the cirrus pouch, and the ovary and the uterus are just at the stage where it is almost impossible to distinguish the one from the other, especially in macerated material. These last two factors seem to show a distinct relationship to the genus *Schizotaenia*. We prefer, however, to leave this species where it is for the present, and await a further supply of material in order to be able to study it further.

Table I.

Species	Author	Year	Length	Width	Diameter of scolex	Dimensions of cirrus pouch	Number of testes	Size of ova	Host	Distri
			mm.	mm.	mm.	mm.		μ		
S. decrescens ...	(Diesing)	1856	296	5	?	0·67 : 0·23	?	?	*Tayassus tajacu, T. albirostris*	Brazil
S. bagmanni ...	Janicki	1904	145	5·8	1·9	0·63 : 0·2	120–140	57	*Hydrochoerus capybara*	Brazil
S. americana ...	(Stiles)	1895	33	6	0·6	0·5–0·63 : 0·19–0·21	70	55–61	*Erethizon dorsatum, E. epixanthum*	U.S.A Can
S. sigmodontis ...	Chandler and Suttles	1922	30–50	2·5–3·5	0·36–0·45	0·6 : 0·19	70	47–53	*Sigmodon hispidus*	Texas
S. anoplocephaloides	Douthitt	1915	30–33	1·7–2	0·39	0·14 : 0·085	70–110	30–40	*Geomys breviceps*	U.S.A
S. erethizontis ...	(Beddard)	1914	20	8·5	0·88	0·49–0·5 : 0·14–0·19	70–110	12–14	*Erethizon dorsatum*	U.S.A

It is interesting to note that the genus *Schizotaenia* is entirely confined for the present, to the New World, where it is found in *Rodentia* and *Suidae*. A point which may be of some importance and which appears very interesting is raised by Scharff (1911). Speaking of the Canadian Tree Porcupine, the author says :

'Yet the species had already come into existence when the sabre-tooth tiger and peculiar kinds of peccaries haunted the forests of Arkansas, for its remains have been found together with these extinct creatures in the Conrad fissure.'

Actually the peccaries have retreated into South America, and the Canadian Tree Porcupine has retreated further north ; both groups, however, harbour the same genus of Cestode parasites. May we

take this as an indication that the genus *Schizotaenia* came into existence during the late Tertiary times, about the Pliocene Period, and has existed ever since ? The collection of further data will show if such an hypothesis is liable to lead to interesting results, or is to be abandoned.

THYSANOTAENIA LEMURIS, Beddard, 1911

Host :—*Lemur macaco*, L. Locality :—Madagascar (Lond. Zoo).

We have only been able to examine a few gravid and much macerated fragments of this worm, and are totally unable to add anything to Beddard's description except that a distinct dorsal excretory vessel is present in all our sections. This genus is, however, not a synonym of *Inermicapsifer*, Janicki, 1910. From the description of the genital organs, and from the aspect of the egg-capsules, we are almost inclined to refer this genus to *Raillietina*, Fuhrmann, 1920, and to the sub-genus *Ransomia*. We would do this in spite of the fact that Beddard states that the scolex is unarmed. All workers who have had to deal with this group know how difficult it is at times to perceive the tiny hooks on the rostellum, especially when the latter is retracted. For the present, however, and until more material has been examined, we retain the genus *Thysanotaenia*, Beddard, 1911, with the type species *T. lemuris*, Beddard, 1911, and place it in the sub-family LINSTOWINAE.

HEMIPARONIA CACATUAE (Maplestone, 1922), n.gen.

Synonym :—

Schizotaenia cacatuae, Maplestone, 1922.

Host :—*Cacatua galerita*, Lath. Locality : North Queensland.

As we have already mentioned above, this species was placed by Maplestone in the genus *Schizotaenia*. Its curiously aberrant anatomy, however, led us to suppose that this might be the type of a new genus. Thanks to the kindness of Professor Warrington Yorke, we have been able to examine the type and also the type material deposited in the collection of the School, and have been able to confirm our first opinion. We also express our sincerest thanks to Dr. Southwell for the loan of this valuable material.

Certain points in Maplestone's description are not very clear; we will therefore briefly redescribe the anatomy.

The *cuticula* is 4μ thick, and beneath this lies the internal longitudinal *musculature*. The latter consists of three layers of stout bundles. As the material is considerably macerated, it is very difficult at times to make out the three layers; these exist, however, throughout the entire strobila. Transverse muscles are hardly developed, whereas the dorso-ventral muscles are very numerous. The cortical parenchyma contains numerous small calcareous corpuscles measuring $7{\cdot}6 : 5\mu$.

The two longitudinal nerve stems lie lateral to the dorsal excretory vessels.

Genitalia. Maplestone estimates the number of *testes* at 100; we believe, however, that this number is too small. The testes lie in two and sometimes three dorso-ventral layers (fig. 11). We should

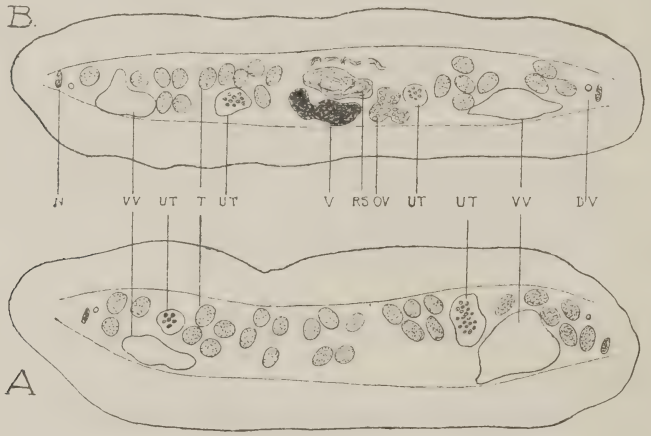


FIG. 11. *H. cacatuae.* Transverse sections: A, through the anterior region of the segment; B, through the posterior region of the segment. D.V.—Dorsal excretory vessel; N.—nerve; OV.—ovary; R.S.—receptaculum seminis; T.—testes; UT.—uterus; V.—vitelline gland; V.V.—ventral excretory vessel.

say that there appear to be about 200 testes. The latter are spherical in the lateral fields, and measure 0·042 mm. in diameter. Towards the centre of the segment they are generally so crowded together that they become egg-shaped, the greatest diameter being dorso-ventral. Laterally the testes pass beyond the ventral

excretory vessels. The vasa efferentia form a distinct and very complicated network, a portion of which is drawn in fig. 12. The

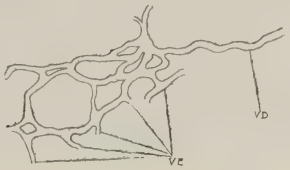


Fig. 12. *H. cacatuae.* Portion of the network formed by the vasa efferentia.

vas deferens soon becomes swollen with spermatozoa. Within the cirrus pouch it forms a few coils also very much distended with spermatozoa; it then enters the cirrus. The latter is covered with minute spines. The *vagina* opens slightly anterior and ventral to the cirrus pouch. Passing ventrally to the vas deferens it forms a large receptaculum seminis situated dorsally (fig. 13). The vagina

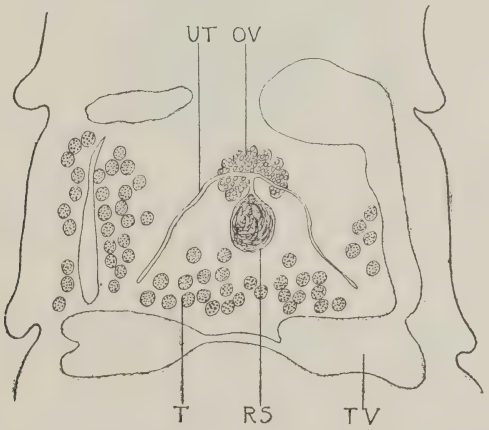


Fig. 13. *H. cacatuae.* A horizontal section through a young proglottid. ov.—ovary; r.s.—receptaculum seminis; t.—testes; t.v.—transverse excretory vessel; ut.—uterus.

very soon becomes so distended with spermatozoa that it is impossible to distinguish it from the receptaculum seminis (fig. 14). The *ovary* and *vitelline gland* are situated one behind the other; the former, fan-shaped, is made up of several lobes, and is situated ventrally to the latter, which is only slightly lobed. This can be

seen in transverse sections passing through this region (fig. 11). We find a slightly different arrangement of the female ducts from that described by Maplestone. The oviduct which is surrounded by numerous glands, receives first of all the duct from the receptaculum seminis, then and on the same side, the vitelline duct, and only then does the shell gland surround the oviduct, the latter passing into the uterus. The *uterus* constitutes the chief character of our new genus. In *anlage* (fig. 15) it appears as a fine horseshoe-shaped tube

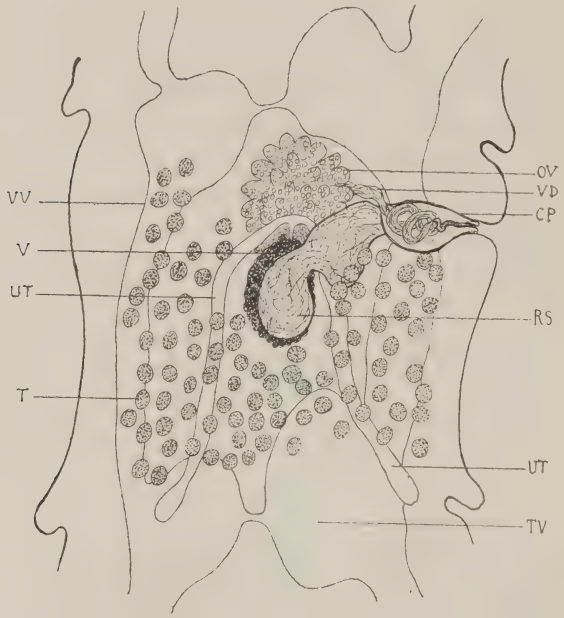


FIG. 14. *H. cacatuae.* A mature segment. C.P.—cirrus pouch; OV.—ovary; R.S.—receptaculum seminis; T.—testes; T.V.—transverse excretory vessel; UT.—uterus; V.—vitelline gland; V.D.—vas deferens; V.V.—ventral excretory vessel.

passing between the ovary and the vitelline gland, and lying in the centre of the segment. Very soon, however, the uterus increases in diameter, and forms several diverticula. In the gravid uterus the two extremities of the horseshoe never fuse together.

This extremely interesting genus from an Australian parrot bears an extraordinary resemblance to the genera *Paronia* Diamare, and *Moniezioides* Fuhrmann (*vide* Fuhrmann, 1918), both of which are also found in Australian parrots. The only difference is that these

two genera possess double genital pores, and a double genital apparatus.

Our new genus possesses unilateral, dextral genital pores, and the vagina lies ventral to the cirrus pouch. We would obtain the same disposition if we were to cut a species of *Paronia* into half, and considered the right half only ; this has led us to propose the name *Hemiparonia*, n.gen., and we define it as follows :—

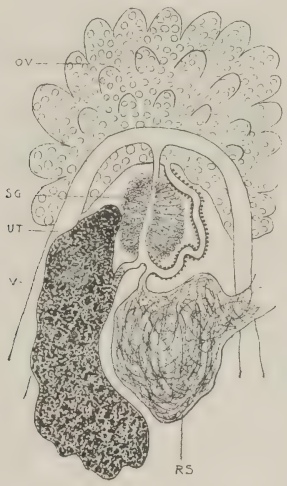


Fig. 15. *H. cacatuae*. The relationship of the female ducts. ov.—ovary ; r.s.—receptaculum seminis ; s.g.—shell gland ; ut.—uterus ; v.—vitelline gland.

Anoplocephalinae *of moderate size. Genital pores unilateral and dextral. A single set of reproductive organs in each segment. Vagina ventral to cirrus pouch. Genital ducts dorsal to excretory ducts and nerve. Testes a single dorsal field extending laterally beyond the ventral excretory vessel. Large receptaculum seminis present. Ovary and vitelline gland in centre of segment, former anterior to latter. Uterus horseshoe-shaped, later forming diverticula ; the two extremities never fuse together. Ova without* (?) *piriform apparatus.*

Adult in Birds. Type : *Hemiparonia cacatuae* (Maplestone, 1922).

REFERENCES

Baer, J. G. (1923). Considérations sur le genre *Anoplocephala*. *Bull. soc. neuchâteloise sc. nat.* Vol. XLVII, p. 1.

——— (1924). Contribution à la faune helminthologique sud-africaine. *Ann. Parasit.* Vol. II, p. 239.

Beddard, F. E. (1911a). Contributions to the Anatomy and Systematic Arrangement of the Cestoidea. I.—On Some Mammalian Cestoidea. *Proc. Zool. Soc. Lond.*, p. 626.

——— (1911b). Contributions to the Anatomy and Systematic Arrangement of the Cestoidea. II.—On two new genera of Cestodes from Mammals. *Ibid.*, p. 994.

——— (1912a). Contributions to the Anatomy and Systematic Arrangement of the Cestoidea. III.—On a new genus of Tapeworms (*Otidiotaenia*) from the Bustard (*Eupoditis kori*). *Ibid.*, p. 194.

——— (1912b). Contributions to the Anatomy and Systematic Arrangement of the Cestoidea. V.—On a new genus (*Dasyurotaenia*) from the Tasmanian Devil (*Dasyurus ursinus*), the type of a new family. *Ibid.*, p. 677.

——— 1914. Contributions to the Anatomy and Systematic Arrangement of the Cestoidea. XV. On a new genus and species of the family Acoleidae. *Ibid.*, p. 1039.

——— (1915). Contributions to the Anatomy and Systematic Arrangement of the Cestoidea. XVI. On certain points in the anatomy of the genus *Amabilia* and *Dasyurotaenia*. *Ibid.*, p. 175.

Douthitt, H. (1915). Studies on the Cestode family Anoplocephalidae. *Illinois Biol. Monographs*, Vol. I, No. 3.

Fuhrmann, O. (1918). Cestodes d'oiseaux de la Nouvelle Calédonie et des Iles Loyalty. Sarasin and Roux. Nova Caledonia, *Zoologie*. Vol. II, p. 399.

Janicki, C. v. (1906). Studien an Saügetiercestoden. *Zeitschr. f. wiss. Zool*, Vol. LXXXI, p. 505.

Maplestone, P. A. (1922). Notes on Australian Cestodes. Part VI. *Ann. Trop. Med. & Parasit.*, Liverpool, Vol. XVI, p. 305.

Meggitt, F. J. (1924a). On two species of Cestoda from a Mongoose. *Parasitol.*, Vol. XVI, p. 48.

——— (1924b). The Cestodes of Mammals. London.

Scharff, R. F. (1911). Distribution and Origin of Life in America. London.

Skrjabin, K. J. (1914). Vergleichende Charakteristik der Gattungen *Chapmania* Mont. und *Schistometra* Cholodk. *Centralbl. f. Bakt. u. Parasit.* 1. Abt. Orig. Vol. LXXIII, p. 397.

Stiles, Ch. W. (1896). A Revision of the Adult Tapeworms of Hares and Rabbits. *Proc. U.S. Nat. Mus.*, Vol. XIX, p. 145.

ON THE VALUE OF THE ESTIMATION OF THE IONIC CALCIUM OF THE SERUM IN THE DIAGNOSIS OF, AND AS A GAUGE OF PROGRESS IN SPRUE

BY

H. HAROLD SCOTT, M.D., M.R.C.P. (London), D.P.H., D.T.M. & H. (Camb.), F.R.S. (Ed.)

MILNER RESEARCH FELLOW IN COMPARATIVE PATHOLOGY, LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE

(*Received for publication* 15 *December*, 1924)

This contribution on the subject of sprue has a two-fold purpose. Firstly, to show the value of the estimation of the ' Ionic ' calcium as a diagnostic criterion ; secondly, to demonstrate that the same test, when repeated at intervals during the course of the disease, affords a valuable, in fact the most reliable gauge of progress.

Vines (1921) has shown that the calcium in the plasma normally exists in two forms. Of the total (10—11 mgm. per 100 c.c.) some 60 per cent. is readily precipitable by its chemical equivalent of ammonium oxalate solution, whereas the remaining 40 per cent., being ' bound,' probably with a lipoid complex, requires nearly three times its corresponding chemical equivalent. This latter, being closely concerned with the clotting of blood, is designated ' coagulative ' or ' combined ' calcium. When coagulation occurs, the latter becomes converted into the former, so that normal serum, as distinguished from plasma, contains all the calcium in the readily precipitable ' free ' or ' ionic ' form. If, on analysis of the serum, the total calcium is found to be normal in amount, whereas the ionic calcium is reduced, some of the calcium must have again become bound, and it is believed that the parathyroid glands, if acting normally, prevent this change.

An examination of this question, which is dealt with in Vines's work—*The Parathyroids in Relation to Disease* (1924), appears to show that ' there is no reason for assigning the control of calcium metabolism to any other endocrine gland ' nor can any other gland

' wholly or partially restore the disorders of calcium metabolism consequent on parathyroid failure or removal.'

It is clear that there may be two types of calcium deficiency, according as the total is decreased owing to there being an excessive excretion and a resultant calcium starvation of the tissues, or, the total being normal, the active or ionic calcium is deficient. In sprue the latter is the case. In very severe forms of the disease the total may be a little diminished, but never, as far as my observations go, to any marked extent. The error, therefore, would appear to be due, not to faulty absorption from the alimentary canal, but either to failure on the part of the tissues to use this calcium, or to errors in the regulation of calcium excretion—' a lowering of the threshold of excretion.' It is believed that in either case the underlying factor is a circulating toxin, which brings about the results found in sprue, by effecting a combination with the calcium of the blood or, perhaps, by damaging or interfering with the function of the parathyroids.

Further, toxaemias are said to stimulate thyroid function, and the antagonistic action of this gland to that of the parathyroid is well exemplified in sprue. If an impure preparation of parathyroid is used in treatment, that is, one containing any thyroid, or if, when progress is being made, parathyroid is stopped and thyroid given in its place, a return of the symptoms can be readily induced.

Dealing first with the value of the calcium estimation in diagnosis, it is particularly in diarrhoeic conditions that a reliable test is needed. We need not, therefore, discuss the diagnosis of diseases which are distinguishable on other clinical grounds. In pellagra, for example, there may be at times watery and offensive stools, with gastric and intestinal flatulence. There may also be a diffuse inflammation of the mouth. But these symptoms are clinically distinguishable from those in sprue, and there is nearly always a history of severe, bilaterally symmetrical ' sunburns ' in the spring, and evidence of pigmentation and roughness (pell' agra) on the back of the hands, the face and neck, to confirm the diagnosis.

In an article published recently in the *Journal of the American Medical Association* the writers Bastedo and Famulener (1923) discuss in full the means available for the diagnosis of sprue, cultural examination of bacteria, yeasts and so forth, and end by stating

'In sprue we have a disease for which no fully reliable laboratory criteria have been established.' I hope to demonstrate in the present paper that this no longer holds good.

In sprue it is the intestinal condition which first shows itself in the vast majority of cases—the early morning call to stool, with increasing bulk and frequency of pultaceous, frothy, fermenting motions. Intestinal disturbances are frequent in the tropics, and it is all-essential that treatment should be undertaken early. Accurate, and, if possible, early diagnosis is therefore most essential. The following is a brief account of a typical example :—

A man, aged 43 years, had spent most of the last twenty years abroad, having been in India, Africa, Mauritius, Ceylon, and the West Indies. For the past eight years he had suffered from loose actions of the bowels, the motions being yellowish or pale, sometimes frothy, and sometimes large. The condition had been diagnosed as malarial in nature, or more frequently merely as 'colitis,' and lastly as 'sprue.' He had submitted to various methods and courses of treatment with little if any benefit, and was finally sent to me definitely as a case of intractable sprue. The stool was certainly somewhat suggestive of that disease, fatty, greasy, unformed and bubbly.

The blood, however, showed a normal total and a normal ionic calcium content, and the faeces nothing particular except fat in excess. A second stool was found to contain *Entamoeba histolytica* cysts, and the probabilities were that the whole trouble was a residual dysenteric condition, the fat being due to the milk diet to which he had been restricted for a long period. Injections of emetine and a course of emetine bismuthous iodide cleared things up. There was no sprue.

Cases like this are comparatively common and I have, therefore, for some months past been examining the blood from patients at the Tropical Diseases Hospital and elsewhere in order to see whether this peculiar condition of the calcium—reduction of the ionic with a normal or nearly normal total—occurred in other diseases in which there was diarrhoea as a prominent symptom especially diarrhoea of a sprue-like nature. The method employed has been that devised by Dr. H. W. C. Vines (1921).

The following Tables will save pages of description :

TABLE I

Showing the Calcium content of the serum of Sprue patients before treatment

Initials	Ionic Ca.	Total Ca.	Initials	Ionic Ca.	Total Ca.
	%	%		%	%
Fr.	7·7	10·1	Ly.	6·9	9·9
Td.	6·8	9·9	J.T.	7·9	10·0
C.	6·9	9·8	R.*	8·1	10·4
L.E.B. ...	6·3	10·1	B.	6·9	9·9
A.W.	7·3	10·4	Bp.	6·6	9·9
E.	7·3	9·9	Bi.	7·9	10·0
Ge.	7·1	10·8	Ln.	6·3	9·9
R.W.	6·3	9·9	Tr.	7·7	10·1
St.	6·6	9·8	Ws.	7·1	10·1
R.P.W. ...	6·9	9·8	Es.	7·9	9·9
McG.	6·1	9·9	H.S.	7·0	9·9
Cr.	7·9	10·1	McC.	6·6	10·0
Ln.	6·6	9·8	Ts.	7·7	10·5
Ct.	6·6	10·1	Hn.	6·6	10·2
Bn.	6 1	10·6	Ee.	6·4	10·4
Rl.	6·7	10·4	Cm.†	6·1	9·4

* A mild case ; had been under treatment outside

† Very ill ; died within twenty-four hours

TABLE II

Showing the Ionic Calcium of the serum of patients other than Sprue

Disease	Initials	Ionic Ca.	Disease	Initials	Ionic Ca.
		%			%
Dysentery Amoebic ...	Ke.	10·6	Dysentery Bacillary ...	P.L.W. ...	11·5
,, ...	Ma.	10·6	,, ...	Ka.	10·6
,, ...	Me.	10·4	,, ...	M.T. ...	10·6
,, ...	Cr.	10·0	,, ...	Wa.	9·9
,, ...	D.S.	10·4	,, ...	S.R. ...	10·4
,, ...	B.S.	10·6	,, ...	W.S. ...	10·4
,, ...	H.E.	10·4	,, ...	M.D. ...	10·6
,, ...	Sn.	9·9	,, ...	T.R.	10·6
,, ...	J.N.R.A. ...	11·0	,, ...	So.	10·6
,, ...	S.N.	9·5	,, ...	B.N.	10·4
,, ...	Co.	10·6	,, ...	Sm.	10·6
,, ...	C.N. ...	10·4	,, ...	Jn.	10·1
,, ...	W.N. ...	10·1	Mucous colitis	O'B	10·4
,, ...	P.T.	10·6	,, ...	N.N. ...	10·4
,, ...	J.N. ...	10·6	,, ...	B.	10·6
,, ...	K.F.	10·4	,, ...	G.R. ...	10·1
,, ...	Mi.	10·7	,, ...	F.N. ...	10·1
,, ...	T.R.	10·0	,, ...	Bi.	11·1
,, ...	Bk.	10·4	Ulcerative colitis ...	Br.	10·6
,, ...	Cr.	10·6	Syphilis ...	A.W. ...	10·6
,, ...	Jn.	10·0	,, ...	W.	10·6
,, ...	Pe.	10·4	,, ...	N.	10·6
,, ...	Ds.	11·1	,, ...	R.	10·1
,, ...	Pt.	10·4	,, ...	Wr.	10·4
,, ...	Sy.	10·2	,, ...	B.	10·6
,, ...	E.	10·4	,, ...	C.H. ...	10·6

TABLE II—*continued*

Disease	Initials	Ionic Ca.	Disease	Initials	Ionic Ca.
		%			%
Syphilis ...	Na.	10·4	Malaria (M.T.)	El.	9·8
,, ...	A.	10·6	,, ,, ...	Ko.	10·2
Malaria (B.T.)	C.	10·4	,, ,, ...	Bo.	10·4
,, ,, ...	C.E.... ...	10·6	,, ,, ...	To.	9·4
,, ,, ...	H.*	9·5	,, ,, ...	La.	10·6
,, ,, ...	D.	9·9	,, ,, ...	H.B.... ...	9·6
,, ,, ...	B.	9·1	,, ,, ...	Dn.	10·2
,, ,, ...	M.	10·1	,, ,, ...	Mn. ...	9·4
,, ,, ...	Eg.	10·4			
,, ,, ...	Bl.	10·4	Kala azar ...	Mo.... ...	10·4
,, ,, ...	Om. ...	10·4			
,, ,, ...	Cr.	9·9	Trypanosomiasis	W.	10·1
,, ,, ...	Ch.	10·6	General Paralysis (treated by malaria)	Ma.	9·9
,, (M.T.)	Wa.* ...	9·1			
,, ,, ...	B.R.... ...	9·9			
,, ,, ...	W.	9·8	Filariasis ...	W.	10·1
,, ,, ...	B.O.... ...	9·9	,, (?) ...	B.	10·6
,, ,, ...	S.	10·4			
,, ,, ...	C.	9·5	Beriberi ...	C.	9·9
,, ,, ...	A.	10·1	,, ...	F.C.... ...	10·4
,, ,, ...	Pr.	9·2	,, ...	C.L.... ...	10·4
,, ,, ...	Cl.	9·1	Jaundice and diarrhoea	D.	10·6
,, ,, ...	Ch.	8·9	,, ...	S.	10·2
,, ,, ...	Hd.	9·7	Undulant fever	B.	10·7
,, ,, ...	F.C.... ...	9·7	Tuberculosis ...	P.	10·4
,, ,, ...	K.	9·7	Endocarditis ...	R.	10·4
,, ,, ...	R.	9·1	Tapeworm ...	S.	10·6
,, ,, ...	L.	10·1	Ankylostomiasis	L.	10·2
,, ,, ...	T.	9·1	Ascariasis, &c....	F.	10·6

* These had Syphilis also

B.T. = *Pl. vivax* infection.

M.T. = *Pl. falciparum* infection.

Table I is a list of cases of sprue whose blood was examined on their first coming to hospital or within a week or so, that is, before sufficient time had elapsed for any treatment to have had an appreciable effect on the calcium content.

From this it will be seen that the total calcium is but very little reduced, whereas the ionic calcium is between 20 and 30 per cent. below the normal.

Table II gives the amount of ionic calcium, as before in mgms. per 100 c.c. in the serum of patients other than sprue, many of them with diarrhoeic symptoms. Others have also been included as a matter of interest. It will be seen that those conditions most likely to be confounded with sprue, namely, the dysenteries and forms of colitis, are all about the normal limit as regards the ionic calcium. It is worthy of note also that the only common tropical affection in which there is a fairly consistent reduction is that of malaria, and in the one case of general paralysis of the insane which was being treated by malaria.* But in none of the malarial patients whose blood was examined was the reduction of the ionic calcium anything like so great as that found in sprue. This, moreover, is not of much importance in practice, for it would only be in those not very common cases in which diarrhoea was associated with malaria as a prominent symptom that the question of diagnosis would arise at all.

It is clear, therefore, that the estimation of the ionic calcium is a very useful factor in diagnosis of sprue from other diarrhoeal conditions, and, since accurate diagnosis forms the basis of rational treatment, a test which will establish the diagnosis of an obscure disease such as is sprue from others with sprue-like symptoms becomes of considerable medical importance.

Passing to the second part of this paper—the value of the estimation of the ionic calcium of the serum in gauging the progress of disease in Sprue.

Important as the test is in diagnosis, it is vastly more important and more useful as an indication of progress. If all is going well and the serum is examined at intervals of a fortnight, or, better still, a week, the percentage of ionic calcium is found to rise steadily to

* This is of interest seeing that cases of chronic malaria have been recorded of late in which marked improvement followed the administration of parathyroid.

the normal. Any return of symptoms, such, for example, as a sore or tender tongue, or the reappearance of a few aphthae in the mouth, is accompanied by a drop in the ionic, not the total, calcium. Such may arise from an attempt to increase diet too rapidly or unduly hurry the convalescence, and may be, usually is, a warning of a relapse, and, if regarded as a mere ' upset from indigestion ' and the warning allowed to pass unheeded, a relapse will certainly take place, necessitating the loss of several days, perhaps weeks, in the cure. If, however, the calcium content is determined and is found to have dropped, a return to milk for a couple of days or so will usually suffice to restore the balance and progress will then continue. If, on the other hand, it is found to have maintained its level, the symptom is of no practical importance and the fuller diet need not be curtailed.

To avoid repetition this aspect of the question may be dealt with briefly under the following headings :—

1. Ionic calcium is always low in the untreated disease, even in the early stages.
2. The ionic calcium increases as the condition improves.
3. The ionic calcium falls again if a relapse occurs.
4. Improvement takes place when calcium is administered alone, but is slower and less stable than when parathyroid is given in addition.
5. Improvement, evidenced by the clinical condition and, more accurately, by the rise in the ionic calcium, is more steady and more rapid when parathyroid is given.

1. This first point need not be further elaborated. It is abundantly proved by what has gone before in showing the value of the test in diagnosis, and the table (Table I) affords many examples.

2. The ionic calcium increases as the patient's condition improves. The following is an illustrative case.

J.T., aged 35 years, had suffered from sprue for eight months. His condition had been diagnosed, first as amoebic dysentery and, later, as colitis, but treatment for these had been unavailing. The symptoms were typical—sore mouth and tongue, with ulceration, flatulence, acidity, large, pale, frothy stools, cramps and loss of weight. At commencement of treatment the ionic calcium was low, 6·8 mgm. per cent. ; three weeks later there was no longer any soreness of the mouth, the stools were less frothy and bulky, and the dyspepsia was less. The ionic calcium was now 7·9 per cent. ; the total had remained about normal, 10·4 per cent. A fortnight later there was great general improvement ; the patient felt

stronger, no longer suffered from lassitude or depression, had only one action of the bowels daily, and that was normal, was on a fairly generous diet and was getting up for three or four hours each day. The ionic calcium was now 8·8 per cent.; in yet another fortnight he 'felt well,' and the calcium was 9·5 per cent., and a week later 10·4, the same as the total calcium. He left the nursing home, taking full diet, and went to Scotland, where he played golf and, in fact, lived a normal life, and ceased to take any medicine. Two months later he wrote to say that he was keeping 'quite fit,' and four months afterwards he was in London and called to show himself. He looked the picture of health, and opportunity was taken to test his blood again. It had more than maintained the previous level, being now at the upper limit of the normal, namely 11·1 per cent. This patient has returned to the tropics.

3. The ionic calcium falls again below the former level if a relapse occurs.

F.W.W., 31 years; duration of typical sprue symptoms six months. When the blood was first examined, after three weeks' treatment, the ionic calcium was 8·1 and the total 10·6 per cent. He made excellent progress and the ionic calcium was found to be 10·6 per cent. three and a half weeks later. He was then allowed full diet and to do more or less as he liked, getting up and going about. In fact, he tried to go along too quickly. In two weeks the mouth began to feel sore, and the stools were a little more bulky and pale. He was, in short, starting to relapse. Another examination of his blood was made and it was found that though the total calcium had remained at 10·6, the ionic had fallen again to 8·5 per cent. The diet had to be reduced and treatment begun again. In another month he was well and able to go out, and there has been no report of any recurrence of symptoms.

This point is important enough to warrant the recording of a second case, which was more severe than the last.

A.C., female, 51 years, had suffered for two years or more from sprue, with frequent relapses. All the typical symptoms were present, the mouth symptoms—soreness, tenderness and ulceration of tongue and buccal mucous membrane—being very pronounced. The ionic calcium before she started treatment was as low as 6·9 mgm. per cent.; three weeks later all the symptoms were much improved. She felt stronger and was getting up for an hour or two daily, and the ionic calcium had increased to 8·1 per cent. In another fortnight it had reached normal, 10·6, and a fortnight later still had increased to what is regarded as the upper limit of the normal, 11·6 per cent. Four weeks later the tongue began to feel tender, two small aphthae appeared, and she began to pass paler motions. The blood was sent up and it was found that the ionic calcium had fallen again to the lower limit of the normal, 10·2 per cent. A return to milk for three days, with a resumption of the medicine, cleared up these symptoms, which, if disregarded, would, as on former occasions, most certainly have been the forerunners of a severe relapse.

4. The mode of treatment has a distinct effect upon the rate of progress, and this is best gauged by the ionic calcium estimation. The disease having been shown to be associated with a deficiency in this substance, treatment by calcium in some form leads to improvement of symptoms, though, if given alone (that is, without

parathyroid) this improvement is slow and not very stable. As an example of the former the following may be very briefly narrated :—

G., male ; a fairly severe case treated on the ordinary lines—diet (chiefly milk) and rest in bed, but without parathyroid. After ten weeks, the ionic calcium was only 7·1 per cent., though improvement had been steady but very gradual. Three weeks later it had risen to 8·3 per cent. only, and in another three weeks (i.e., after sixteen weeks' treatment) it was still below normal, namely 9·1 per cent., though the total was 10·6. Clinically speaking, he was nearly well, was up and about, but on a limited diet still, and shortly afterwards he left hospital. Unless he is very careful he is almost certain to relapse, and that probably before very long.

The following is an illustrative example of the instability of treatment by calcium alone.

H.B., 40 years. After twelve weeks in hospital this patient was up and about, though on a restricted diet, and was to all appearances well, and was on the point of leaving. Without any reason being discovered, and while he was on the restricted diet and under a cautious régime, he again lost weight and expressed himself as ' not feeling quite so well.' His blood was taken and the ionic calcium was found to have fallen to 9·5 per cent., though the total was normal, 10·6 per cent. The diet was therefore again reduced, and he was made to return to bed ; in three weeks the ionic calcium was again normal, 10·4, but he was not considered well enough to leave hospital for another two months.

These two cases are good instances for showing that it is not absorption of calcium which is defective, but the proper regulation of it after it has been absorbed.

5. Lastly, the improvement, as evidenced both by the clinical condition and by the rise in the ionic calcium of the serum, is more rapid when, in addition to the administration of the calcium, parathyroid is also given to regulate its metabolism. Previous papers (see References) afford many examples of this, but to render the present paper more complete the following may be briefly narrated.

(1) A. McG., male, 28 years of age. Ill for ten months with the typical symptoms of sprue. He had lost 28 lbs., in weight, had a sore and tender tongue and mouth, and was passing frothy, pale and rather bulky stools. When admitted to hospital his ionic calcium was down to 6·1 mgm. per cent. He was given calcium in the form of milk and also cachets of the lactate, gr. 15, thrice daily, and parathyroid, gr. 1/10 of the dried extract, twice a day. He reacted wonderfully well. In a week the ionic calcium was 8·1, and in a fortnight 10·1 per cent., the total being close upon normal, 9·9 per cent. on the first two occasions, and 10·1 on the third. He remained in hospital for another five and a half weeks, steadily maintaining his improvement, and left after a stay of less than eight weeks. The ionic calcium when he went out was 10·8 per cent.

(2) S., male, 48 years. This was a more severe case and one of longer standing, having existed for over two and a half years. When admitted to hospital he was 36 lbs. below his normal weight. He was at once given the same treatment as the patient whose case has just been described—calcium lactate gr. 15, three times, and extract parathyroid gr. 1/10, twice daily. The symptoms steadily and rapidly improved and he left hospital thirty-one days after admission, feeling and looking very well.

After one week's treatment the ionic calcium was 7·0 per cent.; after two weeks 7·4; after three weeks 10·8, and it had remained there when he left. More than six months later he came to show himself. He had taken no medicine during that period and had kept perfectly well, and had not had to restrict his diet or smoking in any way. He was a heavy smoker and the soreness of the mouth, by depriving him of this solace, caused him great distress. His blood was taken to see if the calcium content had been maintained, and it was found that his own parathyroids were carrying on their work satisfactorily, and that absorption of calcium was very good. The ionic and the total were the same, 11·1 per cent.

(3) A.B.W., female, 34 years. This case, though not of so long duration, only twelve months, was exceptionally severe. At times she had as many as fifteen copious, loose, frothy, pale stools in the twenty-four hours; her tongue was very sore and ulcerated; she had troublesome cramps, and had lost 42 lbs. in two months. She then started calcium lactate and parathyroid, but both of them in too small doses; of the former gr. 5 thrice daily, of the latter gr. 1/20 twice. When seen two months later, on her arrival from abroad, the tongue and mouth were very sore, red, and tender, and she was obviously ill. The stools, however, had been reduced to four daily. The ionic calcium was at this time 7·3 per cent. She was at once put on an increased dose of calcium, namely gr. 15 of the lactate three times a day, and the dose of the parathyroid was doubled. The change was remarkable; within fourteen days the number of stools was reduced to one daily, no longer frothy, and much less bulky. In another week she had to take liquid paraffin to overcome constipation, and was progressing so well that she was able to sit up for some hours each day. The serum calcium (ionic) had increased to 9·5 per cent.; in another fortnight she was going for walks, was taking a diet comprising milk and milk puddings, bread and butter, eggs, fish, chicken and fruit. The stools were normal in size and colour, and only one in the twenty-four hours. The ionic calcium was now 10·1 per cent. Two weeks later, that is seven weeks after I first saw her, she came to London from Bristol, looking well, and taking all food without discomfort. Another blood examination showed a normal calcium content, 10·6 per cent. It was considered that the food now contained abundant calcium, and, since absorption had not been upset, the total having been practically normal throughout, this element was omitted from the medicine. The dose of parathyroid was reduced in order to test whether her own glands were now capable of carrying on their function and instructions were given that, if no untoward symptoms arose, it, in turn, was to be stopped altogether after another week. This programme was carried out and three weeks later she again travelled up from Bristol, this time to visit the Wembley Exhibition. She had had no treatment of any kind during the previous fortnight, but the calcium content of the blood was fully maintained, being now 11·1 per cent.

I saw her finally four weeks afterwards; she looked the picture of health, stated that she felt better than she had done for years and full of energy, that she was going about all day, eating anything put before her; in fact, living a normal life, and was arranging to return to India.

A last examination of the blood was made and gave ionic calcium 11·1 per cent., no residual, coagulative, combined calcium. This patient wrote five months later to say that she was in perfect health, eating heartily without any restrictions, had more than maintained her weight, was doing hard work (as a missionary) and 'felt full of energy.'

These facts are demonstrated in the accompanying Tables III and IV.

TABLE III

Sprue Cases under Ordinary Treatment
Showing the Gradual Rise in the Ionic Calcium

Initials	Before treatment	Ionic Calcium in mgms. per 100 c.c. serum. Number of weeks after starting treatment											
		1	2	3	4	6	8	10	12	14	16	18	20
F. ...	...	7·7	...	...	9·1	...	...	...	...	...	...	...	...
L. ...	...	...	...	6·9	...	...	9·1	...	...	10·1	...	...	...
Ln. ...	6·6	...	...	...	...	9·9	...	...	...	...	...	...	...
H.W.B.	...	...	...	...	...	...	...	...	9·3	...	10·4	...	...
G. ...	...	...	...	...	...	...	7·1	...	...	8·3	...	...	9·1
L.O. ...	...	6·3	...	...	...	...	...	9·9	...	...	...	...	...

TABLE IV

Sprue Cases Treated by Parathyroid in Addition
Showing the more rapid Return of the Ionic Calcium to Normal

Initials	Before treatment	Ionic Calcium in mgms. per 100 c.c. serum Number of weeks after starting treatment								Remarks
		1	2	3	4	6	8	10	12	
J.T. ...	...	7·9	...	8·8	...	9·5	10·4	...	11·1	Still 11·1 when seen 4 months after ceasing to take any medicine.
M.C....	6·9	...	8·1	...	10·5	...	11·6	...	...	
A.W....	...	7·3	...	9·5	...	10·1	10·6	...	11·1	Maintained when seen 2 months after. Reported as 'perfectly well' 7 months after.
H.E. ...	...	7·3	...	...	...	...	...	10·6	...	
E.J.W.	...	...	8·0	...	8·9	10·4	...	10·8	...	11·0 when seen 3 months after ceasing medicine.
McG.	6·1	8·1	10·1	...	...	10·8	...	...	...	
H.S. ...	...	7·0	7·4	10·8	10·8	...	...	...	...	11·1 when seen 6½ months later.
W.E....	6·9	7·4	8·7	9·7	10·4	...	...	...	...	Living a busy, active life and 'feeling full of energy' 6 months later.
S. ...	6·6	...	7·7	...	9·7	10·4	...	...	...	Gone abroad; keeping well.
E. ...	...	...	7·1	...	10·3	...	11·1	...	...	

I desire to express my thanks to those who either sent or allowed me to take specimens of blood for these tests, especially Professor T. R. Elliott, F.R.S., Director of the Medical Unit, University College Hospital; Dr. G. C. Low and Dr. P. H. Manson-Bahr, Physicians to the Tropical Diseases Hospital; Dr. H. B. Newham, Pathologist to the Tropical Diseases Hospital; Colonel C. Barry, I.M.S. (ret.), and Dr. H. S. Stannus.

SUMMARY

1. Sprue is a disease which is constantly associated with a fall in the amount of ionic calcium in the serum, whereas the total remains at or about normal.

2. A rise and fall of the ionic calcium coincides with improvement and relapse.

3. Absorption of calcium is little, if at all, interfered with, but calcium metabolism is upset.

4. Consideration of this fact and of some of the other symptoms of sprue, especially the cramps and tetany in severe or advanced cases, points to interference with the function of the parathyroid glands.

5. Amelioration of symptoms, followed by cure, is obtained by oral administration of suitable salts of calcium and a pure and active preparation of parathyroid in adequate doses.

6. The period needed for cure by these means is much shorter than by previous methods, the main symptoms clearing up in some cases within a few days. The administration of the parathyroid must, however, be maintained to stabilise the amelioration, but no ill-effects have been found to occur if it be continued longer than is actually necessary to bring about this result.

7. In the light of the above it is interesting to note that most of the old, empiric remedies contain lime as an important constituent.

REFERENCES

Bastedo, Walter A., and Famulener, L. W. (1923). Tropical Sprue. A Critical Study of Cases. *Jl. American Med. Assoc.*, Vol. LXXXI, No. 25, pp. 2102-2108.

Scott, H. Harold (1923). A New Theory as to the Causation of Sprue. *Trans. Roy. Soc. Trop. Med. & Hyg.*, Vol. XVI, No. 8, p.

——— (1923). The Treatment of Sprue. *Lancet*, Vol. II, p.

——— (1923). The Nature and Treatment of Sprue. *Brit. Med. Jl.*, Dec., and in *Proc. Roy. Soc. Med. Trop. Section.*

——— (1924). Recent Advances in the Treatment of Sprue. *Brit. Med. Jl.*, Vol. II, p. 305.

Vines, H. W. C. (1921). The Coagulation of the Blood. Part I. The Rôle of Calcium. *Jl. of Physiology*, Vol. LV, Nos. 1 and 2, p.

——— (1924). The Parathyroids in Relation to Disease. Arnold, London.

MALARIA PARASITES IN THE PLACENTAL BLOOD

BY

B. BLACKLOCK

AND

R. M. GORDON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication* 18 *December*, 1924)

During the months of July, August and September, 1924, the examination of twenty-six placentas for malaria parasites was carried out among native women in Freetown.

EXAMINATION OF THE PLACENTA

The placenta arrived at the Laboratory at variable periods after delivery, sometimes within an hour and almost always within twelve hours. An incision was made through a cauterized area on the placenta, blood was withdrawn from the bottom of the cut by means of a pipette, films were spread, and stained either with Leishman's or Giemsa's stain. Malaria parasites were found in twelve, i.e., 46 per cent of the twenty-six cases.

EXAMINATION OF MATERNAL PERIPHERAL BLOOD

The maternal peripheral blood was examined at the time of the birth in twenty-three of these cases, with the result that only four, i.e., 17 per cent. were found positive. No case of infection of the maternal peripheral blood was found in which the placental blood failed to show a much heavier infection.

Among the twenty-six cases there were four accidents (premature birth, etc.). In the twenty-two cases which had normal labour there were nine, i.e., 41 per cent., in which the placental blood showed parasites, whereas in the four cases which had abnormal labours there were three, i.e., 75 per cent., in which the placenta showed parasites.

EXAMINATION OF THE UMBILICAL CORD

The umbilical cord was cut and films made from the blood in the vessels at two places, one as near as possible to the placenta and one about six inches from the placenta. In none of the twenty-six cases was infection of the umbilical cord blood found, in spite of the fact that in all cases where the placenta was heavily infected several thick films were examined, in addition to thin films ; nor was infection found in the veins of the membranes.

EXAMINATION OF CHILD

Films of the peripheral blood of the new-born child were made in twenty-four of the twenty-six cases ; no infection was found.

In order to obtain information with regard to the possibility of congenital infection in the new-born child having been overlooked in the examination of cord and peripheral blood films, additional examinations were made.

1. In two cases of death of the child where the placenta of the mother was infected, an exhaustive search was made of smears of the heart blood, thymus, lungs, liver, spleen, bone marrow, and omentum with negative result.

   In two similar cases where liver puncture alone was permitted, the smears proved negative.
2. Repeated examination of children (born of mothers with placenta infected) was carried out up to a week after birth and proved negative ; thick as well as thin films being examined.
3. The blood of forty-one children aged one month or under was examined ; infection was found in only one case, a child between three and four weeks old. Such a case as this cannot, for reasons given below, be classified as congenital.

Pezopoulos and Cardamatis (1907) drew attention to the heavy infection which may occur in maternal blood in the placenta at a time when the peripheral blood has only a few parasites. The preponderance of schizogony forms in the placental blood was noted by these observers. Their examinations of the umbilical cord blood, the peripheral blood of the child and organ smears led

them to conclude that the malaria parasite does not pass through the placenta from the maternal into the foetal circulation.

Clark (1915) examined films of the placental blood in a series of 400 cases, taking the blood from the maternal aspect of the placenta after the removal of clots. By this method he found *P. falciparum* in nineteen, i.e., 4·7 per cent. of the cases. Compared with this his findings of parasites in the maternal peripheral blood of the same cases taken at the end of labour were positive in only eight, i.e., 2 per cent. of the cases. In all the cases where the peripheral blood was positive, the placental blood showed a much heavier infection. Among the 400 cases there were forty-four accidents (abortion, still-birth, premature labour). In the 356 cases which had normal labour there were twelve, i.e., 3·4 per cent. in which the placental blood contained parasites, whereas in the forty-four cases which had abnormal labour there were seven cases, i.e., 16 per cent. in which the placental blood contained parasites.

In addition to the placental and maternal peripheral blood, Clark examined the blood in the umbilical cord. The cord was carefully cleaned, cut across and films were made from the foetal blood. In only one case were parasites found in blood from the cord ; in this case the maternal peripheral and the placental bloods were both heavily infected, and the author considers it was due to the complication of an associated accident of pregnancy that the child had congenital infection.

CONGENITAL MALARIA

It is generally acknowledged that congenital malaria is a very rare condition. By congenital malaria is meant malaria which the unborn child acquires from the mother owing to failure of the barrier action of the placenta. The mechanism of the failure on the part of the placental barrier, i.e., whether it is due to the ability of exceptional parasites to penetrate through a healthy placenta, or to disease of, or accident to the placenta during pregnancy, does not here concern us.

The important point to decide is whether, in any given case, there is sufficient evidence to prove that it can only have been acquired owing to failure of this barrier action. Cases in which

parasites are found in the vessels of the umbilical cord at birth or in the peripheral blood or organs of the child at birth clearly belong to this category.

The more remote the period after birth at which parasites are found in the child the less justification have we for speaking of congenital malaria.

There are at least two definite and distinct ways in which a child not infected congenitally may become infected. The first way is by inoculation through abrasions of the skin in the process of delivery, where the maternal blood is mechanically inoculated into the child ; the second way is by the bites of infected mosquitos. Before any case of malaria can be established as congenital, it is clearly essential that the possibility of infection by either of these means should be absolutely excluded. This will be in many cases a difficult task, but the onus of proof is on those who claim cases as congenital. There are many cases standing in the literature to-day as cases of congenital malaria which are represented as congenital on altogether insufficient evidence.

It does not appear permissible to argue that the new-born child of a malarious mother is partially and temporarily tolerant and thus to explain the late development of symptoms and the late discovery of parasites in the child. There is little, if any, evidence to support this belief at present ; on the contrary, there does exist a small amount of definite evidence which tends to disprove it, namely, those cases in which parasites are present in the blood of the umbilical cord at birth, in the peripheral blood of the child at birth, and also some cases in which the parasites were found at the first examination of the child's blood a few days after birth. Such cases are all against the theory of partial immunity of the new-born child.

What is to be our criterion in judging whether a case is or is not congenital ? Our only available criterion is the minimum incubation period for the parasite, whether after inoculation of blood or after the bite of infected anophelines.

For example, in Yorke and Macfie's series of experimental infections with *Plasmodium vivax* by blood inoculation and mosquito bite infection, the shortest parasitic incubation found was after inoculation of infected blood, and was six days. We do not know whether, in the case of new-born children, this incubation period

might not be less. But accepting, for the present, six days as a minimum incubation period for *P. vivax*, it is not legitimate to assume to be congenital any case of infection with *P. vivax* in which parasites are first found more than six days after birth, unless other proof is supplied sufficient to show that the placental barrier has broken down.

When we consider the massive sporulating infections which the placental blood frequently shows (see Table I) and the absence of parasites in the cord and in the peripheral blood of the child, it appears certain that the walls of the villi are very efficient safeguards against the passage of merozoites even when these are present in enormous numbers. From the cases studied here, as well as from the literature dealing with congenital malaria, it appears that this condition is of great rarity.

THE PLACENTA AS AN INTERNAL ORGAN

In relapsing malaria, the parasites, in the intervals during which they cannot be discovered in the peripheral blood, are considered by most observers to be present in the internal organs. Of these, the spleen has always been the organ chiefly incriminated, the liver and bone marrow to a less extent have also been considered reservoirs. In the case of the spleen, however, it is known that even after removal of the organ, malaria may occur without re-infection. Obviously, in these cases, the spleen is not the only if even one of the chief reservoirs of the parasite. Acton, Knowles and Gupta (1923) punctured the spleen in fifteen cases and found splenic puncture to be ' a method of no diagnostic value in chronic malaria.'

In the present series an examination of the blood of the placenta has proved of striking value as a diagnostic of malaria. We recall that whereas examination of the peripheral blood revealed only 17 per cent. positive in twenty-three cases examined, the examination of the placenta revealed 46 per cent. positive in twenty-six cases.

NUMBERS AND STAGES OF PARASITE FOUND IN THE PLACENTA

In some of the blood films taken from infected placentas the number of parasites present is, as previously noted, quite remarkable. It might be approached rarely by the peripheral blood in fulminating cases of malaria, or more frequently by the capillary blood in

cerebral cases. We have never seen anything comparable to it in smears of spleen, liver, lung, kidney or bone marrow. Examination of the placenta at the time of child-birth, provided means of obtaining in women a malarial infection rate far higher than that obtained by even frequently repeated examinations of the peripheral blood.

The forms of parasites found in the placenta represent many stages not normally found in the peripheral blood. In all of the twelve placentas, parasites in the sporulating stage were present. In six cases sporulating forms were predominant, and in some of these, such forms constituted about 90 per cent. of all parasites found. In four of the other six cases there were about equal numbers of sporulating forms and young and medium-sized trophozoites. In two, the form of parasite present was almost exclusively the young trophozoite.

Double infection of cells was frequent, and in some instances two parasites in the same cell were sporulating at the same time.

COMPARISON OF INFECTION IN PERIPHERAL AND PLACENTAL BLOOD

The relative number of infected red blood corpuscles in the maternal peripheral and placental blood were determined in each of the four cases in which both the peripheral and placental blood contained parasites Ten thousand red corpuscles in the peripheral blood were counted and five hundred in the placental. The results are shown in Table I.

Table I.

Showing the ratio of the numbers of parasites in the peripheral to those in the placental blood, and the percentage of infected cells in each, and the ratio thereof.

	Peripheral blood	Placental blood	Percentage of red cells infected		Ratio
			Peripheral	Placental	
Case I	1	1292	0.05	65.0	1 : 1300
Case II	1	44	0.05	2.5	1 : 50
Case III	1	20	0.03	0.6	1 : 20
Case IV	1	1395	0.04	56.0	1 : 1400

CRESCENTS

It was notable that in none of the placenta infections were crescents seen, nor were they found in the peripheral blood where infected. When it is borne in mind that enormous numbers of parasites were examined in the placental blood in both thin and thick films, it seems reasonable to conclude that however favourable the conditions present in the placental blood may be for the development of asexual forms, they are, for some reason, unfavourable to the development of mature sexual forms.

That this absence of crescents was not a seasonal phenomenon affecting equally all cases is shown by the fact that, during the same period, crescents were present in 17 per cent. of ninety-six specimens of the peripheral blood examined in the children's clinic. The absence of crescents in the series of placentas examined here may be compared with the rarity of crescents recorded in the series of placentas examined by Clark.

ENUMERATION OF MEROZOITES PRODUCED BY PARASITES IN PLACENTAL BLOOD

Forms in which the process of sporulation was judged to be complete were chosen for counting; viz., those in which each merozoite was definitely separated from its neighbours. The maximum, minimum and average number of merozoites produced by the parasites of each case was thus determined. The highest count obtained was thirty-eight, but this was excluded owing to the possibility of its having a double infection of the red cell. The results are set out in Table II.

Table II.

Numbers of merozoites produced by parasites in different cases.

	Total number of sporulating forms counted	Maximum number of merozoites produced	Minimum number of merozoites produced	Averages
Case I	36	20	10	15
Case II	36	26	15	19
Case III	36	30	21	25
Case IV	36	33	20	26

It will be observed that Cases III and IV, as contrasted with Cases I and II, show a far higher average number of merozoites per parasite when division is apparently completed, and also that the maximum and minimum figures obtained in each of these two cases are at a higher level. Attempts were made to ascertain by measurement the size of the merozoites, but this proved unsatisfactory. Even where the largest numbers were produced, e.g., thirty-three merozoites, there was much variation in the size of individuals, and such merozoites appeared on the average to be equal in size to those occurring in parasites producing a much smaller number. It appears possible that certain varieties of *P. falciparum* produce a larger number of merozoites than others ; if this were so, it might have some bearing on the rapidity of the onset and the course of an attack.

Very little evidence is procurable as to the bio-chemical conditions prevailing in the placental blood ; while it is generally admitted that the blood of the placenta differs in some respects both from the maternal peripheral and foetal bloods, the differences do not appear to have been accurately determined.

Regarded purely from the standpoint of the suitability for the development of *P. falciparum*, the placental blood appears to afford conditions which are not paralleled in the maternal peripheral blood. These conditions appear extremely favourable for the asexual phase of development, but not favourable for the sexual phase. The placental blood fulfills the following conditions known to be necessary for the culture *in vitro* of *P. falciparum*.

1. Stagnation of blood.
2. Limitation of oxygen.
3. Presence of glucose.

Yoshida and Ko (1920) have shown that in all types of malaria the blood sugar is increased during the pyrexial period ; the maximum figure obtained was in infection with *P. falciparum*. Wells (1920) says glycogen is most abundant in the uterus at the time of childbirth and is abundant in the placenta.

It is interesting to recall the observation of Bass and Johns (1913) that the blood sugar of diabetics who have malaria renders the addition of dextrose to the culture medium unnecessary. It is perhaps relevant to remark that crescents have never been seen *in vitro* in media fulfilling the above-mentioned conditions.

SUMMARY

1. Of twenty-six placentas of native women examined in Sierra Leone twelve, i.e., 46 per cent., were infected with *P. falciparum*.

2. The infection in many of these placentas was massive.

3. Examination of the peripheral blood of twenty-three of these cases revealed only four, i.e., 17 per cent., infected.

4. In the placental blood, sporulating parasites were numerous and also young and half-grown forms.

5. Definite differences were observed in the number of merozoites produced by the parasites of different cases ; these differences may influence the rapidity of onset and the course of the disease.

6. Crescents were never found in the placental blood in any of the cases examined.

7. No case of congenital malaria was encountered.

8. Some evidence is produced which suggests that malaria infection of the mother predisposes to accidents during pregnancy or at birth.

REFERENCES

Acton, R., Knowles, H. W., Gupta, S. A. S., and Bihaj Mohan Das (1923). *Ind. Med. Gaz.*, Vol. LVIII, No. 5, pp. 211-213.

Bass, C. C., and Johns, F. M. (1913). *Amer. Jl. Trop. Dis. & Prev. Med.*, Vol. I, No. 3, pp. 246-9.

Clark, H. C. (1915). *Jl. Exper. Med.*, Vol. No. IV, p. 427.

Pezopoulos, N., and Cardamatis, J. (1907). *Centralb. f. Bakt.* 1. Abt. Orig. Vol. XLIII, p. 181.

Wells, H. G. (1920). *Chemical Pathology*, 4th Ed., p. 434.

Yorke, W., and Macfie, J. W. S. (1924). *Trans. Roy. Soc. Trop. Med. & Hyg.*, Vol. XVIII, Nos. 1 and 2, p. 13.

Yoshida, T., and Ko., K. (1920). *Jl. Formosa Med. Soc.*, Nos. 206-7, p. 70 (Ex. *Trop. Dis. Bull.*, Vol. XVI, p. 345).

A NOTE ON TWO VESICANT BEETLES BELONGING TO THE FAMILY *STAPHYLINIDAE*

BY

R. M. GORDON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication 22 December*, 1924)

PLATE I

The vesicant properties possessed by beetles belonging to the family CANTHARIDAE are well recognised in medical literature, but references to the lesions produced by members of the family STAPHYLINIDAE are less common ; it, therefore, appears of interest to record the relatively severe ulcerations which have been observed to follow contact with two members of the latter family.

PAEDERUS AMAZONICUS (Sharp)

This beetle was first encountered at Manáos, Amazonas, in January, 1921, and appeared to be common in this locality at all seasons of the year. It was most frequently seen close to the river banks and sometimes proved a positive pest on the river steamers. It is a small insect only eight to ten millimetres long, the head, wing-cases and last two segments of the abdomen are of a blue-black colour, the thorax, legs and remaining segments of the abdomen being of a bright orange. The insect was well known to the natives under the name of ' Poto ' (pronounced Potā) ; they stated that if it alighted on the bare skin it produced a blister which sometimes developed into a slowly healing ulcer, the most common site of the attack being the face and neck ; they believed that it sometimes produced permanent blindness in young children when it found its way into the eye.

The writer was not able to verify any such injury, but as *Paederus amazonicus* is a small, free-flying and intensely active insect it seems quite probable that it may sometimes alight on the conjunctiva and there produce similar lesions to those later described in the text as probably caused by *P. sabaeus*. Göldi (1913) draws attention to a similar species *Paederus goeldii* (Wasmann, 1905) taken by him on the Rio Purus, also known locally as 'Poto' and possessed of similar vesicant properties, while da Silva (1912) describes another member of the same genus *P. columbinus* as causing dermatitis in Bahia. The only reference to *P. amazonicus* that the writer has noted is that of Bequaert (1921) who mentions it amongst a list of vesicant STAPHYLINIDAE compiled from various parts of the world.

The irritant powers of *P. amazonicus* were frequently tested on two Europeans and the results may be summarised as follows. When the insect was allowed to wander freely over the bare arm it produced no reaction, either immediate or delayed; if, however, it was irritated, or rubbed against the skin, after an incubation period of eighteen to twenty-four hours, a series of bullae made their appearance; these usually coalesced to form a single blister which burst, leaving an intensely raw and tender area which did not heal for about ten to fourteen days; the most severe reaction always followed vigorous rubbing of the beetle against the skin. When one of these insects was gently compressed in a live-box and examined under a dissecting microscope, minute drops of fluid could be seen exuding from the labial orifice and drying with extreme rapidity on the glass; no fluid was observed to be extruded from the anus or leg joints.

PAEDERUS SABAEUS

During May and June, 1924, Dr. E. J. Wright, of Freetown, called the attention of the Laboratory to several cases of ulceration of the face and neck which he thought might be caused by some vesicant insect; during June of the same year the writer observed a beetle which appeared to resemble closely the Amazonian species already referred to and which has subsequently been identified as *Paederus sabaeus*.

Rodhain and Houssiau (1915) give a description of the lesions produced by a vesicant beetle of the genus *Paederus* in Léopoldville. Bequaert (1921) states that this insect is *P. sabaeus*. Ross (1916) records similar reactions from Nairobi, due in this instance to *P. cribipunctata*, and Eysell (1913) records the vesicant properties of *P. peregrinus* in Malaysia.

P. sabaeus—unlike the Amazonian species—appeared to be quite unfamiliar to the local inhabitants and none of them associated its handling with any subsequent ill effects ; Professor Blacklock took some specimens with him during a tour of the Protectorate and showed them to many of the natives, all of whom he informs me failed to recognise it. The insect appeared to be fairly common in Freetown during June, July and August ; it disappeared during September and October, but re-appeared in the middle of November. Most of the specimens were taken at night-time in the Laboratory where they appeared to be attracted to the artificial light ; usually as many as half-a-dozen beetles could be captured in the course of a single evening, whereas during the four months they were present in Freetown only three were taken in daylight. The periodicity of the insect is decidedly curious ; thus, though careful search was made, not a single specimen was captured during September, October, or the first half of November, yet on November 21st no less than twenty-four specimens were taken in a space of two hours round a single electric light ; the same locality the following night yielded only two of these beetles. The experimental results obtained with this beetle were very similar to those recorded with *P. amazonicus*, but of a slightly milder nature ; also the incubation period appeared to be longer, no trace of any reaction occurring for a full twenty-four hours and blisters not appearing till after the lapse of two days, the subsequent course of these blisters being similar to that already recorded for *P. amazonicus :* they leave a well-marked cicatrix, some of the scars being still clearly visible five months after the experiment. It has already been noted in the case of *P. amazonicus* that on compressing the insect, fluid (apparently of a volatile nature) could be observed exuding from the labial orifice ; on testing *P. sabaeus* in a like manner no such result was noted. By means of a razor one of the beetles was divided into three separate portions consisting of the head, thorax and abdomen ; each portion

was then rubbed into a different part of the forearm ; a well-marked reaction subsequently developed on the areas smeared with the thorax and abdomen, but none where the head had been applied. Göldi (1913) refers to an enteritis occurring in the Marshall Islands under the name ' Toddy-Krankheit,' which is supposed to be due to the swallowing of fluids into which some vesicant beetle has previously fallen ; in order to test the toxic nature of *P. sabaeus* in this respect one of the beetles was ground up in two c.cs. of tap water and the fluid injected down the oesophagus of an adult guinea-pig ; no results followed the injection, the animal remaining well and the stools formed.

Dr. Wright has recently brought to my notice an interesting case in which the lesions would appear to be due either to this insect, or else to some similar species possessed of equally strong vesicant powers. Mr. G. was motoring in Freetown and about seven in the evening was struck in the eye by some small object which he took to be an insect ; he rubbed it out of his eye and only suffered temporary inconvenience. The next day the eye was slightly inflamed and sore, the following day it was considerably worse and he consulted Dr. Wright, who was at once struck with its similarity to the cases he had previously observed on the face and neck ; at this time the eye was intensely inflamed and discharging freely ; a circle of inflammation and oedema extended all round the eye and involved the eyebrow and the cheek. On examining this latter area with a lens, numerous minute bullae could be seen precisely as in the case of the experimental lesions already referred to. The following morning—i.e., sixty hours after the injury—the blisters were greatly increased in size and a further crop had made their appearance on the left ear, which was swollen and tender ; these latter blisters were in just such a position as would be caused by a person brushing some inflammatory substance across the face from the eye to the ear.

I am indebted to Mr. K. G. Blair, of the British Natural History Museum, for the identification of both these insects.

Since the above was written the writer's attention has been drawn to an article by Strickland (1924) which gives an account of the vesicant properties of *P. fuscipes*, as studied in India. In both morphology and habits—as regards attraction to light, etc.—this insect appears to resemble closely *P. sabaeus*. The dermatitis and preceding incubation period being also similar.

REFERENCES

Béquaert, J. (1921). À propos des Staphylinides vésicants du Bas-Congo. *Ann. Soc. Belge de Méd. Trop.*, Brussels, Vol. I, No. 2, pp. 227-229.

Da Silva (1912). As quoted by Castellani and Chalmer's *Manual of Tropical Medicine*. p. 2204. 1919.

Eysell, A. (1913). Handbuch der Tropenkrankheiten, Vol. I, p. 247.

Göldi, E. A. (1913). Die Sanitarisch-Pathologische Bedeutung der Insekten.

Rodhain, J., and Houssiau, J. (1915). Dermatite vésiculeuse saisonnière produite par un coléoptère. *Bull. Soc. Path. Exot.*, Vol. VIII, No. 8, p. 587.

Ross, P. H. (1916). As quoted by Castellani and Chalmer's *Manual of Tropical Medicine*, p. 2204. 1919.

Strickland, C. (1924). On Spider-lick. A dermatozoosis. *Ind. Med. Gaz.*, Vol. LIX, No. 8, p. 385.

EXPLANATION OF PLATE I

Fig. 1. Showing dermatitis produced by *P. amazonicus*. Photo. taken twenty-four hours after infection.

Fig. 2. Showing dermatitis produced by *P. sabaeus*. Photo. taken three days after infection. The lesions shown at 1 and 3 were produced respectively by rubbing in the thorax and abdomen; no reaction followed the rubbing in of the head at 2.

Fig. 3. Showing dermatitis involving the eye and ear, probably caused by *P. sabaeus*. Photos. taken two and a half days after infection.

FIG. 1

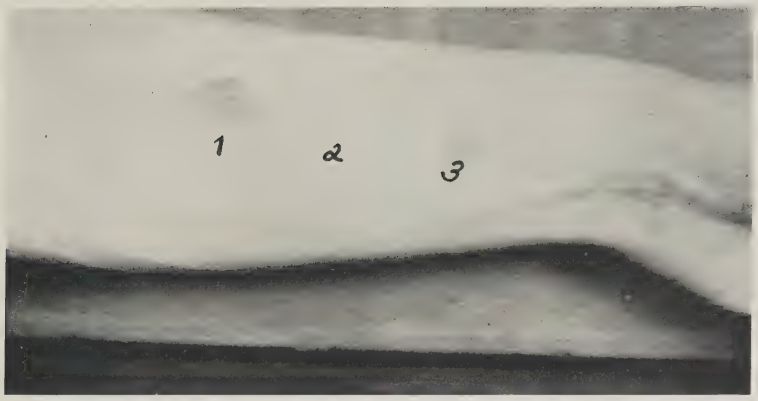


FIG. 2

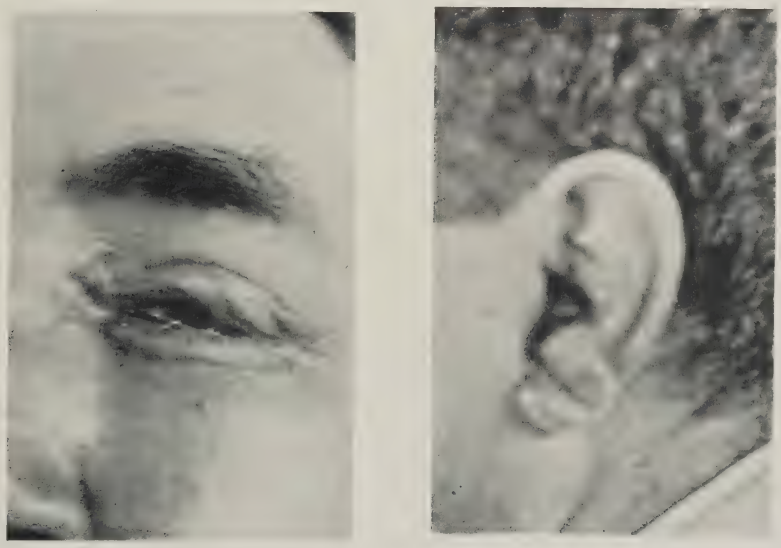


FIG. 3

C. Tinling & Co., Ltd., Imp.

THE GENUS *KILULUMA*

BY

E. L. TAYLOR

(*Received for publication* 24 *December*, 1924)

This genus was the subject of a recent paper by Thapar (1924), who divided it into six new species: unfortunately, he does not give any key to assist in placing a member of the genus in its proper species, nor does he give any list of differences of specific value, but only a detailed description of each type, in which he singles out but very few points considered by him to be of specific importance. A complete list of the measurements and morphological differences given by Thapar was, therefore, drawn up with the idea of preparing a key to be used in the classification of worms of this genus in the Museum of the Liverpool School of Tropical Medicine. On perusal of this table of differences there appeared to be small reason for the subdivision of the genus to such an extent, and the subsequent examination of the large amount of material at my disposal has brought me to the conclusion that the individual differences noted by Thapar are only sufficient to divide the genus into two, or possibly three species. The Museum of the Liverpool School of Tropical Medicine contains some eight hundred worms of the genus *Kiluluma*, collected in Rhodesia from five rhinoceroses; measurements were made from a number of worms picked at random; details of morphology noted in a still greater number, while general characters of the whole collection were also noted for the purposes of this paper.

Of the six species named by Thapar, I should consider the following four synonymous:—*K. rhinocerotis*, *K. africana*, *K. pachyderma* and *K. solitaria*, since in the same individual I have found varying combinations of the supposed specific differences. These supposed differences in morphological characters are very small. As an example, two definite points, in which the presence or absence of a character is involved, may be singled out, namely

the presence or absence of a second wing to the spicules, and of a small branch to the externo-dorsal ray. Although, according to Thapar, the presence of the branch to the ray should only coincide with a one-winged spicule (*K. pachyderma*), I have frequently found it to coincide in the same individual with a two-winged spicule.

Differences between these four species in the matter of the detailed measurements given by Thapar are also very small, and similar measurements made from material at hand have in no single instance fitted one of the four species to any marked degree more than the rest ; where measurements of one part of an individual might coincide with those of *K. rhinocerotis*, measurements of other parts might fit *K. pachyderma*, or *K. solitaria*, or *K. africana*. In my opinion, Thapar attaches too much importance to small differences in measurement : for example, in the text, attention is especially drawn to the larger spicule in *K. africana* as a difference from *K. rhinocerotis*, yet this difference is only between spicules 2·1 mm. and those 2·25 mm. in length, where the male of the first species measures 13 mm. and of the second 13 to 14 mm. in length.

Differences made on the position of the so-called ' filiform process of the lips ' and the narrow, or the swollen appearance of the anterior end of the ' lips ' do not seem to hold, since this internal leaf-crown appears to be pliable and liable to be fixed in varying positions. Although by far the greater number of worms examined by me showed the ' lips ' in the position seen in Thapar's drawings of *K. pachyderma* and *K. macdonaldi*, I came across several with ' lips ' approaching the shapes shown in the drawings of *K. africana* and *K. rhinocerotis*. I did not, however, come across any with ' lips ' in the positions seen in the drawing of *K. solitaria*.

The reasons for making the species *K. macdonaldi* do not seem to be much stronger than those for making the four other species mentioned above ; but two characters are described as not occurring in these four ; firstly, the cervical papillae are said to be anterior to the excretory pore ; and secondly, the preventral ray in the bursa of the male is stated to be moved forward to the position of a prebursal papilla. The first of these two differences does not seem to be of great importance, since in common with other species the papillae are at about the same level as the excretory pore. The second point may be of more importance, although I have come

across some remarkable variations from the normal in the arrangement of bursal rays ; two males actually showed asymmetrical lateral lobes, the postero-lateral and extra-lateral rays being present on the one side only.

The sixth species, *K. magna*, shows some outstanding differences, the most marked of which is in the much greater length of the oesophagus, the excretory pore and cervical papillae being on that account in the oesophageal region of the body : the general size of the worm is greater than in the five preceding species, the uterus is much larger and the eggs are double the size. I did not find any worm belonging to this species, but the differences given by Thapar clearly set it apart from the other five.

In my opinion, *K. rhinocerotis*, *K. africana*, *K. pachyderma* and *K. solitaria* are one and the same species to which *K. macdonaldi* may also belong, while *K. magna* only has distinctive specific characters.

REFERENCE

THAPAR, G. S. (1924). On *Kililuma* skrjabin, a genus of Strongylid nematodes parasitic in the African Rhinoceros. *Jl. of Helminthology*, Vol. II, pp. 209-238.

NOTES ON SOME NEMATODES IN THE MUSEUM OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE.—II

BY

E. L. TAYLOR

(*Received for publication* 28 *December*, 1924)

SPIRONOURA CONGOLENSE, n.sp.

Material :—Specimens collected in the Congo from a fish.

This worm presents all the characters typical of the genus with the exception of the arrangement of caudal papillae in the male, which appears to be somewhat variable in this species.

The body tapers towards the extremities in both sexes and is covered with exceedingly fine cross striations. The head (figs. 1 and 2) presents the usual globular outline and is followed by a well-marked neck. The male measures 13 to 17 mm. in length, by 0·45 to 0·72 mm. in greatest diameter; the head has a diameter of 0·183 to 0·21 mm., the small cervical papillae are placed 1·47 and

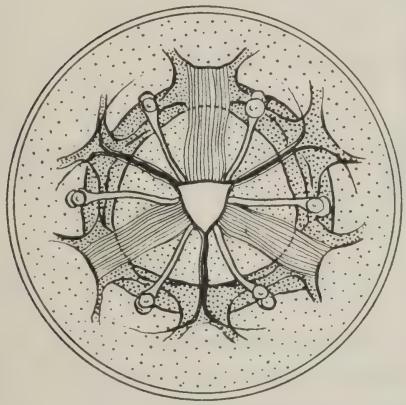


FIG. 1. *Spironoura congolense*, n.sp. Head, anterior view. × 250.

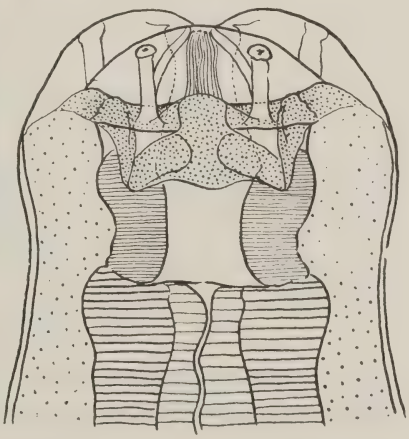


FIG. 2. *Spironoura congolense*, n.sp. Head, dorsal view. × 250.

1·72 mm. from the anterior extremity and the excretory pore 1·95 to 2·1 mm. from the same point. The pharynx joins the second part of the oesophagus at a point 0·1 to 0·116 mm. from the anterior extremity, from which point the oesophagus continues as a cylindrical muscular tube to the double bulb at its extremity ; the complete length of the oesophagus (fig. 3) is 2·85 to 3·00 mm. and the length

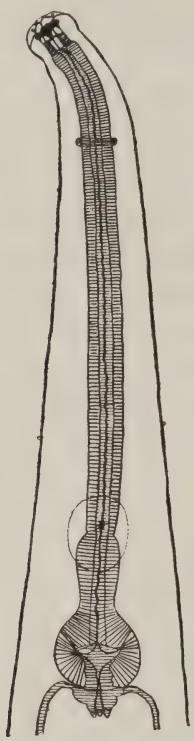


Fig. 3. *Spironoura congolense*, n.sp. Anterior extremity. × 37.

of the bulb 0·58 to 0·60 mm. ; the anterior narrow portion of the bulb has a maximum diameter of 0·18 mm. and the second portion of 0·36 mm. The nerve ring surrounds the second part of the oesophagus at a point 0·52 to 0·66 mm. from the anterior extremity. The caudal extremity (fig. 4) is ventrally curved and terminates in a sharp point ; the special caudal muscles are well developed and continued for a distance of about 3·3 mm. forward along the ventral aspect, but the fan-like formation of muscular fibres forming the pseudo-sucker, seen in some species, is entirely unrepresented. The

spicules are short, measuring only 0·51 to 0·6 mm. in length, and having a maximum diameter of 0·073 to 0·10 mm. The gubernaculum is a distinct and well chitinised organ. The preanal papillae number three pairs and are very small, the unpaired preanal papilla is present. The postanal papillae vary from seven to nine in number : of the four males present two showed eight postanal papillae on the right-hand side and seven on the left, one showed nine on the right and seven on the left, and the remaining specimen showed eight on either side. A further variation from the generic type is

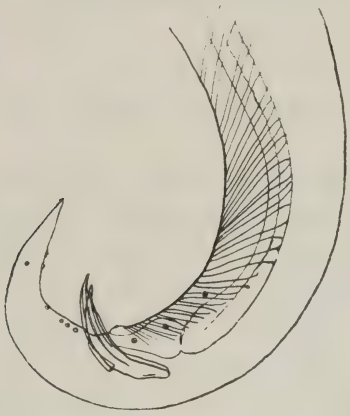


Fig. 4. *Spironoura congolense*, n.sp. Male. Caudal extremity. × 27.

seen in the absence in some specimens of the second lateral postanal papilla—Baylis (1922), points out the constant presence in this genus of at least two pairs of lateral papillae postanal—two of the four males examined had only one pair of these papillae as seen in the figure ; when the second pair was present it was placed half-way between the arc seen in the figure and the cloaca.

The female measures 13 to 22 mm. in length by 0·45 to 0·795 mm. at its greatest diameter ; the head is 0·2 to 0·232 mm. in width, the distance from the anterior extremity of the excretory pore is 1·95 to 2·55 mm. and to the cervical papillae 1·65 to 1·85 mm. The oesophagus has a complete length of 2·55 to 3·06 mm., the bulb measures 0·57 to 0·69 mm. in length and 0·3 to 0·36 in diameter at the second portion. The distance from the caudal extremity to the anus is 1·125 to 1·375 mm., the vulva is placed posterior to the middle of the body, being 5·25 to 10 mm. from the posterior extremity,

the vagina has a length of 0·6 to 1·2 mm. and is directed antero-dorsally ; it divides into the two divergent branches which describe the usual loops before reaching the ovaries. The eggs are large and filled with a granular mass when laid ; they measure 106 by 73 μ.

In general form and measurement this worm closely resembles *S. barbi* (Baylis, 1922), but differs in the arrangement of the papillae on the caudal extremity of the male, in the length of spicules and in the absence of the pseudo-sucker. *S. barbi* has spicules about twice the length of the spicules of this species, has a well-developed pseudo-sucker, and presents three preanal and seven postanal papillae.

HABRONEMA MAGNA, n.sp.

Material :—Two bottles of worms collected from the air-sacs of *Trachurus declivis*, there being twelve females and eight males in one bottle and seven females and five males in the other. A third bottle contained four damaged females collected from the sub-peritoneum of *Sparus* sp. The three lots were collected in Australia by Dr. P. A. Maplestone.

Compared with other species of the genus *Habronema* this worm is large, measuring up to 94 mm. in length and 1·2 mm. in width. The body is of a dirty yellowish-white colour and of a fairly even thickness throughout its length, tapering a little towards the two extremities. In either sex the cuticle in the anterior part of the body shows a rather coarse transverse striation, but posteriorly the markings are different in the two sexes as described below. Cuticular alae are bilateral in both sexes and in cross section are seen to be as thick as broad. The head may be described as having two large lateral lips and two smaller median lips continuous with the lateral lips by means of a cuticular fold (figs. 5, 6 and 7). Seen anteriorly the two lateral lips appear as triangular pieces base to base. The median lips are much smaller structures, and in viewing the head dorsally appear as thickenings in the level fold of cuticle joining the lateral lips : viewing the head laterally these median lips are seen to have considerable thickness, and an anterior view of the head shows them to project in a wedge-shaped manner into the space between the outer edges of the two lateral lips.

Gendre (1923) makes a general distinction between the type of head seen in species belonging to the genus *Habronema* from birds and from mammals. It is only a very general difference and cannot be strictly adhered to, but he points out that species parasitic in birds have large triangular lateral lips with a broad extremity and narrow base, and two median lips on a broad base, each composed of two lateral globular masses, with a median conical piece, and carrying the two papillae, on the contrary the type parasitic in mammals presents lateral lips of a more quadrangular shape, joined on either side merely by a cuticular fold in place of the two median lips. The type of head seen in the species here described may be regarded as intermediate between the two; the lateral lips show the

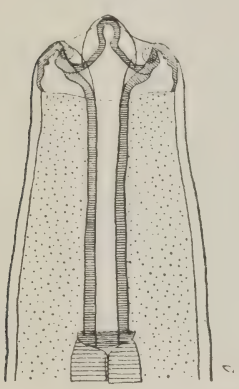


Fig. 5. *Habronema magna*, n.sp. Head, lateral view. × 125.

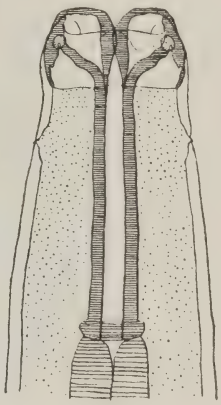


Fig. 6. *Habronema magna*, n.sp. Head, dorsal view. × 125.

triangular form of the avian type, while the median lips, although showing the wedge-shaped point are without the two lateral globular masses and are not separated by any dividing cleft from the lateral lips, in which two points the head resembles the mammalian type.

The whole head structure is strengthened with chitin which forms an outer subcuticular capsule as well as lining the mouth parts, where it is continuous with the thick chitinous wall of the pharynx. There are four, large, flat, submedian papillae, placed just below the margin of the cuticular folds between the lateral and median lips. The cervical papillae are small and situated far forward, one-third the distance down the pharyngeal portion of the

body. The pharynx is thick-walled, long and cylindrical in form. The oesophagus is long and divided into two portions ; the anterior, muscular portion is narrow and about one-third the length of the second part ; at a short distance from its anterior end it carries the nerve ring. After the junction of muscular and glandular portions the oesophagus rapidly widens to twice its former diameter and from this point it continues at an even width to its junction with the intestine.

The male measures 23·25 by 0·45 mm. to 25 by 0·6 mm. ; the head has a diameter of 0·15 to 0·17 mm., the pharynx has a diameter of 0·04 to 0·043 mm. and terminates a distance of 0·33 to 0·345 mm.

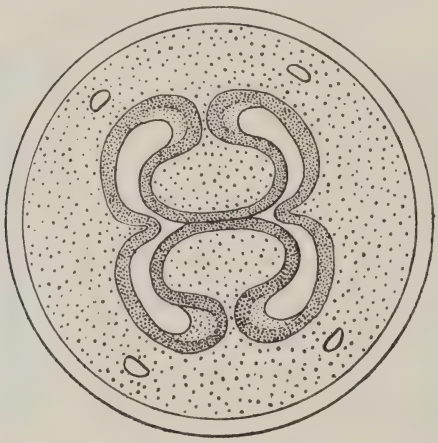


Fig. 7. *Habronema magna*, n.sp. Head, anterior view. × 250.

from the anterior extremity. Cervical papillae are situated at a distance of 0·133 mm. and the excretory pore 0·70 to 0·75 mm. from the same point. The length of the first part of the oesophagus is 1·5 mm. and of the second part 3·9 mm. ; the nerve ring surrounds the first portion at a distance of about 0·43 mm. from the anterior extremity. The distance between the cuticular striations increases from about 5·5μ at the anterior end to 25μ near the caudal extremity. These transverse striations are only continued to the caudal extremity on the dorsal side of the lateral alae ; the ventral aspect of the worm for the posterior 9 mm. of its length presents a series of parallel longitudinal folds in the cuticle, each about 30μ wide, these are

continued up to a point just anterior to the cloaca. The caudal extremity is spirally coiled and describes two or three complete turns. Towards the cloaca the lateral alae widen in each dimension, reaching a maximum width just in front of this orifice where they are broad and semi-cylindrical in shape ; posterior to this point they diminish in size to the extremity of the tail. The pedunculated papillae number eight pairs (figs. 8 and 9) ; there are four large pedunculated preanal pairs, the posterior three of which are in a line subventrally placed, while the anterior pair is more laterally placed, a little in advance of the second papilla. Posterior to the anus and a short

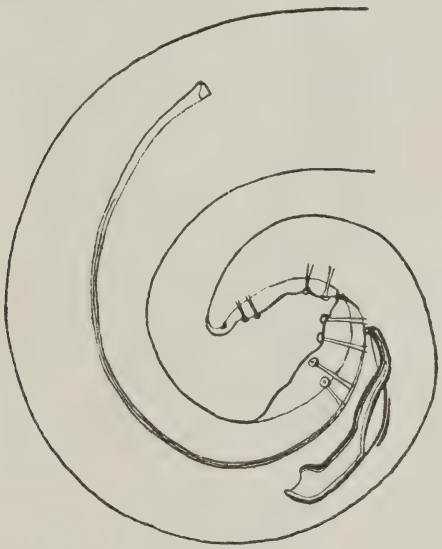


Fig. 8. *Habronema magna*, n.sp. Caudal extremity of male, lateral view. × 55.

distance behind, are two pairs of similar large pedunculated papillae sub-ventrally placed, while two much smaller pedunculated pairs occupy a subventral position nearer the extremity. Near to the extreme end and ventrally placed are two broad sessile papillae, each carrying three points. The spicules are very unequal in size, but differ from those of other species of *Habronema* in that the long, delicate spicule is placed on the right side of the worm, while the short one is the left spicule. The long spicule varies in length from 1·7 to 1·8 mm. and in average width of shaft from ·013 to ·023 mm., being broader at the proximal end and tapering to a very fine point at the

extremity ; the spicule appears to carry a lateral flange in its posterior three-quarters. The short left spicule is very short and of a peculiar shape ; the proximal end is in the form of a wide bulb and is bent ventrally ; this is followed by a stout cylindrical shaft which leads to a narrow portion, that describes a gradual dorsal curve followed by a decided ventral bend ; a short distance from the extremity it bends forwards to the side and terminates in two divergent points :

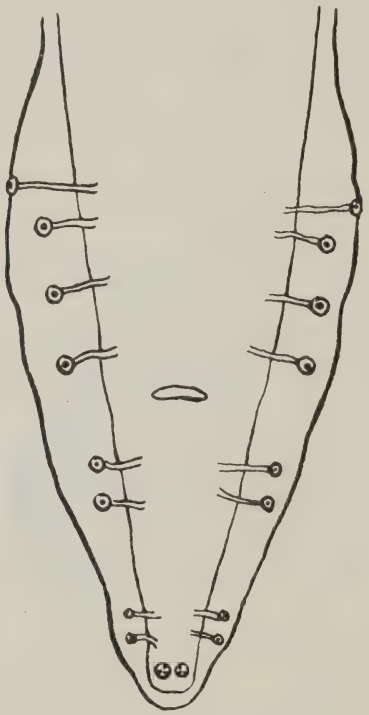


Fig. 9. *Habronema magna*, n.sp. Caudal extremity of male, ventral view. × 80.

there is also a keel-like piece which, commencing dorsally in the middle of the shaft, winds round the outer side to terminate ventrally near the extremity ; this spicule varies in length from 0·39 to 0·45 mm. and in greatest thickness from 0·053 to 0·063 mm. The gubernaculum is a small, well-defined piece about 0·093 mm. long and lies immediately behind the shaft of the short spicule. The tail measures 0·27 to 0·31 mm. from cloaca to extremity.

The female measures 19 by 0·30 to 94 by 1·2 mm. The diameter of the head is 0·13 to 0·285 mm., the cervical papillae are placed at

0·1 to 0·166 mm. from the anterior extremity and the excretory pore is 0·52 to 1·05 mm. from the same point. The cuticle in the anterior part of the body is striated at intervals of about 4μ near the head, increasing to intervals of 22μ just anterior to the vulva; here the narrow grooves which cross the raised portion between the striations are more in evidence than they are near the head and the raised portion is seen to be composed of numerous more or less oblong shaped elements; posterior to the vagina these elements rapidly increase in size and near the caudal extremity the striations are seen to be at a distance of 99μ apart, rather irregular in appearance and the raised intermediate portion composed of projecting pieces of varying shape and size, roughly twice as long as broad. The lateral alae are stout structures commencing about 0·75 mm. from the head in a mature female and projecting at their maximum width a distance of 0·076 mm.; they are almost as thick as they are broad and have a rounded, striated edge; they are prolonged to the caudal extremity, where they gradually diminish in width, and present a rather broken outline. The pharynx has a diameter of 0·025 and 0·066 mm. and terminates a distance of 0·226 to 0·45 mm. from the anterior extremity. The first part of the oesophagus measures 1·005 to 2·4 mm. in length and the second part 3·3 to 7·5 mm. The vulva (fig. 10) is placed ventrally about the junction of the anterior and middle third of the body length, being 8·5 to 23·5 mm. from the anterior extremity; it is surrounded by a prominent muscular ring which in a small female measured 0·18 mm. deep and 0·28 mm. in diameter. In the gravid female the anterior and posterior parts of this ring meet one another to form two prominent muscular lips. The vagina opens on the inner side of the anterior lip, from which place it may be seen to take an immediate turn backwards. After leaving this muscular ring, the vagina is continued backward as a long, straight muscular tube 0·04 to 0·07 mm. in diameter and up to 22 mm. long; at its extremity it divides into the two divergent branches of the uterus. In one immature female the vagina was found to run back a distance of 0·9 mm., then bend forwards to a point 1·5 mm. in front of the vulva, then double back again for a distance of 0·75 mm. where it divided. The caudal extremity of the female is a short, blunt cone and is usually bent dorsally; the anus is about 0·1 to 0·21 mm. from the extremity.

The eggs are about 37 by 23μ in size, thick shelled and have a small 'button' arrangement at either end, from each of which proceed two very delicate flagella each of about the same length as the egg. The contents of the egg are segmented when laid.

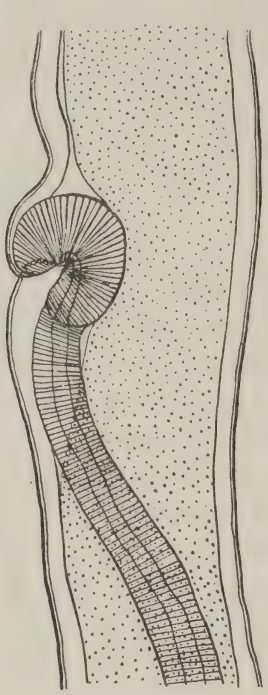


Fig. 10. *Habronema magna*, n.sp. Genital opening of female. × 190.

CONTRACAECUM CLAVATUM (Rud., 1809)

Material :—Seven bottles of worms from *Gadus morrhua*, two from *G. merlangus*, one from *Solea* sp., one from *Raja* sp. and one from *Belone belone*.

Most of the bottles contained a large number of worms in a good state of preservation.

Two clearly related species of *Contracaecum* have been described in these hosts. *Contracaecum clavatum* (Rud., 1809) and *Contracaecum pedum* (Deslgch, 1824), the only difference apart from a slight difference in total length being in the length of spicules; the male *C. pedum* is described as 32 mm. long, with spicules 2·4 mm.

long ; and *C. clavatum* as 33 and 46 mm. long with spicules measuring 1·25 mm. This difference in spicule length is quite a considerable one and when worms were found in the material at hand showing this variation, it was at first thought that two species were present ; on making further examinations, however, and taking a large number of measurements of worms picked at random from various bottles, it was found that only one species was represented. Without any reference to size or relative maturity of worm in some forty specimens measured, the ratio of spicule length to total length showed an even variation between the extremes of $\frac{1}{9{\cdot}5}$ to $\frac{1}{27}$. In view of the absence of any further difference between the two species, it seems that the two worms are synonymous and refer to a species which shows a rather remarkable variability of spicule length, so that *C. pedum* (Deslgch, 1824), falls as a synonym of *C. clavatum* (Rud., 1809).

PROCAMALLANUS LAEVICONCHUS (Wedl, 1862)

Material :—Four females collected from a silurid fish in the Congo.

These specimens conformed in every way to Baylis's (1923) description, but as there do not seem to be in existence any clear drawings of the rather peculiar mouth capsule, it has been thought advisable to produce some from the well-preserved specimens available (figs. 11 and 12).

The mouth parts may be described as follows :—The buccal capsule is deep and of a dark brown colour and has a particularly thick wall at the bottom where it joins the oesophagus. At the anterior opening of this capsule are six inwardly curving plates directed forward ; these plates have rounded extremities and are adjacent towards their free ends, but at their bases are separated by spaces in the chitinous wall of the capsule ; the spaces are both broader and deeper between the two subventral and between the two subdorsal plates, so that although the mouth is not actually in the form of a dorso-ventral slit—which is the type met with in the family *Camallanidae*—it still has a bilateral symmetry which approaches the type. Anteriorly the mouth is bounded by an

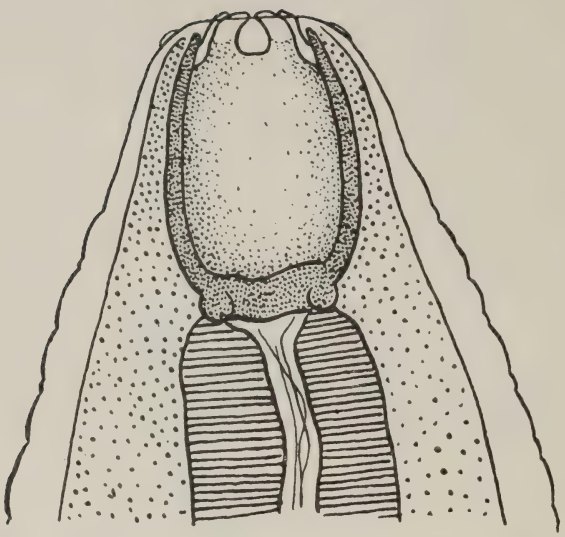


FIG. 11. *Procamallanus laeviconchus.* Head, dorsal view. × 500.

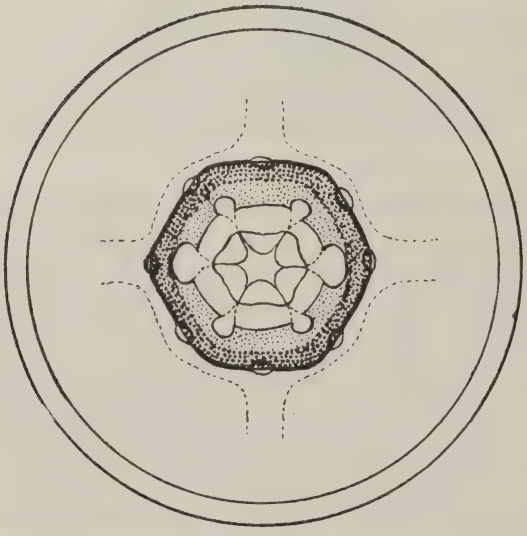


FIG. 12. *Procamallanus laeviconchus.* Anterior view. × 500.

external membrane which is merely a continuation of the integuments of the body ; the orifice formed is somewhat hexagonal, corresponding with the hexagonal shape of the buccal capsule. Eight head papillae are present, one dorsal, one ventral, two subdorsal, two subventral and two lateral.

ECHINOCEPHALUS SOUTHWELLI, Baylis, 1920

Material :—Male and female specimens collected from *Urogymnus* sp. in Ceylon.

ECHINOCEPHALUS SPINOSISSIMUS (v. Linstow, 1905)

Material :—Male and female specimens collected from *Trygon sephen*. Pearl Banks, Ceylon.

PROLEPTUS OBTUSUS, Duj., 1845

Material :—Numerous specimens collected from *Acanthias vulgaris, Scyllium caniculum* in Ceylon, and *Coronilla scillicola* in South Africa.

REFERENCES

Baylis, H. A. (1923). Report on a Collection of Parasitic Nematodes. Mainly from Egypt. Part III. *Parasitology*, Vol. XV, pp. 26 and 27.

Baylis, H. A., and Daubney, R. (1922). *Memoirs of the Indian Museum*, Vol. VII, No. 4, pp. 305 and 309.

Baylis, H. A., and Lane, Clayton (1920). A Revision of the Nematode Family *Gnathostomidae*. *Proc. Zool. Soc., Lond.*, pp. 277-289.

Dujardin, M. F. (1845). Histoire Naturelle des Helminthes, pp. 105, 208, 209, 211 and 212.

Gendre, E. (1923). Sur quelques espèces d'*Habronema* parasites des Oiseaux. *Procés-Verbaux de la Société Linnéenne de Bordeaux*, pp. 4 and 5.

ON THE GENUS *TETRACAMPOS*, WEDL, 1861

BY

T. SOUTHWELL

(*Received for publication* 17 *January*, 1925)

Genus *Tetracampos*, Wedl, 1861.

SYNONYMS :—*Ophryocotyle*, Southwell, 1913.
Gangesia, Woodland, 1924.

In 1861, Wedl described a cestode from a fresh-water fish under the name *Tetracampos ciliotheca* ; but he gave no definition of the new genus which he erected. Braun (1900) described the characters of the genus as follows :—

Head with four bothridia. Rostellum in the form of a cupola. On this rostellum there are four groups of nine hooks. The hooks are of unequal length, slightly curved, ending in a claw ; the longest hook is in the middle, the shortest hooks are at the sides of each group. Neck of average length ; four excretory canals to each segment. Genital pores on the flat sides. Egg thin shelled, containing a ciliated onchosphere.'

Type species :—*Tetracampos ciliotheca*, Wedl, 1861, from *Heterobranchus anguillaris*.

The following is an abstract of Wedl's description of the worm :—

Tetracampos ciliotheca (fig. 1).

Specimens of the above were found in the mucus from the intestine of *Heterobranchus anguillaris* (from the Nile) immediately below the stomach. They are delicate, thread-like worms 10 mm. to 15 mm. in length. The button-like head measures 0·2 mm. in breadth and is of a remarkable structure, recalling, by virtue of its ' lobes ' that of *Tetrabothrium*. Each ' lobe ' consists of parenchyma and is thin-walled and contractile, projecting as a flat disc. Anteriorly these lobes (bothridia of van Beneden) approach one another and encircle (surround) a projecting cupola-shaped armed papilla. The hooks, which may be differentiated into a long stalk with a short, slightly curved, pointed, sickle-shaped process or continuation, form four groups and are not arranged in circles or rows as in species of the genus *Taenia*. Each group generally consists of nine hooks, with the longest hook in the middle, and the shortest hooks at the outside of the group. A line drawn through the points of the hooks would describe an arc.

For a short distance behind the head, the segments are delicate, transparent, rounded off laterally, and connected to one another by well-developed longitudinal muscles. Two pairs of parallel vessels with transverse anastomosing branches run through the segments, and, in the head region, divide up into a complex network.

The last segment is cone-shaped (strobiliform) and possesses a distinct so-called ' porus excretorius.'

The genital pores are situated on the middle of the flat surface of each sexually mature segment. The eggs enclosing the hexacanth embryo are peculiar. Eggs were taken from the last segments and kept under observation, and it was noticed, after the external egg-skin had burst open, that the internal covering was furnished with comparatively long cilia, which were in rapid movement; these produced not only a rotatory but also a forward movement.

(Wedl at first doubted this phenomenon, but after observing many eggs, satisfied himself that the embryophore was ciliated.)

The anatomy of the worm was not described, but the essential features of the species are the presence of an armed rostellum and the fact that the embryophore is ciliated. It is impossible to decide from Wedl's figure and descriptions whether the so-called ' bothridia ' are really outgrowths from the head, or whether they are true acetabula.

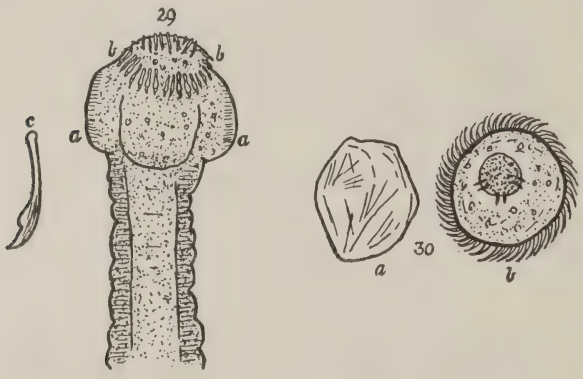


Fig. 1. *Tetracampos ciliotheca*, after Wedl. Magnification unknown.
(29) Head. *a.a.*—bothridia; *b.b.*—the four groups of hooks; *c.*—an isolated hook;
(30) *a.*—external egg-shell; *b.*—internal ciliated egg-shell with embryo.

Wedl states that the genital pores are situated on the flat surface of the worm (i.e., ventrally), but it appears probable that the sexual apertures are situated laterally, and that the apertures to which he refers are secondary pores, caused by the dehiscence of the gravid uterus.

It is to be noticed that the worm was obtained from the intestine of a fresh-water cat-fish (*Heterobranchus anguillaris*). The adult

cestode parasites most common in fresh-water fishes belong to the genus *Proteocephalus* (Weinland, 1858) La Rue, 1914.

La Rue (1914) ascribed the following characters to the family PROTEOCEPHALIDAE :—

'Heads small. Suckers sessile and without accessory areola. Fifth sucker functional, vestigial, or lacking. No rostellum. Genital organs as in other Tetraphyllideans. Genital pores marginal, irregularly alternating. Vitellaria lateral, follicular, follicles closely grouped about a central conducting tubule. Ovary bilobed, posterior. Oöcapt, oötype, shell gland, uterine passage present. Uterus with lateral outpocketings and one or more preformed, ventral, uterine openings. Vitellaria, testes, ovary and uterus *within* the inner longitudinal muscle sheath.

'Habitat.—In fresh-water fish, amphibia, and aquatic reptiles.'

La Rue defined the genus *Proteocephalus* as follows :—

'With the characters of the family.

'Head globose or conical, flattened dorso-ventrally. No rostellum. No spines or hooks. No fold of tissue encircling base of head or enfolding suckers. Suckers circular or oval. Fifth sucker functional or vestigial, rarely lacking. Testes in a broad field between vitellaria. Parenchyma with close meshes. Musculature well developed. Eggs with three membranes. Habitat :—In fresh-water fish.'

It is clear that, owing to the presence of an armed rostellum, *Tetracampos ciliotheca* cannot be referred to the genus *Proteocephalus* as defined above, but, as other authors have since recorded similar worms with an armed rostellum and possessing an internal anatomy typical of the genus *Proteocephalus*, it is most probable that the internal anatomy of Wedl's specimen was also typical of the genus *Proteocephalus*.

As a result, it is necessary to emend the characters of the family so as to include within it a genus with an armed head.

The writer in 1913 described as follows a worm which should clearly be referred to Wedl's genus *Tetracampos* :—

Ophryocotyle bengalensis, Southwell, 1913 (fig. 2)

'Over sixty specimens of this worm were obtained from the intestine of *Ophiocephalus striatus*, and a few were also obtained from the intestine of *Labeo rohita*. Both fish were caught at Berhampur Court, Bengal, in a fresh-water tank. This genus of tapeworm usually occurs in birds, and considerable interest attaches to the presence of these adult forms in Teleosts. The average length of the worms was 7·5 mm. Greatest breadth (at posterior end), 0·8 mm. These latter segments were from four to five times broader than long. The head consists of four cup-shaped suckers, directed slightly forward. Anteriorly the head terminates in an umbrella-shaped protrusible rostral disc whose circumference is armed with a large number of hooks arranged in two rows. The exact number could not be determined,

as, in removing the parasites from the intestine of the fish, many of the hooks had been torn away. The exact number counted in three specimens is given in the following table :—

(i) One row of twenty-five hooks.
(ii) Two rows with a total of fifty-three hooks.
(iii) Two rows with a total of fifty-two hooks.

'The hooks appear to be all similar. They have broad bases and are sharply recurved in profile. Viewed end on, they appear elongated.

'The suckers are armed with exceedingly minute spines which appear to be limited to their anterior borders. The head measures about 0·5 mm. broad. The neck is fairly long, measuring 2·7 mm. Dots of black pigment are scattered about over the whole worm. The first proglottides are exceedingly shallow, and *all* proglottides are broader than long. The lateral margins are wrinkled in such a way that in young specimens the true strobilization can only be determined under a lens. The genital apertures are lateral and are almost all on one side.

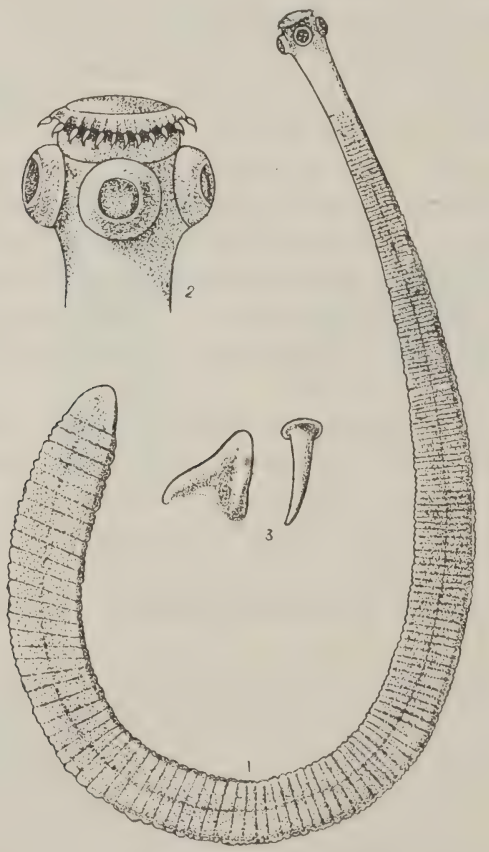


FIG. 2. *Tetracampos bengalensis* (Southwell) = *Gangesia wallago*, Woodland. (1) Entire worm. × about 30. (2) Head. × about 180. (3) Hooks greatly enlarged. After Southwell.

'The uterus appears to be made up of a number of rounded egg capsules scattered about the proglottid.

'Habitat :—The intestines of *Labeo rohita* and *Ophiocephalus striatus*, Berhampur Court, Bengal, June, 1912. About sixty specimens.

'Amongst the worms just described were two large specimens measuring 27 mm. and 22 mm. respectively. They differ from the smaller forms only in having the neck very much shorter and in being much larger. Two rows of about fifty hooks were counted round the circumference of the rostral disc.'

A re-examination of a single specimen of this species (the only one now in the writer's possession) has brought to light the fact that the internal anatomy of the worm is exactly similar to that found in species of the genus *Proteocephalus*.

The hooks on the head are all alike and are in a *single* crown as originally figured; they measure about 35 μ. The uterus is rudimentary and does not contain egg capsules as suggested in the above description.

Although the species is clearly to be referred to the genus *Tetracampos* it is undoubtedly different from *T. ciliotheca*; for, in *O. bengalensis*, the rostellum is armed with a crown of hooks, all of which are similar, whilst in *T. ciliotheca* the rostellum is armed with four groups of hooks which are not uniform in size.

Woodland (1924) has just described two species of cestodes, viz., *Gangesia wallago* and *G. macrones* from India, obtained from the intestines of *Wallago attu* and *Macrones seenghala* respectively, for which he erects a new genus with the following characters :—

'*Gangesia* :—with the characters of the family PROTEOCEPHALIDAE but emended to include forms with armed rostella. With a scolex possessing a globose muscular rostellum armed or unarmed, and no fifth sucker. The suckers may or may not bear spinelets. Testes in a single broad field between vitellaria. Eggs with three membranes. Habitat :—In fresh-water fish.'

Woodland described the two species as follows :—

G. wallago, Woodland, 1924 (fig. 3).

'Length of strobila usually not exceeding 40 mm., with a maximum breadth of about 1·5 mm. Proglottides numerous, well over 100 in number in mature forms, narrow antero-posteriorly in front but square or elongated posteriorly. Segmentation distinct. Scolex 0·166 to 0·232 mm. long and 0·298 to 0·448 mm. broad. Suckers with projecting edges, 0·120 to 0·172 mm. broad, and in part bearing numerous closely-set spinelets. The globular rostellum bears a single circle of hooklets, all of one kind, 29·28 to 43·92 microns long and twenty-eight to forty-two in number. A very short neck is present, but is only visible in specimens with the scolex torn off or in flattened specimens, and gradually increases in diameter up to the first traces of segmentation. Genitalia like those of *Proteocephalus*. Uterine diverticula twenty to twenty-eight in number. Testes over 100 in number,

65·8 to 109·8 microns in length and maximum breadth of 28 microns. Genital openings lie a little in front of the middle transverse line of the proglottid, and the cirrus sac and vaginal openings vary as to which is anterior. The uncontracted cirrus sac extends over about one-third of the distance across the proglottis. Eggs provided with three membranes, the outermost being 91·5 to 98·8 microns in diameter and the spherical embryo measuring 18·30 to 21·96 microns. Habitat :— intestine of *Wallago attu* Bleek (and probably *Ophiocephalus striatus* and *Labeo rohita*), rivers of India.'

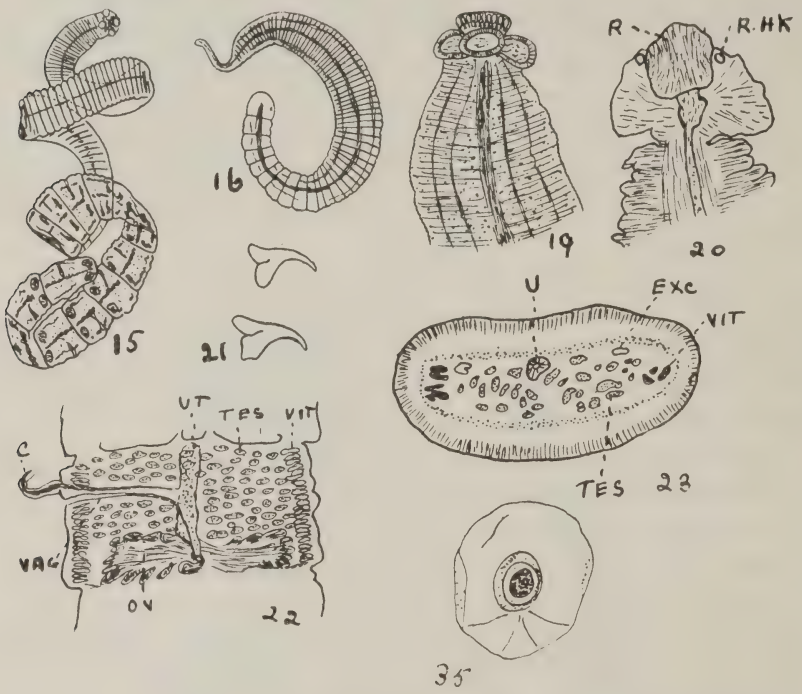


FIG. 3. *Tetracampos bengalensis* (Southwell, 1913)=*Gangesia wallago* (Woodland, 1924). (15) A mature worm (actually about 12 mm. long) with scolex. × 12. (16) A small worm (actually about 4 mm. long) with scolex torn off and showing the drawn-out short neck. × 12. (19) Scolex with rostellum and suckers protruded and a slight indication of the short contracted neck. × 39. (20) Longitudinal section through scolex to show the limits of the muscular rostellum. × 87·5. (21) Two hooks from the rostellum. × 260. (22) Flattened mature proglottid. × 27·5. (23) Transverse section through a mature proglottid just behind the level of the cirrus sac. × 56. (35) Egg (fully developed) from contents of the fish intestine. × 180. *r.*—rostellum ; *r.h.k.*—rostellar hooks ; *u.*—uterus ; *exc.*—excretory canal ; *vit.*—vitellaria ; *tes.*—testes ; *vag.*—vagina ; *ut.*—uterus ; *ov.*—ovary ; *c.*—cirrus. After Woodland.

G. macrones, Woodland, 1924 (fig. 4).

' Length of strobila does not exceed 60 mm. in length, with a maximum breadth of about 1·2 mm. Proglottides numerous, between 150 and 200 in number in mature worms, very narrow antero-posteriorly in front but square or elongated posteriorly. Segmentation distinct. Scolex measures about 109 microns long and 193 microns broad. Suckers small and thick-walled, about 67 microns broad

and bearing numerous closely-set spinelets on their upper edges and adjacent internal surfaces. The globular rostellum (about 109 microns in diameter) bears a single circle of hooks, of two kinds, large (11·0 to 14·6 microns long) and small (about 6 microns long) alternating. Neck absent. Genitalia like those of *Proteocephalus*. Uterine diverticula twenty to thirty in number. Testes over 100 in number. The genital apertures lie in front of the middle transverse line of the proglottid and usually the cirrus sac opening is anterior to the vaginal but the reverse condition also occurs. The uncontracted cirrus sac in flattened specimens extends over only from one-sixth to one-quarter of the breadth of the proglottis. Habitat :— Intestine of *Macrones seenghala* Sykes, from rivers of India.'

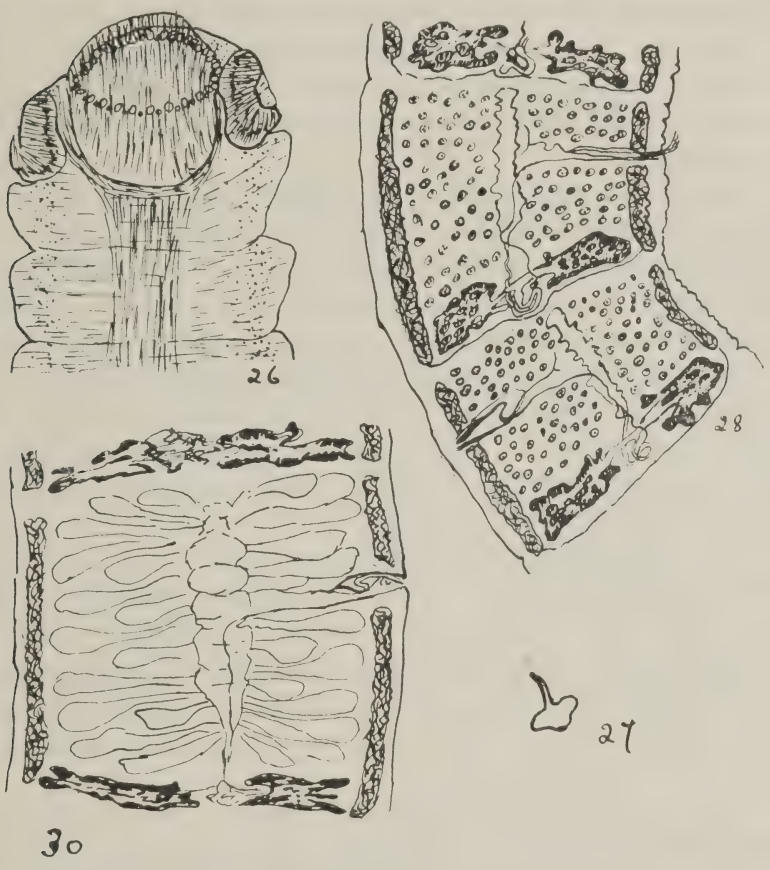


FIG. 4. *Tetracampos macrones* (Woodland, 1924)=*Gangesia macrones*, Woodland, 1924. (26) Scolex viewed in optical section. × 180. (27) Rostellar hook. × 530. (28) Mature flattened proglottids (both cirrus sacs have been drawn too long, judging from later measurements). × 17·5. (30) Sketch of a gravid proglottid with outlines of fully developed uterine diverticula (full of eggs in actual preparations). The central stem of the uterus is considerably flattened. × 17·5. After Woodland.

As all the preceding species possess an armed rostellum and are found in fresh-water cat-fish there can be no doubt that they are all to be referred to Wedl's genus *Tetracampos*, which, as Braun's description is inadequate, is now redefined as follows:—

PROTEOCEPHALIDAE. *Body segmented; head with four suckers, and armed with hooks. Internal anatomy as in the genus Proteocephalus, La Rue. Genital pores marginal and irregularly alternate. Adults parasitic in fresh-water fishes.*

Woodland proposed emending the characters of the Order *Tetraphyllidea*, Lühe, 1910, and of the Family PROTEOCEPHALIDAE, La Rue, 1914, in order to include the forms which possess an armed rostellum. Apparently it did not occur to him that cestodes whose heads are armed with four suckers probably belong to the Order *Cyclophyllidea*.

Prior to the appearance of Woodland's paper, the writer had already made an exhaustive study of the worms included in the Order *Tetraphyllidea* and had arrived at the conclusion that the order should be limited so as to include only species in which the head bears four bothridia (lappet-like outgrowths from the head).

The family PROTEOCEPHALIDAE, which possesses four suckers, or acetabula, falls naturally into the *Cyclophyllidea*, but differs from most other families of that order in having numerous vitelline glands situated laterally, instead of being condensed into a single mass in the vicinity of the ovary.

The *Cyclophyllidea* were accordingly split up by the writer into two sub-orders, viz.: (1) the *Univitellata*, comprising all those forms with four suckers, and in which the vitelline glands are condensed into a single mass; and (2) the *Multivitellata*, comprising all other forms with four suckers, in which the vitelline glands are either situated laterally or extend over the dorsal and ventral surface of the worm.

Wedl's genus *Tetracampos* is referred to the sub-order *Multivitellata*. As Braun, however, stated that the head was armed with four bothridia, the writer in his Monograph dealt with it under the Family ONCHOBOTHRIIDAE. The genus clearly belongs to the Family PROTEOCEPHALIDAE, La Rue, 1914, which is emended accordingly as follows:—

Body segmented; head small, bearing four suckers (acetabula) and either armed or unarmed. Fifth sucker functional, vestigial or

lacking. Genital pores marginal and irregularly alternate. Vitellaria lateral or extending over the dorsal and ventral surfaces ; Uterus with lateral outpocketings and with one, or more, preformed, ventral uterine openings. Habitat :—In fresh-water fish, amphibia and aquatic reptiles.

The genus *Tetracampos*, Wedl, 1861, is thus referred to the Family PROTEOCEPHALIDAE (La Rue, 1914) emended ; this family is placed in the sub-order *Multivitellata*, Southwell, 1925, of the Order *Cyclophyllidea*, Southwell, 1925.

The genus at present contains three species, viz. :—

(1) *Tetracampos ciliotheca*, Wedl, 1861.

(2) *Tetracampos bengalensis* (Southwell, 1913).

SYNONYMS :—*Ophryocotyle bengalensis*, Southwell, 1913.
Gangesia wallago, Woodland, 1924.

(3) *Tetracampos macrones* (Woodland, 1924).

SYNONYM :—*Gangesia macrones*, Woodland, 1924.

Woodland points out that the specimens of *O. bengalensis* Southwell, 1913, are ' almost certainly examples of *G. wallago*,' but

' I think Southwell's wholly insufficient description of his *Ophryocotyle bengalensis* justifies me in not adopting his specific name for my type species of *Gangesia*. Only an adequate statement of distinctive characters can justify claim to priority.'

As the description of *O. bengalensis* was sufficient to enable Woodland to state that his *G. wallago* is almost certainly the same, there is obviously no justification whatever for burdening the literature with other names. Under the ordinary rules of nomenclature, *Gangesia wallago* becomes a synonym of *Tetracampos bengalensis* (Southwell, 1913).

The writer is indebted to the Editor of *Parasitology* for permission to reproduce the descriptions and figures of *Gangesia wallago* and *G. macrones*.

REFERENCES

LA RUE, G. R. (1914). A Revision of the Cestode Family *Proteocephalidae*. *Illinois Biological Monographs*, Vol. I, Nos. 1 and 2. July-October.

SOUTHWELL, T. (1913). Notes from the Bengal Fisheries Laboratory. Indian Museum. No. 1. *Rec. Ind. Mus.*, Vol. IX, Pt. II, pp. 79-103.

WEDL, K. (1861). Zur Helminthenfauna Aegyptens. *Sitzgsb. d. K. Acad. d. Wiss. Wien.* (Math.-nat. Cl.), Vol. XLIV, I Abth., pp. 225-240, 463-482.

WEINLAND, D. F. (1858). Human cestoides., 93 pp., 12 figs. Cambridge, Mass.

WOODLAND, W. N. F. (1924). On a new *Bothriocephalus* and a new genus of *Proteocephalidae* from Indian fresh-water fishes. *Parasitology*, Vol. XVI, No. 4, pp. 441-451.

ON THE BIONOMICS OF *HIPPOBOSCA EQUINA*

BY

J. ISGAER ROBERTS, B.Sc. (Wales)

(*Laboratory of Agricultural Zoology—Department of Agriculture—The University, Bangor*)

(*Received for publication 27 January*, 1925)

Distribution in Wales

Hippobosca equina is very localised in its habits, being more abundant on the Continent than in Great Britain; records of infestation of horses and cattle are found in most European countries, and also from Palestine, where it is extremely common on horses and dogs during spring and summer (Buxton, 1924). In Britain, the New Forest in Hampshire and certain of the sheltered valleys of South Carnarvonshire and North Merionethshire in North Wales, appear to be the only known areas where the fly is abundantly found.

H. equina occurs in but a few localised areas in North Wales, and chiefly in the secluded and protected valleys of the southern spur of the Snowdonian mountains. On making an investigation of most of these valleys and taking notes of the approximate numbers found, a striking disparity was remarked in the prevalence even in neighbouring valleys. The reason for this limited distribution has been one of the chief objects of the investigation. The fly is distributed as follows:—in the valley which runs from Portmadoc to Beddgelert it occurs, but not very commonly, and also in the smaller lateral valleys. Somewhat further to the West lies the deep winding valley of the river Dwyfawr, known as the Pennant Valley; here the fly is abundant. This valley is chiefly given over to sheep farming, but on the lower lands a number of cattle are kept, and owing to the habit of milking the cows out of doors in summer, the cattle were more readily approachable and the work of the observation thus rendered easier. Most of the field work, therefore, was carried

out at this valley, and chiefly about the little village of Llanfihangel-y-Pennant, which is conveniently situated near the main road leading from Tremadoc to Garn Dolbenmaen, and above which the valley runs for a distance of five miles into the hills. Another smaller valley running almost parallel to the Pennant, and known as Cwm Ystradlyn, has also supplied data. All these valleys lie in the main north-east to south-west direction. The upper reaches are steep ascents merging into mountain, while the lower portions are wooded. The valley bottoms are wide and level and well suited to the typical hill farming of cattle and sheep rearing. There is a mean annual rainfall of 45 to 50 inches.

The village of Beddgelert and district is commonly supposed to be the real habitat of *H. equina*, and the insect is locally known as the Beddgelert Fly, but it has not been found so abundantly there as in the Pennant valley. *H. equina* is also found on the northern slopes of the Snowdonian mountains, in the valley running up from Llanberis, about a small village known as Nant Ucha, an important route for stage coaches in the past, but now almost exclusively used for motor traffic. Many of the older inhabitants speak of the way horses used to be worried and terrified, but its occurrence there is now rare. The fly is also known to occur about Bala and Llanuwchllyn in North Merionethshire.

From an historical point of view it is rather disappointing not to be able to record what is felt would have been interesting reading, dealing with the molestation of coach horses of which frequent mention was heard ; but the information gleaned seems unreliable, and the passing of time has in no way helped to enhance its value. Suffice it to say that the replacing of horse by motor traffic has caused the fly to become of less economic importance and the old coach roads are particularly free from the pest, which is now concentrated about the cattle lands.

Factors Controlling Occurrence

A succession of wet summers and the distance of the valleys named from Bangor has added considerably to the task of observing the natural habits of *H. equina*. The nights are invariably cold, with heavy dews. When the sun shines the flies are to be abundantly obtained from both horses and cattle, but during cold or rainy days

very few are to be noticed. The entrance to the Pennant Valley is well wooded and cattle grazing in these parts are not so much infested as those higher up the valley, where there are fewer trees. The trees are mostly Ash and Oak, giving way to a dense covering of Bracken (*Pteris aquilina*) which dominates the western side up to where the valley ends ; this side is also steeper than the eastern, which has a more gradual rise and better pasture. Animals grazing on the western side were found to harbour a greater number of flies than those of the opposite side.

This relative prevalence of *H. equina* in one localised area was most fortunate, greatly facilitating the work of comparison and analysis of factors which were supposed to be conducive and favourable to its existence. The geological formation of the area covered is chiefly Cambrian, with its associated shales and slates, this same rock formation being dominant throughout the range. The presence of trees seems in no way necessary to the activities of the flies, and the number obtained was smaller when cattle were sheltering in their cool shade ; there was, also, not much difference in the facies of either side of the valley in their lower and wooded regions. Climatic conditions claimed greater attention, it being well known that *H. equina* is more active during sunshine than at other periods. The western side receives the sun's rays earlier, and thus has a greater period of warmth daily than the opposite slopes which remain cold up to mid-day—this factor may well be of importance. But the most striking difference lies in the abundance of bracken on the western and its comparative scarcity on the eastern slopes. Several farmers had previously suggested an association between *H. equina* and bracken, and acting upon this information a close observation of the habits of the fly has revealed an association which is the main factor for localisation, namely a dependence upon the presence of bracken. During the day *H. equina* are only occasionally found settling on the fronds sunning themselves, but at sunset, or when a spell of cold weather or rain is imminent, they generally leave the cattle and settle on the undersides of the fronds, such a position offering shelter and protection during the night. The main association, however, appears to be during the period of pupation, which will be discussed later.

Hosts

The chief hosts of *H. equina* are horses and cattle, though in their absence the flies are stated to attack other domestic animals, or even man (Neveu-Lemaire, 1912). They usually occupy a position safe from disturbance by the host, generally clustering together under the tail of cows, along the perinaeum, and occurring even as low as between the thighs and on the udder. The skin at these places is thinner than at other parts and without a dense hairy covering, but, the inaccessibility of the parts chosen demonstrate the necessity for freedom from molestation during long spells of feeding. When disturbed, *H. equina* scatter in all directions and exhibit their marked capacity of varied and rapid movements. Many try to conceal themselves in the positions indicated, and only resort to flight as a final alternative. Animals which have been reared in these valleys are so accustomed to the presence of the fly that little or no resentment is shown at their presence. In the country investigated *H. equina* has only been observed on cattle and horses ; but a very interesting report has been received from one of the chief sheep owners who states that he has observed the pest on dogs. Young sheep dogs (not having completed their training) are stated to have been attacked in each of three cases noted ; the older dogs are described as running through the bracken with their heads held high, whilst these young dogs, through keeping their heads too near the ground were attacked. The infested dogs, holding their heads to one side, make efforts to rid themselves of the pest with their paws, the insects being eventually found on the inner surface of the pinna of the ear. This evidence is borne out by the statements of both Neveu-Lemaire and Buxton (*op. cit.*) in their records of the observation of *H. equina* on dogs. A closely related species *H. capensis*, v. Olf. (*H. canina*, Rond), is always found in large numbers on the head and neck of pariah dogs in the near East (see Buxton, *op. cit.*). Unfortunately the writer is unable to confirm these statements from personal observation.

In most of the districts, cattle are sent out to pasture in early summer, and are not brought indoors again until the cold weather sets in. The majority of the cattle kept in the hilly districts are Welsh Blacks or crosses of that breed, and tables of occurrence of the

pest were kept to ascertain whether the lighter coloured animals were more liable to infestation. Little difference was found in the aggregate, and the following data from a typical farm show the colour of cattle, and the number of *H. equina* found. This indicated that both light and dark coloured cattle are similarly infested. These figures were obtained on August 2nd, six cattle yielding the following : Black, 17 flies ; Black, 9 ; Black, 4 ; Blue Grey, 15 ; Roan, 10 ; Blue Grey, 4 ; giving a rather low average of 9·8.

Mode of Dispersal

Residents of the infested areas state that *H. equina* does not migrate of its own accord, but is distributed by host animals, and all evidence points to this view being correct. The mountain ranges are almost impassable barriers to insects that seldom fly more than a few yards, and unless strong winds carry them (which is very improbable) they are entirely dependent for their distribution on the movement of cattle and horses. When cattle are driven or taken away from their pasture, some of the flies present upon them may adhere and accompany them for considerable distances. This is well known to the local farmers. One of the chief reasons for the failure of the fly to extend its range, appears to be the fact that such flies are practically invariably caught and destroyed. The presence of one fly is sufficient to terrify animals not inured to it, the cattle racing wildly to be rid of it. Stallions from the infested areas, travelling the countryside, have been known to introduce the fly to new districts, but owing to the scrupulous care taken in destroying the pest, and the unsuitability of new environment, they soon die off. The following instance was brought to the notice of the writer. A farm outside the infested area was visited by a stallion from the Pennant Valley, carrying with it some of the flies. This led to trouble, mares becoming excited and restless, until every fly had been destroyed. Another instance of the annoyance caused by their presence was given by a blacksmith who had been shoeing a horse from an infested area, and which had left this some time previously. When a horse from a non-infested area was brought in, a fly that had left its host and was present in the smithy, rendered the shoeing impossible until the insect was detected and destroyed.

COLLECTING, ETC.

H. equina is easily caught by hand, there being little risk of damaging the flies by this method owing to the tough and leathery consistence of the integument ; another method is to place a wide-mouthed bottle over a cluster. A forceps net eventually proved most successful for collecting them in numbers. Great difficulty was experienced in attempting to keep the fly in the live state for laboratory experiments, the failure of which necessitated relying entirely upon field observation. When the flies were transferred direct to breeding cages, they died within 48 hours, a few only surviving that period. All the gravid females (distinguished by their swollen abdomens) were placed in separate breeding cages, but clustering together deposited their larvae prematurely. When a single gravid female is placed in a tube there is the same premature deposition, within less than an hour in many cases. A final trial was made by arranging a layer of peat covered with young bracken, and transferring gravid females direct from the host into the cage containing it. The deposition in this instance was delayed and the larvae obtained turned black in colour but no imagines were bred out. Massonnat and Vinet (1913) also complain of the great difficulty that attended their efforts to produce adults.

These unsuccessful attempts at rearing left but one course open for the observation of the deposition of larvae and their development—a prolonged investigation in the field during periods of suitable weather. Well advanced gravid females were caught and a little cotton wool fixed to the underside of the abdomen with a drop of gum. This enabled a close watch to be kept on the activities of those marked, and to follow their movements when they left the cattle. Here may be noted the great advantage obtained in having one or more cows which will not heed the presence of the observer. After marking a few females in the morning it was found necessary to remain with the cattle for the greater part of the day.

HABITS

For the greater part of the day *H. equina* rarely leave the host animal, but during cold or wet weather they are often found on the undersides of the bracken fronds. Occasionally they have been noticed sunning themselves either on the bracken fronds or on the

slabs which abound in the valley, but their activities are chiefly confined to blood sucking. Ormerod (1900) states that *H. equina* feeds on 'the perspiration given off by cattle during the period of their activity in the summer months' besides blood sucking. The nature of the mouth parts, with their narrow piercing stylet curving downwards and forwards and terminated by a distinct cutting apparatus, would seem to leave no doubts as to the nature of the chief food supply. The length of the proboscis is of some importance, that of *H. equina* being about the longest met with during an examination of the mouth parts of other Hippoboscidae; this is, no doubt, a necessity for the successful penetration of the hides of horses and cattle. It is still a matter of opinion whether the flexibility of the proboscis and its sweeping of surfaces allow the admissibility of Miss Ormerod's assumption, and moreover it is a very difficult matter to prove. In the writer's opinion the act of sweeping the surface is thought to be for the locating of a suitable spot for puncturing; and the length is an essential adaptation for reaching the blood-vessels.

Flight

The wings of *H. equina* are well developed, and it is a strong flier, but rarely makes flights of longer duration than is necessary to reach the bracken.

Numbers

The number of flies seen on any one animal varies considerably; as many as thirty may be obtained in some cases, though generally they range from ten to twenty. They are to be found in their greatest numbers on the part immediately below the genitalia and are only occasionally met with in the inguinal region, on the udder and the perinaeum.

The first appearance of *H. equina* is variable, being dependent to a great extent on weather conditions; they have been known to appear as early as April, but their usual time is May. The height of infestation is towards the middle of August and early September, when new individuals are appearing; there is then a sharp falling-off in numbers during the latter end of September, although a few persist into October.

Proportion of Sexes and Copulation

There is no great disparity in the proportion of sexes, the females being in a very slight majority. Copulation has always been observed to take place on the host animals; the male, without any preliminaries, grasps the female and remains in this position for but a short time.

Breeding Habits

The gravid females are readily distinguishable by their distended abdomen. When the larva is mature the females leave the host, but it is a matter of great difficulty to observe the act of deposition, which takes place among the organic débris that collects at the base of the stems of bracken (*Pteris aquilina*). After leaving the host the females settle on a frond of bracken and, dropping to earth, choose a situation in the decaying humus where the larva is deposited. The writer has observed this on five separate occasions all during early August. The larva is partially buried in the humus as soon as extruded. It is of a globular shape and creamy white in colour at extrusion, with a black cap and two conical projections at that pole. It is incapable of any individual movement and has little or no trace of segmentation. It pupates after the passing of a few hours, the larval integument simply becoming chitinised to form the pupal casing, whilst a gradual darkening of the integument takes place until the puparium is black. It is not essential for the larva to be placed in suitable surroundings for pupation as this will take place, in many instances, in a glass tube or other receptacle. In addition to the five cases of actual larval deposition in decaying humus noted above, the writer discovered twelve pupae among organic débris beneath the bracken. Further, two pupae have been discovered on pasture land (not far from bracken) lying in crevices of twisted roots of grass. It is believed that this is an unusual occurrence. The nature of the decaying humus beneath the bracken lends support to the assumption that this is the normal habitat of the pupae, as nearly all observed have been found here, and the actual deposition of larvae has been observed to occur here. In this position there is shelter from heavy rains, while moisture drips down from the fronds. The sun does not penetrate strongly,

and a continuous moisture is assured. The decay of the humus possibly supplies a certain amount of warmth during decomposition.

Climatic conditions may play an important part in determining the duration of the pupal instar, for during periods of hot weather newly hatched individuals were noted on the cattle. This is possibly due to a shortening in the duration of the instar owing to the warmth. The numbers are always greatest on cattle during such times.

In no case has the writer succeeded in hatching out any imagines under artificial conditions, despite a number of attempts, using breeding cages and varying temperatures and conditions.

SUMMARY

1. *Hippobosca equina* has a limited distribution in North Wales, and is restricted to certain valleys in South Carnarvonshire and Merionethshire. Its distribution seems to be governed by two factors :—(1) Presence of bracken, on which depends successful pupation, (2) The amount of sunshine available.

2. The chief hosts of *H. equina* are horses and cattle, although evidence is available to show that it attacks dogs.

3. Extension of range seems to be kept in check by a policy of destruction when stock are removed from its haunts or when an individual is noted in a new district.

4. As an economic pest it is a source of annoyance in terrifying animals not accustomed to its presence, and may then give rise to grave consequences. This factor appears of less importance since the diminution of horse transport. It now occurs almost entirely on cattle and confines its activities to blood sucking.

5. It was found impossible to keep the fly alive and conduct breeding experiments under laboratory conditions.

6. The fly is generally found from May to August.

7. Copulation takes place on the host animals.

8. From observation it seems probable that the decaying humus beneath growing bracken is the normal habitat for deposition of larvae.

ACKNOWLEDGMENTS

It gives the writer much pleasure to acknowledge his indebtedness to Dr. C. L. Walton, Adviser in Agricultural Zoology, University College of North Wales, Bangor, at whose suggestion these observations were carried out, for most willing and helpful guidance and advice. Also, to Professor P. J. White, M.B., F.R.S.E., Department of Zoology, for his stimulating interest and suggestions during the conduct of the work.

REFERENCES

*Brumpt, E. (1922). Précis de Parasitologie. Paris.

Buxton, P. A. (1924). 'Applied Entomology of Palestine.' *Bull. of Entomological Research*, Vol. XIV, p. 289.

*Massonnat, E. (1909). 'Contributions à l'Étude des Pupipares.' Fascicule 28.

Massonnat, E., and Vinet, C. (1913). 'Ethologie et Pupation chez les Diptères Pupipares et les Oestrides.' *C. R. Soc. de Biol.*, Paris, Vol. LXXV, pp. 49-51.

Neveu-Lemaire (1912). Parasitologie des Animaux Domestiques.

Ormerod (1900). Flies injurious to Stock.

* Not quoted in the Paper.

REPORT ON THE INVESTIGATION INTO THE DESTRUCTION OF VERMIN BY HYDROGEN CYANIDE, WITH ESPECIAL REFERENCE TO BED BUGS

BY

R. NEWSTEAD, F.R.S.

ALWEN M. EVANS, M.Sc.

AND

W. H. POTTS, B.A.

(*Received for publication* 28 *January*, 1925)

PLATE II

This investigation was carried out at the request of the Liverpool Port Sanitary Authority.

The work was done with the collaboration of Professor W. H. Roberts, Drs. W. Hanna and F. C. White.

The object of the enquiry was to determine the efficacy of various strengths of Hydrogen Cyanide in the destruction of vermin, especially bed bugs, under natural conditions on board ship.

Preliminary experiments with this gas were conducted in a lethal chamber on the roof of the Public Health Laboratory, the final experiments on board ship.

A summary of the experiments is given on p. 117, and recommendations as to the use of Hydrogen Cyanide on p. 118.

HAUNTS OF THE BED BUG

Certain bug-infested houses and quarters on board ship were inspected for the purpose of ascertaining the various conditions under which these insects live. The conditions observed were, as far as possible, reproduced in the test experiments with the hydrogen cyanide.

Bed bugs, as their name suggests, are to be found chiefly in bedrooms and sleeping quarters, places which will afford them an

opportunity of feeding on their host (man) during the night. Being insects which shun light, they withdraw during the day to any retreat which will give them shelter from the light. From these places they come out only at night for the purpose of feeding. The eggs are laid in their day-time haunts.

In houses, the situations favoured by these insects are :—cracks between woodwork fittings and the wall, such as are afforded by brackets or racks nailed or screwed on to the wall ; by badly fitting door frames and mantel-pieces ; behind pictures, especially underneath the paper backing where this is broken ; behind old wall-paper which is peeling off the walls ; cracks in plaster ; hangings, such as curtains, or mantel-covers ; bed-frames, especially in the case of bedsteads with hollow or tubular iron frames.

In ships similar conditions will afford shelter to the bugs, but one or two special ones require attention. Thus the tongue and groove boarding, which so often covers the partitions, or two thicknesses of which form the actual partition, forms a very good refuge, especially when there is a certain amount of air space behind the tongue and groove boarding, into which the bugs can penetrate.

The frame-work of the bunks also seems to be of some importance. In one ship that was investigated, the bunks were put up in sections, and the joints were furnished with collars with slots (see Plate II, fig. 1), in which accumulations of cast skins and living bugs were found. Of greater importance, however, was the fact that the frames of certain types of bunks were hollow tubes with small openings at the ends (fig. 3, B). In the case of the upright stanchions (fig. 3, C), the top end fitted loosely into a socket, whilst the bottom end was let into the deck. The loose fitting socket at the top was of such a nature as to allow the bugs easy access into the tube, whilst the gas, owing to its lightness, would penetrate down the tube only slowly and with difficulty.

A third form of refuge on board was found in a pile of life-jackets observed on one ship. In the folds of the canvas covering of these, bugs were found, and it was thought possible that the insects might penetrate to their interior. Piles of bedding, old clothes, and other such articles might form a similar refuge.

Most of the situations which have been mentioned—crevices in wood-work, cracks in plaster, etc., do not afford the bugs efficient

protection against the gas. Three cases, however, required special attention :— (1) match-boarding with a cavity behind, into which the bugs could retreat ; (2) tubular iron bunk frames ; (3) life-jackets, piles of bedding, old clothes, etc. The first of these cases was investigated by means of a specially constructed box which will be described below ; the second, by means of glass tubes, as will also be described below ; and the third, by using similar life-belts.

DESCRIPTION OF APPARATUS USED

Pill Boxes (card-board). Those used were about 5 cms. in diameter, and 3·5 cms. in height. They proved to be readily permeable to the Hydrogen Cyanide, and appeared to afford no protection to the bugs.

In the first experiments, in order that some sort of protection from the gas should be afforded, the bugs were placed between two layers of felt in the bottom of the pill box, which was then loosely packed with cotton wool, flannel, paper, etc. This packing appeared to make no difference to the efficacy of the gas.

Glass Jars (fig. 1). In many of the experiments the pill boxes were placed inside glass jars of the type used for preserving fruit. These jars had a capacity varying between 930 and 960 c.cs., having

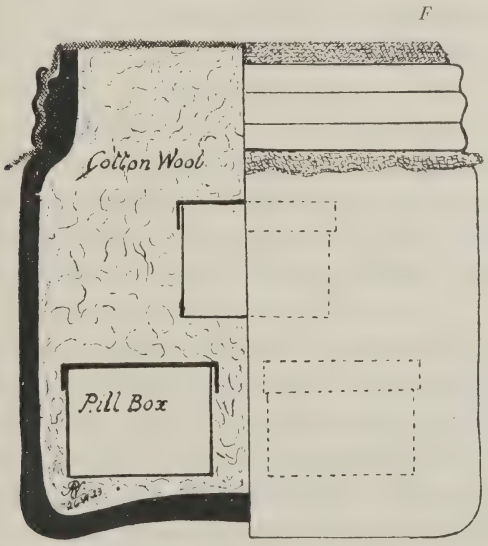


Fig. 1. Section and elevation of the glass jar used in the experiments. *F.*—Flannel covering.

a height of about 12 cms. and a diameter of about 10 cms. In ordinary use the grooved metal ring clamps down a flat metal disc over the top of the jar, thus hermetically sealing it ; for the purpose of the experiment, the flat metal disc was replaced by a piece of flannel readily permeable to the gas.

The jars were used in two ways :—

(1) The pill boxes containing the bugs were placed on the bottom of an empty jar, the mouth of which was closed with flannel as described above.

(2) The pill boxes were placed in the middle of cotton wool which filled the jar, as is shown in fig. 1.

Lethal Chamber. In the Experiments I-VIII, the exposure to the Hydrogen Cyanide was effected by placing the various pieces of apparatus containing the bugs in a strong wooden chest, referred to as the ' Lethal Chamber.' For a description of this and details of the strength of gas used and method of generation see the Chemist's report (p. 118).

The apparatus so far described, was designed for a preliminary test of the efficacy of the gas, and the protection afforded to the bugs in no way closely imitated the protection available to them in their natural haunts on board ship. The pieces of apparatus to be described below were designed especially to imitate certain of the refuges available under natural conditions.

The Tongue and Groove Board Box (figs. 2, 2, A). This box was made in order to test as far as possible the protection afforded to insects by the tongue and groove boarding which is used more particularly in ships, either covering portions of the ' skin ' of the vessel, or forming actual partitions ; the grooves afford a certain amount of shelter for the bugs, but of more importance is the possibility of their congregating in the space behind the boarding.

The box was 14 inches long, 10 inches wide, and 12 inches high, having thus a cubic capacity of a little less than 1 cubic foot. One of the sides of the box only consisted of match-boarding, and on the opposite side of the box was a glass window. In Experiments V and VII the match-boarding consisted of three pieces placed horizontally, whilst in Experiment VIII it consisted of four pieces placed vertically.

The lid was heavily weighted, so that it fitted down closely on the top of the box, and the grooves formed practically the only means by which the gas could penetrate to the interior of the box.

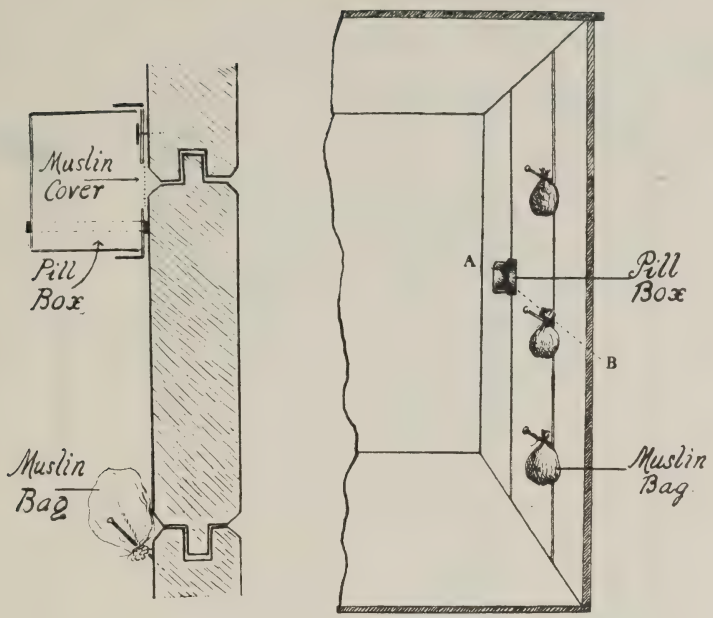


FIG. 2. Tongue and groove board box: section of part of the interior showing the relative positions of the pill box and muslin bags containing bugs. 2A.—section of the tongue and groove boarding (scale $\frac{1}{2}$), showing the positions of the pill box and muslin bag in relation to the joints of the timber.

The Glass Tubes (fig. 3). Two glass tubes, I and II, were arranged so as to imitate the conditions found in certain bunks (see fig. 3, B). Tube I was roughly 48 cms. long, and 4 cms. in diameter, having a volume of about 530 c.cs.; the tube was closed at each end by corks, pierced by two short pieces of glass tubing about 8 mm. in diameter. The corks were sealed with wax, so that gas entered the tube only through the two small pieces of glass tubing. This gave the bugs such shelter as would have been afforded by those bunk tubes with an opening at the ends.

Tube II had a length of about 51 cms., a diameter of about 4 cms., and a volume of about 590 c.cs. The bottom end was closed by a cork covered with wax; on the top end was fitted the lid of a pill box, having a slightly larger diameter than the tube, and raised from it by a small piece of plasticine on each side. There was thus a small inlet for the gas, such as was afforded by the loosely-fitting socket of the upright stanchion of the bunk (fig. 3, C).

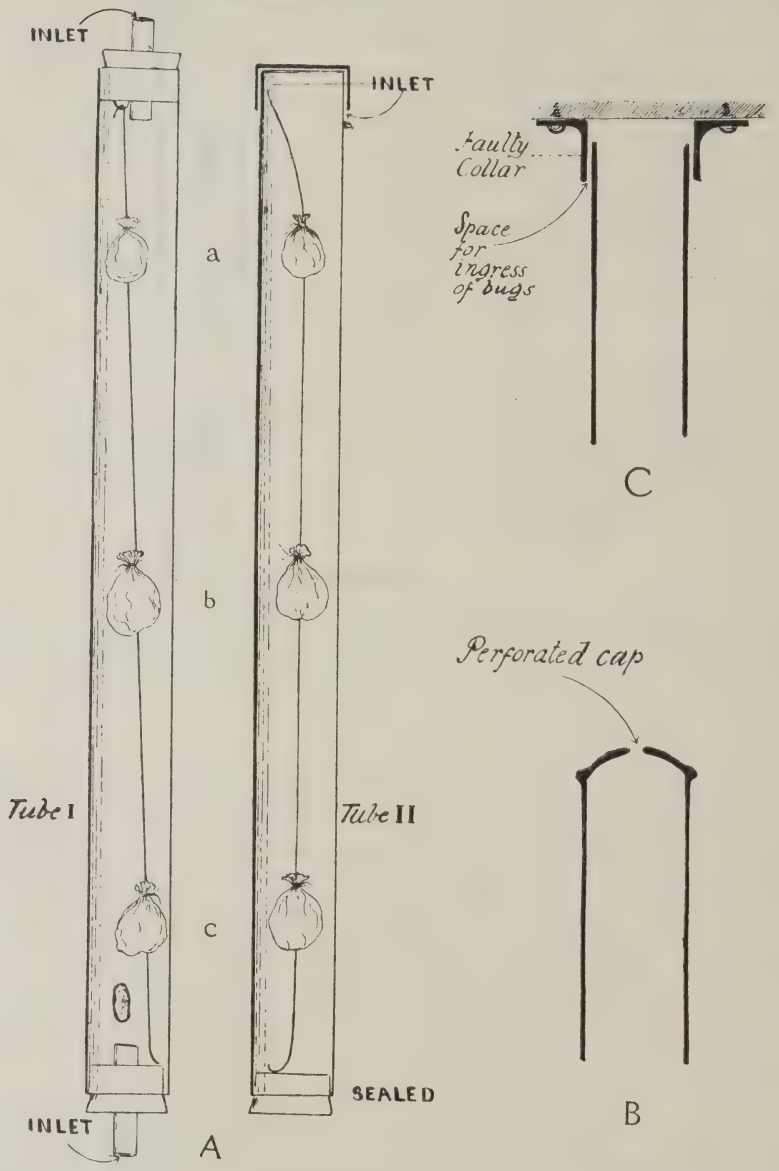


FIG. 3. *A*.—The glass tubes used in the experiments to test the viability of the bugs and the powers of diffusion of the gas under conditions illustrated in *B* and *C*. *a*, *b*, *c*—muslin bags containing bugs. *B*.—Schematic section of the end of a tubular iron bedstead (faulty type), showing the perforated cap through which the bugs gain access to the tube. *C*.—Schematic section of the tubular stanchion with loose-fitting (faulty) collar, leaving space for the ingress of bugs.

Life-Jackets, Bedding, etc. As a preliminary test of the amount of protection such objects might afford, in Experiments Vc, VIIc, and IXb, a pill box was wrapped in a roll of flannel and cotton wool (a cross section is shown in fig. 4) so that the pill box was protected in

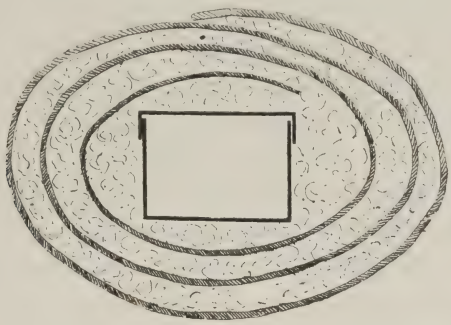


Fig. 4. Schematic section of the roll of cotton wool and flannel showing the position of the pill box containing bugs.

all directions by several thicknesses of alternate cotton wool and flannel. This protection being proved to be of no value to the bugs, sterner tests were carried out.

In Experiment VIIf, a life-jacket (Plate II, fig. 2), 2 ft. $8\frac{1}{2}$ in. long, $10\frac{1}{2}$ in. wide, and 3 in. in height was used. This life-jacket was stuffed with tightly-packed Kapok (a vegetable product resembling raw cotton). The pill box was placed inside the middle portion (Plate II, fig. 2x), and the end portion tied firmly over the middle portion, the resultant being a compact roll, 10 in. by $10\frac{1}{2}$ in. Even this failed to prevent a fatal concentration of gas reaching the bugs.

In Experiment IXk, a third test was carried out, the pill box being inserted in the interior of a straw-stuffed mattress. This, too, failed to protect the bugs against the gas.

LABORATORY METHODS OF DEALING WITH BUGS

In the Laboratory the bugs were kept between layers of dark-green baize in glass jars $3\frac{1}{2}$ in. by 2 in., or tubes 3 in. by 1 in., covered with a layer of cotton voile, and the jars and tubes placed in an incubator at a mean temperature of 25° C. At intervals of about a week the bugs were given an opportunity of feeding on the shaved abdomen of a rabbit. The voile-covered jars were applied to the

host's skin, and the bugs had no difficulty in feeding to repletion in this way. We are indebted to Dr. J. W. Scott Macfie for conducting these operations. In addition many batches of larval bugs were fed on the investigators.

Great care had to be taken in deciding whether bugs which had been exposed to the action of the gas had been permanently affected or not. It was found that after the experiments, bugs could be divided roughly into four categories according to their condition :—

(1) Bugs motionless and apparently dead immediately after the experiment, and never recovering their powers of movement.

(2) Bugs motionless immediately after the experiment, after the lapse of 24 hours regaining imperfectly their powers of movement, but after this becoming feebler daily and finally dying.

(3) Bugs motionless immediately after the experiment, but after about 24 hours completely regaining their powers of locomotion, after which they continue to live in a normal way.

(4) Bugs which are quite active and apparently healthy immediately after the experiment and remain so.

There was no doubt that bugs belonging to categories 1 and 2 were killed by the gas, but it was less easy to decide whether those in 3 and 4 had not suffered some permanent injury which would result in their ultimate destruction. The surviving bugs of categories 3 and 4 were, therefore, kept under observation for several days and tested to see whether they were able to feed, and produce fertile eggs, before they were considered to have recovered completely from the effects of the gas. Several batches of eggs obtained from the survivors were allowed to hatch and the larvae which emerged were quite healthy and fed readily when placed upon a host. It was abundantly evident from these observations that the bugs were in no way injured by the gas and would have been quite capable of continuing the infestation of either houses or ships.

At the beginning of the investigation controls were used for both adults and eggs of *C. lectularius*. These were put into receptacles similar to those containing the experimental specimens and taken to the place where the experiment was conducted, remaining near the lethal chamber till the close of the experiment. This was done in order to test the effect of the sudden lowering of temperature upon the bugs. As the control bugs remained entirely unaffected, we

considered it unnecessary to use them for the adults and nymphs in later experiments, the stock lot of bugs serving for comparison with the survivors during the period of observation. In the case of eggs, however, control eggs laid on the same day were kept under observation to insure that there was no defect inherent in the eggs to prevent their hatching.

EXPERIMENT I. 7.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularius*).

A and *C*. 12 bugs in each. *Controls:* for *A* and *C*. 12 bugs.
B and *D*. 12 eggs in each. *Controls:* for *B* and *D*. 13 eggs.

Conditions.

General. The material was exposed for two hours to a concentration of Hydrogen Cyanide of about 0·3 per cent.

Details. A and *B*. The specimens were placed between two layers of green felt in chip-boxes, and the latter filled loosely with flannel. The boxes were placed in a glass jar, covered with flannel.

C and *D*. The specimens were placed between two layers of green felt in chip boxes and the latter packed with cotton wool. The boxes were placed in a glass jar which was also packed with cotton wool and covered with flannel.

Results.

A, *B*, *C*, and *D*. Both bugs and eggs were all killed in each case.

Controls. Bugs. All were alive on the next day and had laid seven eggs during the night after the experiment. Eggs. 10 out of 13 (77 per cent.) hatched between 10th and 12th June.

EXPERIMENT II. 8.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularius*).

A. 12 eggs. *Controls:* 12 eggs (laid same day).
B. 6 bugs. *Controls:* 6 bugs.
C. 9 eggs. *Controls:* 9 eggs (laid same day).

Conditions.

General. The material was exposed for one hour to a concentration of Hydrogen Cyanide of about 0·3 per cent.

Details. A. The specimens were placed between layers of green felt in a chip box, and the latter loosely filled with flannel, and placed in an empty jar.

B and *C.* The specimens were placed between layers of green felt in a chip box, the latter packed with cotton wool, and placed in a jar also packed with cotton wool.

Results.

A. All the eggs were killed.
Controls. All hatched.

B. Of the 6 bugs, 3 survived.
Controls. All were quite normal on the morning after experiment.

C. All the eggs were killed.
Controls. 5 out of 9 (55 per cent.) hatched.

EXPERIMENT III.

Head louse (*Pediculus capitis*).
A. Adults and eggs. *Controls:* eggs.

Body louse (*Pediculus corporis*).
B. Adults.

Conditions.

General. The material was exposed for two hours to a concentration of Hydrogen Cyanide of about 0·3 per cent.

Details. The specimens were placed in chip boxes in petri-dishes and the dishes filled up with tightly-packed cotton wool and covered with one layer of flannel.

Results.

The eggs and adults were all killed by the experiments.
Controls. The eggs hatched normally.

EXPERIMENT IV. 13.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularis*).

A. 30 bugs. *Controls:* 6 bugs.
B. 6 bugs. *Controls:* 4 bugs.
C. 45 eggs. *Controls:* 12 eggs.
D. 11 eggs. *Controls:* 10 eggs.

Head lice (*Pediculus capitis*).

E. Eggs. *Controls:* eggs.

Body lice (*Pediculus corporis*).

F. Adults. *Controls:* adults.

Conditions.

General. The material was exposed for three hours to a concentration of Hydrogen Cynaide of about 0·2 per cent.

Details. A and *C*. The specimens were placed between two layers of baize in chip boxes and the boxes filled up tightly with cotton wool; the boxes were placed in jars and the jars packed with cotton wool and covered with a layer of flannel (see fig. 1).

B and *D*. The specimens were placed between two layers of baize in chip boxes and the boxes filled up with flannel and then placed in otherwise empty jars covered with flannel.

E. The eggs attached to the hairs on which they had been laid, were placed in glass-bottomed pasteboard boxes with tight-fitting pasteboard lids.

F. The lice were among the folds of a garment which was rolled up and placed in a glass jar covered with flannel.

Results.

A. 10 out of 30 bugs were killed.
B. 2 out of 6 bugs were killed.
C. 12 out of 45 eggs hatched (i.e., 27 per cent.).
Controls: 8 out of 12 hatched (i.e., 67 per cent.).
D. 6 out of 11 eggs hatched (i.e., 54 per cent.).
Controls: 7 out of 10 hatched (i.e., 70 per cent.).
E. All the eggs hatched.
F. None of the adults was killed.

EXPERIMENT V. 19.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularius*).

A. 20 bugs. *Controls:* 12 bugs.

B. 48 eggs. *Controls:* 29 eggs.

C. 20 bugs. *Controls:* those used for *A*.

D. 20 bugs. *Controls:* those used for *A*.

Black Rats.

E. 3 living rats from ship.

Conditions.

General. The material was exposed for three hours to a concentration of Hydrogen Cyanide of about 0·2 per cent.

Details. A and *B*. The specimens were placed between folds of green baize, in a chip box with two holes in the lid, over which had been pasted voile. The box was then pinned inside the match-boarding box (fig. 2, A), so that the two holes in the lid of the former were lying opposite the groove between the match-boarding of the latter.

C. The specimens were placed between folds of baize in a chip box, and the latter wrapped up in cotton wool and flannel (see fig. 4), forming a roll of about 4 inches in diameter, in the centre of which was the chip box.

D. The specimens were placed in a chip box between folds of baize. The lid of the chip box was perforated with small holes, and the chip box placed on the floor of the lethal chamber.

E. The rats were placed in a small cage on the floor of the lethal chamber.

Results.

A. Bugs. The whole number (20) recovered by the next morning.

B. Eggs. 37 out of the 48 (77 per cent.) hatched.

Controls. Of the 29 eggs, 28 hatched.

C. Bugs. 19 of the 20 completely recovered by the next morning.

D. Bugs. All were killed.

A, *C*, and *D*. *Controls*. Bugs. All 12 quite normal on the next morning.

E. The three rats were stiff when taken out of the lethal chamber. From them were collected the following :—9 ♂ ♂ and 4 ♀ ♀ of the plague flea (*Xenopsylla cheopis*), all of which were dead.

EXPERIMENT VI. 22.6.23.

Material used in this experiment.

A. 15 rats from a warehouse ' black rats.'

B. One rat's nest.

C. 10 larvae of the rat flea (*Ceratophyllus fasciatus*).

Conditions.

General. The material was placed in the lethal chamber for three hours and the concentration used was about 0·2 per cent. of Hydrogen Cyanide.

Details. A. The 15 rats were placed in lethal chamber in a stout unbleached calico bag.

B. The rat's nest was wrapped in paper, which was pierced with slits.

C. 10 larvae of *Ceratophyllus fasciatus* were placed in a tube, the mouth of which was closed by cotton wool, in the rat's nest.

Results.

A. The 15 rats were quite dead, and from them were collected :— 3 ♂ ♂ and 5 ♀ ♀ of *Ceratophyllus fasciatus*, also dead.

B. From the rat's nest we obtained :—One adult *Ceratophyllus fasciatus*, dead, and two larvae, dead.

C Of the 10 larvae in the tube, all were dead when examined on the 22nd and 23rd of June, whilst control larvae were still alive.

EXPERIMENT VII. 25.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularius*).

A. 20 bugs.

B. 50 eggs. *Controls:* 60 eggs (laid same day).

C. 20 bugs.

D. 10 eggs.

E. Tube I. (*a*), (*b*) and (*c*), 10 bugs. (b^1), 25 eggs.
Tube II. (*a*), (*b*) and (*c*), 10 bugs. (b^1), 25 eggs.

Control: for *D* and *E*, 23 eggs.

F. 20 bugs.

Conditions.

General. The material was exposed for two hours to a concentration of Hydrogen Cyanide of about 0·3 per cent.

Details. A and *B.* The specimens were placed between layers of green baize in a chip box, which was put inside the tongue and groove board chest as in Experiment Va.

C and *D.* As in Experiment Vc.

E. The specimens were placed in muslin bags, (*a*), (*b*), (*b*^{1}) and (*c*), and these suspended, (*a*) at the top, (*b*) and (*b*^{1}) in the middle, and (*c*) at the bottom, of two glass tubes, I and II (see fig. 3, A and description of apparatus, p. 95).

F. The specimens were placed in a chip box in the usual manner, and the chip box placed inside a life-belt (see Plate II, fig. 2, and description of apparatus, p. 97).

Results.

A. Bugs. 14 out of 20 (70 per cent.) survived.
B. Eggs. 42 out of 50 (84 per cent.) hatched.
Controls. 47 out of 60 (78 per cent.) hatched.
C. Bugs. All were killed.
D. Eggs. All were killed. *Controls.* All except 2 hatched.
E. Tube I. Bugs. (*a*), (*b*), and (*c*). All were killed in each case.
Eggs. (*b*^{1}). All were killed.
Tube II. Bugs. (*a*) All (10 out of 10) were killed.
(*b*) 9 out of 10 were killed.
(*c*) 1 out of 10 was killed.
Eggs. (*b*^{1}) 17 out of 25 (68 per cent.) hatched.
Control. Eggs. All hatched except 2 (91 per cent.).
F. Bugs. All were killed.

Experiment VIII. 13.6.23.

Material used in this experiment.

Bed bugs (*Cimex lectularius*).
A, *B*, (*a*), (*b*), (*c*) ; *C* (*a*), (*b*), (*c*). 10 bugs in each.
B (*b*^{1}) and *C* (*b*^{1}), 20 eggs in each. *Controls :* 8 eggs (laid on same day).

Conditions.

General. The material was exposed for three hours to a concentration of Hydrogen Cyanide, of about 0·3 per cent.

Details. A. The specimens were placed in an open voile bag in a chip box as used in Experiment Va, and the box placed under the same condition as in that experiment.

B. The specimens were placed in voile bags (*a*), (*b*), (*b*¹) and (*c*), in a glass tube (see fig. 3, A), (*a*) and (*c*) being at each end and (*b*) and (*b*¹) in the middle. The tube was supported horizontally in the lethal chamber in such a position that bag (*a*) was nearest to the point of evolution of the gas.

C. The specimens were placed in voile bags (*a*), (*b*), (*b*¹) and (*c*), which were pinned against the groove on the inside of the match-boarding box (see fig. 2), (*a*) being at the top, (*b*) and (*b*¹) in the middle and (*c*) at the bottom.

Results.

A. 4 out of the 10 bugs were killed.

B. (*a*), (*b*), (*c*). All were killed.

B. (*b*¹). None of the eggs hatched. *Controls:* all hatched.

C. (*a*) and (*b*). 5 out of 10 bugs were killed in each case.

C. (*c*). 9 out of 10 bugs were killed.

C. (*b*¹). 12 out of 20 eggs hatched. *Controls:* all hatched.

EXPERIMENTS ON BOARD SHIP

Experiment IX. 20.7.23. *Fumigation of the s.s. 'Lady Emerald.'*

Material used in this experiment.

A-K. Ten voile bags, each containing 10 bugs, were used.

L and *M.* Two cages of rats, containing 4 and 3 respectively.

Conditions.

General. The material was exposed for two hours to a concentration of Hydrogen Cyanide produced by 8 oz. of Sodium Cyanide per 1,000 cubic feet (i.e., about 0·3 per cent. Hydrogen Cyanide). The gas was generated in tubs, in the usual manner, in the two places fumigated (the seamen's and the firemen's quarters); the position of these tubs in relation to the dispositions of the material may be seen quite readily from figs. 5 and 6.

There were no traces of bugs in the ship, but the food-lockers were very much infested with mice.

Details (see figs. 5 and 6). *A-K.* The voile bags containing the bugs were, for convenience, placed in chip boxes, and these were disposed as follows :—

In the Seamen's quarters (see fig. 5).

A, B and *C* were placed, in the match-boarding box used in previous experiments, on a bench, raised about two feet off the floor.

D was placed in a roll of cotton wool and flannel (as used in Experiment Vc.) on the floor beneath the bench mentioned above.

I was placed near to it, to serve as a control.

E was placed in the food-locker, about four feet from the ground.

F was placed on the table in the mess-room.

G was placed on a beam just under the roof.

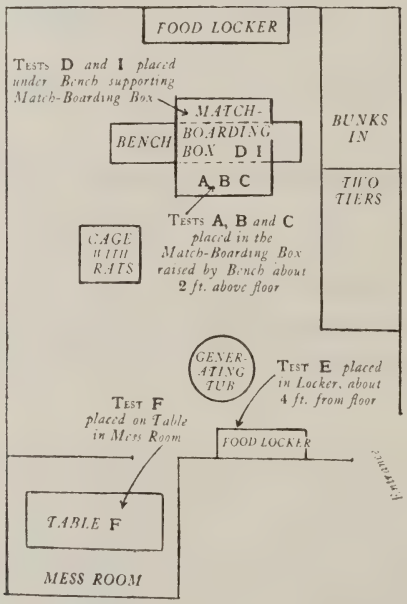


FIG. 5. Schematic plan showing the relative positions of the tests placed in the Seamen's quarters, Experiment IX.

In the Firemen's quarters (see fig. 6).

H was placed in a situation similar to that of G.

K was placed in the straw stuffing of a mattress lying on the bottom bunk on the left-hand side.

L and *M*, the two cages of rats, were placed, one on the floor of the seamen's quarters (see fig. 5), and the other on a bench, raised about 1½ feet above the ground in the firemen's quarters (see fig. 6).

Results.

A. All the bugs survived except one.

B. All the bugs survived except two, though this lot showed a quicker rate of mortality, subsequently, than did either *A* or *C*.

C. All the bugs survived.

D. All the bugs were killed.

E. 3 bugs only out of the 10 survived.

F. 1 bug only (a 3rd stage larva) survived.

G, *H*, *I*, and *K*. All the bugs were killed.

L and *M*. All the rats (7) were killed, and from them were taken 2 specimens of *Ceratophyllus fasciatus* (♀ ♀) and a number of lice, also dead.

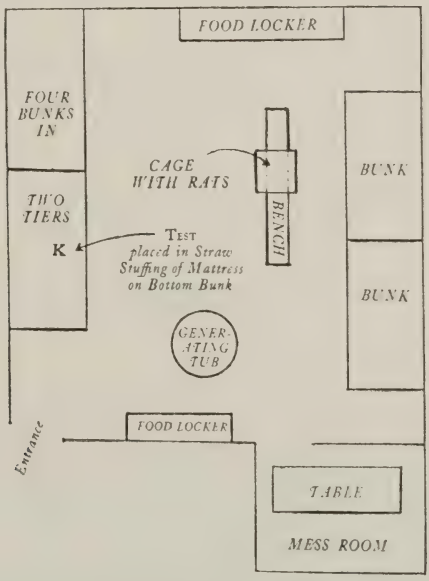


FIG. 6. Schematic plan showing the relative positions of the tests placed in the Firemen's quarters, Experiment IX.

EXPERIMENT X. 23.2.24. *Fumigation of the s.s. 'Montcalm.'*

Material used.

Bed bugs (*Cimex lectularius*).

A, *C* and *D*. 10 bugs.

B. 14 bugs.

Conditions.

General. The passenger accommodation only was fumigated. The fumigation was effected by spraying liquid Hydrogen Cyanide, $2\frac{1}{2}$ oz. per 1,000 cubic feet, giving an average concentration of about 0·27 per cent. HCN. The duration of exposure was from 3-$3\frac{1}{2}$ hours.

Details. The bugs were placed in chip boxes loosely packed with pieces of green felt.

A. The chip box was placed behind the skirting-board of a cabin in the stewards' quarters, amidships. The skirting-board was open underneath, and had a couple of large circular holes, the one directly in front of the chip box being closed by means of a pillow.

B. This chip box was used as a control for *A*, being placed on a table in the cabin, to test the concentration of the gas outside the skirting-board.

C. The chip box was placed in a cupboard in a cabin in the passengers' starboard quarters.

D. This was used as a test of the concentration of the gas outside the cupboard in which C was, being placed on a table in the same cabin.

Results.

A, *B*, *C* and *D*. All the bugs were killed in each case.

EXPERIMENT XI. 13.3.24. *Fumigation of the s.s. 'City of Paris.'*

Material used.

Bed bugs (*Cimex lectularis*). *A-G*. 7 lots of 10 bugs.

Conditions.

General. As in the last experiment, fumigation was effected by spraying liquid Hydrogen Cyanide, the concentration produced being about 0·2 per cent. The fumigation lasted for 3-$3\frac{1}{2}$ hours.

Details. The bugs were placed in chip boxes with a little green felt and newspaper packing.

A and *B*. The two chip boxes were placed in the rudder post locker, in the firemen's fo'castle. This structure was formed by tongue and groove boarding, about one and a quarter inches thick, with a closely-fitting door, which was closed. The capacity was about 50 cubic feet. The temperature in the fo'castle was fairly high.

C and *D*. These two chip boxes were placed on chests in the firemen's fo'castle, near the rudder post locker. They served as tests of the concentration of gas outside the locker.

E. This chip box was placed in the space under a chest of drawers, in a passenger cabin on the port side of the bridge deck. As this structure was built into the side of the cabin, the only way the gas could penetrate into the space was by means of the crack between the bottom drawer and the framework of the chest of drawers.

F. This chip box was placed in the bottom drawer of the above-mentioned chest of drawers.

G. This chip box was placed on the shelf of a toilet apparatus in the same cabin as *C*. It formed a test of the concentration of the gas in the air outside the chest of drawers.

Results.

A. 1 bug only survived out of 10.

B. 3 out of the 10 survived in a healthy condition.

C and *D*. All the bugs were killed.

E. All the bugs were killed.

F. 2 bugs survived in a healthy condition.

G. All the bugs were killed.

DISCUSSION OF RESULTS AND TABLES

In all the experiments, the action of the gas was not considered to be satisfactory unless every bug was killed. This attitude was adopted because one bug, if it happened to be a fertilised female, would be quite capable of starting a fresh infection.

TABLE I.

Summary of experiments on Bed Bug (*Cimex lectularius*).

Experiments I-IV.

No. of experiment	Conditions of experiment			Experimental material		Control material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed	Number of specimens	Percentage died
	%				%		%
IA ...	0·3	2 hours	In pill boxes in empty glass jar, unprotected	12 bugs	100	...	...
IB ...	0·3	2 hours	In pill boxes in empty glass jar, unprotected	12 eggs	100	13 eggs	3
IC ...	0·3	2 hours	In pill boxes in glass jar, protected by cotton wool	12 bugs	100	...	...
ID ...	0·3	2 hours	In pill boxes in glass jar, protected by cotton wool	12 eggs	100	13 eggs (as used for IB)	3
IIA ...	0·3	1 hour	In pill boxes in empty glass jar, unprotected	12 eggs	100	12 eggs	0
IIB ...	0·3	1 hour	In pill boxes in glass jar, protected by cotton wool	6 bugs	50	...	...
IIC ...	0·3	1 hour	In pill boxes in glass jar, protected by cotton wool	9 eggs	100	9 eggs	45
IVA ...	0·2	3 hours	In pill boxes in glass jar, protected by cotton wool	30 bugs	33	...	...
IVB ...	0·2	3 hours	In pill boxes in empty jar, unprotected	6 bugs	33	...	...
IVC ...	0·2	3 hours	In pill boxes in glass jar, protected by cotton wool	45 eggs	73	12 eggs	33
IVD ...	0·2	3 hours	In pill boxes in empty jar, unprotected	11 eggs	46	10 eggs	30

Table I summarises the results obtained in the preliminary experiments where the only protection afforded to the bugs was that of the cotton wool in which they were packed. A concentration of 0·3 per cent. of gas, acting for two hours, was sufficient to kill both bugs and eggs, whether protected or not (Experiment I). It did not, in one hour, kill bugs when protected by cotton wool (Experiment II). A concentration of 0·2 per cent. of gas, even though allowed to act for three hours, failed to kill either bugs or eggs whether protected or not (Experiment IV).

In the following table, which for convenience has been divided into three sections, are seen the results obtained in the lethal chamber, when forms of protection resembling more nearly those of their natural conditions were afforded to the bugs.

TABLE II.

Summary of the Experiments on Bed Bugs (*Cimex lectularius*).

Section A. Experiment V

No. of experiment	Conditions of experiments			Experimental material		Control material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed	Number of specimens	Percentage died
	%				%		%
VA ...	0·2	3 hours	Pill box pinned inside tongue and groove board box	20 bugs	0	...	...
VB ...	0·2	3 hours	Pill box pinned inside tongue and groove board box	48 eggs	23	29 eggs	3
VC ...	0·2	3 hours	Pill box inside cotton wool and flannel roll	20 bugs	5	...	...
VD ...	0·2	3 hours	Pill box with perforated lid on floor of lethal chamber	20 bugs	100	...	...

From this section it will be seen that a concentration of 0·2 per cent., acting for three hours, failed to kill bugs protected by tongue and groove boarding, or by the flannel and cotton wool roll; it succeeded, however, in killing them completely when they were exposed without any means of protection (Experiment V).

TABLE II—*Continued*

Section B, Experiment VII

No. of experiment	Conditions of Experiments			Experimental material		Control material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed	Number of specimens	Percentage died
	%				%		%
VIIA ...	0·3	2 hours	Pill box pinned inside tongue and groove board box	20 bugs	30	...	...
VIIB ...	0·3	2 hours	Pill box pinned inside tongue and groove board box	50 eggs	16	60 eggs	2
VIIC ...	0·3	2 hours	Pill box inside cotton wool and flannel roll	20 bugs	100	...	...
VIID ...	0·3	2 hours	Pill box inside cotton wool and flannel roll	10 eggs	100	23 eggs	9
VIIE, I(*a*)	0·3	2 hours	Voile bag suspended at top of Tube I	10 bugs	100	...	...
VIIE, I(*b*)	0·3	2 hours	Voile bag suspended in middle of Tube I	10 bugs	100	...	...
VIIE, I(*b*^{1})	0·3	2 hours	Voile bag suspended in middle of Tube I	25 eggs	100	23 eggs (same as used in VIID)	9
VIIE, I(*c*)	0·3	2 hours	Voile bag suspended at bottom of Tube I	10 bugs	100	...	...
VIIE, II(*a*)	0·3	2 hours	Voile bag suspended at top of Tube II	10 bugs	100	...	...
VIIE, II(*b*)	0·3	2 hours	Voile bag suspended in middle of Tube II	10 bugs	90	...	...
VIIE, II(*b*^{1})	0·3	2 hours	Voile bag suspended in middle of Tube II	25 eggs	32	23 eggs (same as used in VIID)	9
VIIE, II(*c*)	0·3	2 hours	Voile bag suspended at bottom of Tube II	10 bugs	10	...	...
VIIF ...	0·3	2 hours	Pill box inside life-belt	20 bugs	100	...	...

This section shows that even 0·3 per cent. of the gas failed to kill bugs behind match-boarding in two hours. This concentration also failed, in that period, to penetrate even to the middle of Tube II

in sufficient quantity to kill the bugs. It did, however, succeed in killing completely all those bugs placed inside the flannel and cotton wool roll, in Tube I (the tube open at both ends), and in the interior of the Life-Jacket (Experiment VII).

TABLE II—*Continued*

Section C, Experiment VIII

No. of experiment	Conditions of experiments			Experimental material		Control material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed	Number of specimens	Percentage died
	%				%		%
VIIIA ...	0·3	3 hours	Pill box pinned inside tongue and groove board box	10 bugs	40	...	...
VIIIB (*a*)...	0·3	3 hours	Voile bag at end of Tube II furthest from the gas	10 bugs	100	...	...
VIIIB (*b*)...	0·3	3 hours	Voile bag in middle of Tube II	10 bugs	100	...	...
VIIIB (*bl*)	0·3	3 hours	Voile bag in middle of Tube II	20 eggs	100	8 eggs	0
VIIIB (*c*)	0·3	3 hours	Voile bag at end of Tube II nearest the gas	10 bugs	100	...	...
VIIIc (*a*)	0·3	3 hours	Voile bag pinned at top of groove of tongue and groove board box	10 bugs	50	...	...
VIIIc (*b*)...	0·3	3 hours	Voile bag pinned in middle of groove of tongue and groove board box	10 bugs	50	...	...
VIIIc (*bl*)	0·3	3 hours	Voile bag pinned in middle of groove of tongue and groove board box	20 eggs	40	8 eggs (used in VIIIB (*bl*))	0
VIIIc (*c*)...	0·3	3 hours	Voile bag pinned at bottom of groove of tongue and groove board box	10 bugs	90	...	...

Here it is shown that a period of three hours, even, was not sufficient for 0·3 per cent. of the gas to kill the bugs behind tongue and groove boarding, although it did allow the gas to penetrate into Tube II, when placed horizontally, in sufficient concentration to kill all the bugs in it (Experiment VIII).

An interesting fact, brought out by the experiments summarised in the above two tables, is that the eggs of bugs are not more resistant to the action of hydrogen cyanide than are the other stages.

TABLE III.

Summary of Experiments on Bed Bugs (*Cimex lectularius*).

Experiments IX-XI.

No. of experiment	Conditions of Experiments.			Experimental material	
	Average concentration of gas	Length of exposure	Conditions	Number of specimens	Percentage killed
	%				%
IXA ...	0·3	2 hours	Pill box at top of groove of tongue and groove board box	10 bugs	10
IXB ...	0·3	2 hours	Pill box in middle of groove of tongue and groove board box	10 bugs	20
IXC ...	0·3	2 hours	Pill box at bottom of groove of tongue and groove board box	10 bugs	0
IXD ...	0·3	2 hours	Pill box in roll of cotton wool and flannel	10 bugs	100
IXE ...	0·3	2 hours	Pill box in a food-locker	10 bugs	70
IXF ...	0·3	2 hours	Pill box on a table in small mess-room	10 bugs	90
IXG ...	0·3	2 hours	Pill box on a beam under ceiling	10 bugs	100
IXH ...	0·3	2 hours	Pill box on a beam under ceiling	10 bugs	100
IXI ...	0·3	2 hours	Pill box unprotected, near to IXD	10 bugs	100
IXK ...	0·3	2 hours	Pill box in straw stuffing of mattress	10 bugs	100
XA ...	0·27	3-3½ hours	Pill box behind skirting-board of cabin	10 bugs	100
XB ...	0·27	3-3½ hours	Pill box on table in same cabin as XA	14 bugs	100
XC ...	0·27	3-3½ hours	Pill box in cupboard in cabin	10 bugs	100
XD ...	0·27	3-3½ hours	Pill box on table in same cabin as XC	10 bugs	100

TABLE III—*continued*

No. of experiment	Conditions of Experiments			Experimental material	
	Average concentration of gas	Lengths of exposure	Conditions	Number of specimens	Percentage killed
	%				%
XIA ...	0·20	3-3½ hours	Pill boxes in locker	10 bugs	90
XIB ...	0·20	3-3½ hours		10 bugs	70
XIC ...	0·20	3-3½ hours	Pill boxes on chests near locker	10 bugs	100
XID ...	0·20	3-3½ hours		10 bugs	100
XIE ...	0·20	3-3½ hours	Pill box in space under bottom drawer of chest of drawers	10 bugs	100
XIF ...	0·20	3-3½ hours	Pill box in bottom drawer of chest of drawers	10 bugs	80
XIG ...	0·20	3-3½ hours	Pill box on shelf in same cabin as XIE and XIF	10 bugs	100

The experiments summarised in this table were carried out on various ships ; in the first one, fumigation was effected by the dumping method, and in the second and third ones, by using liquid Cyanide with a spray.

In Experiment IX, the concentration was calculated at about 0·3 per cent. of the gas, and the fumigation lasted about two hours ; this failed, again, to kill the bugs behind the tongue and groove boarding ; it failed, also, to penetrate into a food-locker. This experiment illustrates the disadvantage of the dumping method, in that the concentration of the gas is not distributed uniformly throughout the space to be fumigated ; thus, whilst bugs in various positions, including the interior of a straw-stuffed mattress, were killed, some exposed on a table in a small mess-room just off the main one (see fig. 6, p. 107) were not killed.

In Experiment X, a concentration of about 0·27 per cent., acting for three to three-and-a-half hours, was completely successful, all the bugs being killed; the protection afforded was not very great, the skirting-board being open at the bottom, and the cupboard (*C*) not very air-tight.

Experiment XI shows, again, that a concentration of 0·2 per cent., even when acting for three to three-and-a-half hours, is too low to kill bugs if any kind of protection is afforded (e.g., *A*, *B* and *F*), although it does kill those exposed.

TABLE IV.

Summary of Experiments on Lice, Fleas and Rats.

No. of experiment	Conditions of Experiments			Material	Results
	Average concentration of gas	Lengths of exposure	Conditions		
IIIA ...	% 0·3	2 hours	In pill box in petri-dish filled with cotton wool	Head lice and eggs	All were killed. *Control.* Eggs were normal
IIIB ...	0·3	2 hours	In pill box in petri-dish filled with cotton wool	Body lice	All were killed
IVE ...	0·2	3 hours	In glass-bottomed paste-board pill-boxes	Head louse eggs	None were killed
IVF ...	0·2	3 hours	In garment stuffed into glass jar	Body lice	None were killed
VE ...	0·2	3 hours	In an iron cage	3 black rats	All killed—13 dead fleas found
VIA ...	0·2	3 hours	In a stout calico bag	15 black rats	All killed—8 dead fleas found
VIB ...	0·2	3 hours	Rat's nest wrapped up in paper pierced by slits	Fleas and larvae	1 flea and 2 flea larvae found dead
VIC ...	0·2	3 hours	In a small glass tube plugged with cotton wool, inside the rat's nest	10 flea larvae	10 larvae all dead. *Controls* remained alive
IXL ...	0·3	2 hours	In a cage on the floor	4 black rats	All were killed, and 2 fleas and a number of lice were found dead on the rats
IXM ...	0·3	2 hours	In a cage on a bench	3 black rats	

From this table it appears that, in the case of lice, a concentration of 0·3 per cent., for one hour, was sufficient to kill both adults and eggs (Experiment III), whilst a concentration of 0·2 per cent., for three hours, was not sufficient (Experiment IV). It is interesting to note that a few of the lice used in this experiment, on the 13th of June, were still alive on the 20th, having spent the seven days off the human body at ordinary room temperature, without any food. They were all dead on June 21st.

Both fleas (adults and larvae), and rats, are killed by a concentration of 0·2 per cent., for three hours, (Experiments V, VI, and IX).

SUMMARY

1. A concentration of 0·2 per cent. of Hydrogen Cyanide does not, even if allowed to act for as long as three hours, with certainty kill every bug.

2. A concentration of 0·3 per cent. of the gas, acting for only one hour, is not sufficient to kill every bug.

3. A concentration of 0·3 per cent. of the gas, acting for three hours, will kill all the bugs present, except where they can retire behind tongue and groove boarding.

4. Eggs of bugs are not more resistant to Hydrogen Cyanide than are the adults.

5. A concentration of 0·3 per cent. of the gas, acting for one hour, is sufficient to kill lice, both adults and eggs ; but a concentration of 0·2 per cent. of gas, even acting for three hours, does not do so.

6. A concentration of 0·2 per cent. of the gas, acting for three hours, is sufficient to kill both fleas (adults and larvae), and rats.

7. Spraying with liquid Cyanide gives better results than does the dumping method, in that it tends to give a more uniform concentration throughout the area, although not ensuring this absolutely.

RECOMMENDATIONS

1. That a concentration of 0·3 per cent. of Hydrogen Cyanide, acting for a period of three hours, should be used.

2. That where match-boarding is present, one or two boards should, if possible, be removed, in order to allow the gas easy access into the cavity behind.

3. That where bunks with hollow metal frames are present, they should be taken to pieces, when this is practicable, and the tubular portions laid horizontally, so that the gas can penetrate easily into their interior. Or better, as a preventative, the ends of the tubing should be hermetically sealed, as illustrated on Plate II, fig. 1.

APPENDIX I

NOTE ON LETHAL CHAMBER AND CHEMICAL METHODS EMPLOYED

BY

W. H. ROBERTS, M.Sc., F.I.C.

CITY ANALYST

The material to be treated was placed inside a rectangular wooden chamber of internal dimensions 100 × 60 × 60 cms., closed with a lid, which, when clamped in position, rendered it air-tight. On the floor of the chamber and in one corner was placed a large porcelain dish containing dilute sulphuric acid. The lid was closed and the necessary quantity of potassium cyanide solution was run into the dish from a dropping funnel through a bent glass delivery tube passing through the wall of the chamber.

Two minutes later, sodium carbonate solution was run in from the same funnel, this being in order to expel all dissolved hydrogen cyanide gas from solution.

In the top of the opposite wall of the chamber was a glass delivery tube connected to a long length of india-rubber tubing. This and the inlet tube were now firmly clamped and the material left exposed for the time of the experiment.

In opening up the chamber the inlet tube was attached to a foot-bellows and slight pressure applied. Both clamps were now removed and air blown through for 15 minutes. At the end of this period the box could safely be opened.

To give a concentration of 0·3 per cent. HCN gas in the chamber, the following reagents were used :—

15 c.cs. H_2SO_4 (1 in 3 by volume).

3·2 grms. KCN (98 per cent.) dissolved in about 20 c.cs. of water.

followed by :—

20 c.c. of a 10 per cent. Na_2CO_3 solution.

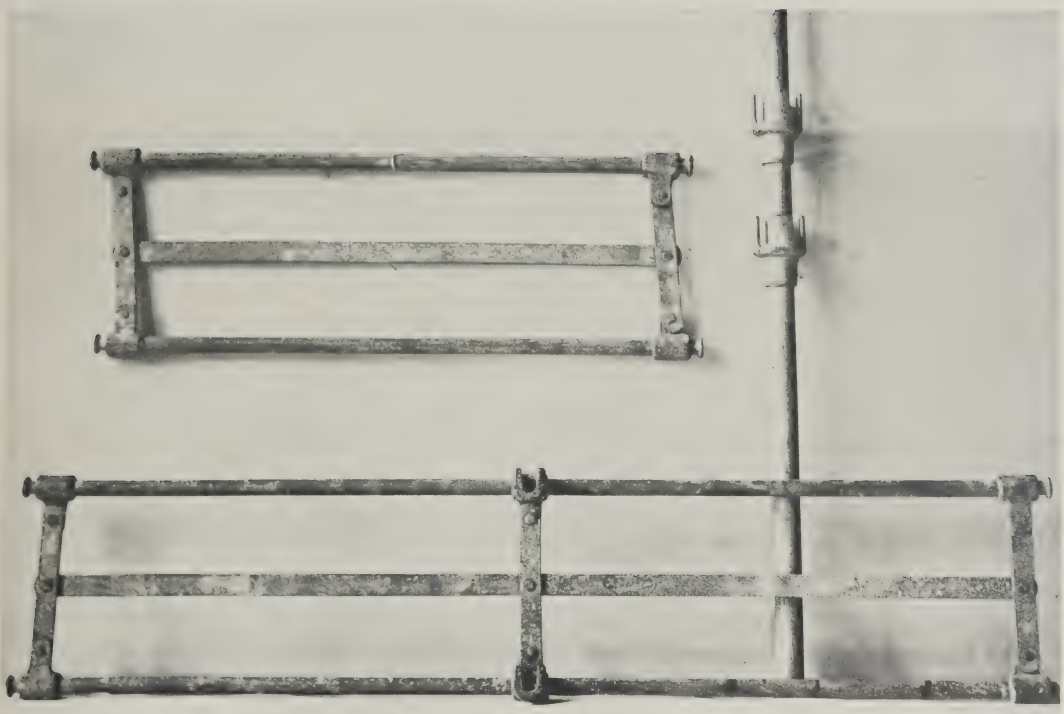


FIG. 1

FIG. 2

C. Tinling & Co., Ltd., Imp.

NOTES ON CULICIDAE COLLECTED IN SIERRA LEONE, WITH DESCRIPTIONS OF A NEW SPECIES AND A NEW VARIETY

BY

A. M. EVANS.

(*Received for publication* 12 *February*, 1925)

PLATE III

Professor Blacklock has recently made numerous collections of larvae of Culicidae from very varied situations at Daru, and on the Cape Lighthouse Peninsula, near Freetown. These situations included many 'small enclosed collections of water' such as rot-holes in trees, and it may be interesting to compare these findings with those recorded by Macfie and Ingram (1923) from the Gold Coast.

The adults reared from these larvae were submitted to the writer for identification and have been found to comprise twenty-four species, seven of which do not appear to have been recorded from the Colony hitherto; one species is an undescribed member of the *apicoargentea* series of '*Stegomyia*,' and another a new variety of *Aedes* (*Aedimorphus*) *cumminsi*, Theo. The following is a list of the species contained in the collection; the material from Daru was collected in the latter half of September, 1924, and that near Freetown on the 17th and 18th August in the same year.

The types and co-type specimens described in this paper are in the collections of the Liverpool School of Tropical Medicine.

Anopheles costalis, Loew.

Daru: Stream, 1 ♂; 'Swamp A,' 14 ♂♂, 12 ♀♀; Stream to 'Swamp B,' 1 ♀; Moa River, 6 ♂♂, 2 ♀♀; Hospital drain area, 1 ♂; Cape Lighthouse Peninsula, Freetown: Rock-pool, 1 ♀.

Anopheles nili, Theo.

Moa River, Daru, 14 ♂♂, 12 ♀♀.

Professor Blacklock observed that during the short period of investigation this species did not enter houses frequently. The

following observation tends to show a striking contrast in this respect between *A. nili* and *A. costalis*. He noted that in a native's house that was only fifty yards away from the edge of the Moa river, where *A. nili* was breeding in large numbers, only one adult specimen of this species was to be found. At the same time more than one hundred *A. costalis* were captured in this house, although its breeding-ground was ' in a marsh (Swamp A) further away.'

Anopheles mauritianus, Grandpré.
' Swamp B,' Daru, 1 ♀.

Anopheles umbrosus, Theo.
' Swamp A,' Daru, 1 ♀.

Anopheles rhodesiensis, Theo.
Rock-pool, Cape Lighthouse Peninsula, near Freetown: 4 ♂♂, 6 ♀♀.

Uranotaenia fusca, Theo.
Daru: stream to ' Swamp B,' 3 ♂♂, 3 ♀♀; tree-hole, 1 ♀.

Uranotaenia nigripes, Theo.
Daru: in a pineapple found in an empty bungalow, 1 ♂, 2 ♀♀; in a tree-root in ' native S.M.'s yard,' 1 ♀.

Mimomyia hispida, Theo.
' Hospital drain area,' Daru, 2 ♂♂, 1 ♀.

Aedes (*Stegomyia*) *argenteus*, Poiret.
Daru: latrine washing-bucket, 1 ♀; Cape Lighthouse Peninsula, Freetown; rock-pool, 1 ♀; tree-hole, 3 ♂♂, 1 ♀.

Aedes (*Stegomyia*) *africanus*, Theo.
Daru: Between forks of three-stemmed tree at ground level, 1 ♂, 4 ♀♀; tree-hole, 2 ♂♂; banana tree, near bungalow, 1 ♂, 3 ♀♀; stream, 1 ♂.

Aedes (*Stegomyia*) *simpsoni*, Theo.
Daru: hole in root of tree, in ' native S.M.'s yard,' 1 ♀.

Aedes (*Stegomyia*) *apicoargentea*, Theo.
Daru: stream near river, 1 ♂, 1 ♀; latrine washing-bucket, 3 ♀♀.

AEDES (STEGOMYIA) BLACKLOCKI, sp.n. (Pl. III, fig. 1).

FEMALE.

Head and *palpi* with black and silvery-white scales arranged as shown in figure. *Thorax.* Mesonotum with silvery-white anterior patch formed of broad, flat scales in front and narrow curved scales behind. Middle line (largely denuded in the type) of narrow-curved, pale-yellow scales broadening posteriorly, the scales becoming silvery and forking to form two lines surrounding the ante-scutellar space; large paired silvery spots formed of broad curved scales; smaller silvery spots over wing roots of long curved scales; the scales forming the paired posterior lines very pale yellow, narrowly spindle-shaped. Scutellum with a few black scales behind the silvery ones on the median lobe, and one or two black scales internally on the right lateral lobe. Pleurae without lower mesepimeral bristles, a large patch of flat silvery scales on the upper part of the mesepimeron.

Abdomen. Dorsum of third segment with silvery-white scales forming an irregular and asymmetrical basal band. Fourth and fifth segments with well-developed silvery basal bands. Sixth and seventh segments with broad basal areas of silvery scales reaching to their distal borders in the middle. Third to seventh segments with large, basal, lateral, rectangular silvery spots, and ventrally with narrow basal silvery bands.

Legs. Front femora with a narrow line of white scales on the basal two-fifths anteriorly, and a line of silvery-white scales at the outer third, internally, not extending to the apex. Mid-femur with basal white spot; on the external face a median silvery spot, and a broad, apical, silvery patch continuous with a narrow, internal line of white scales extending backwards for nearly one-third the length of the segment. Hind femur creamy white at base beneath, with a conspicuous, silvery-white apical spot and a small, sub-median, external, silvery stripe. Front tibia with a narrow, basal, silvery-white ring, broadest beneath, mid tibia with a white basal patch beneath, hind tibia with a white spot near the base externally and a creamy-white stripe at the base beneath. Front tarsi with narrow, white bands on first two segments; mid tarsi with a basal, white band on the first segment, second segment creamy-white with a narrow, apical, black ring; hind tarsi with basal, white rings

about one-fourth to one-fifth the length of the segments, fourth segment white with a narrow, apical, black ring, fifth segment with a very small basal white band. Wing with dense black scales; length: 3·5 mm.

MALE.

Palpi with silvery-white scales forming a ring at about the middle of the long segment, a small, dorsal, sub-basal patch; small spots beneath the bases of the last two segments; occiput with several golden, upright forked scales behind; coloration of thorax and abdomen as in the female; but legs with mid tibia entirely dark, hind tibia with external white spot continuous with ventral stripe, last hind tarsal segment with basal half white. *Hypopygium* (Plate III. fig. 2) as in *C.* (*Stegomyia*) *apicoargentea*, but lobe of side-piece very narrow and furnished with three long bristles.

Co-type ♂♂ (2) and type ♀ bred from larvae taken from a tree-hole, Daru, Sierra Leone, 24.ix.1924, by Professor B. Blacklock and 1 ♂ and 1 ♀ taken from a tree-hole on the Cape Lighthouse Peninsula, near Freetown, 18.ix.1924.

In the specimens from the neighbourhood of Freetown, the third abdominal segment is without a white basal band, in the male this segment is completely dark-scaled, but in the female one or two whitish scales occur in the basal region.

This species is obviously one of the *apicoargentea* series of *Stegomyia* (Edwards, 1925) and appears to come nearest to *A.* (*S.*) *poweri*, Theo., from which it differs in having:—the silvery margin to the eyes not interrupted by dark spots; the large silvery areas on the mesonotum very broadly oval, not crescent-shaped; the basal abdominal bands not dull white, but markedly silvery; the fourth hind tarsal segment not all white and the fifth not all black.

Aedes (*Finlaya*) *longipalpis*, Grünb.

Daru: banana fibre, Mailemma, 1 ♂; tree-root, 3 ♂♂, 1 ♀; hole in tree-root, 5 ♂♂; tree-hole, 1 ♂, 1 ♀; stream, 1 ♀.

Aedes (*Aedimorphus*) *apicoannulatus*, Edw.

Tree-hole, Cape Lighthouse Peninsula, Freetown, 1 ♂, 1 ♀.

Aedes (*Aedimorphus*) *domesticus*, Theo.

Daru : ' Swamp A,' 1 ♀ ; ' Swamp B,' 1 ♂, 1 ♀ ; Moa River, 1 ♂.

Aedes (*Aedimorphus*) *tarsalis*, Newst.

Daru : tree, 1 ♂, 1 ♀ ; Moa River, 2 ♂♂ ; ' Swamp A,' 8 ♂♂, 18 ♀♀ ; ' Swamp B,' 3 ♂♂, 7 ♀♀ ; stream to ' Swamp B,' 1 ♂.

AEDES (*AEDIMORPHUS*) *CUMMINSI* var. *DARUENSIS*, n.var.

This variety differs from typical *A. cumminsi* as follows :— *Head* with the narrow curved scales creamy white. *Mesonotum* with very pale, brassy, rather long, narrow-curved scales and with short, dark-brown, almost hair-like scales concentrated in certain areas as follows :—a narrow, median stripe extending from the anterior border for about two-thirds the length of the mesonotum ; a pair of broadly ovate patches just in front of the sutures ; an inner and outer pair of stripes extending from the ante-scutellar region to beyond the posterior extremity of the middle stripe. *Abdomen* with small, but well-defined median, basal, pale spots on the third to seventh segments. Tibiae with well-marked white, apical spots, femora with apices narrowly pale. *Male hypopygium*—clasper as shown in Plate III, fig. 3.

Type ♂ and type ♀ reared from larvae, Moa River, Daru, Sierra Leone, 18.ix.1924, Professor Blacklock. One other female from the same locality.

The abdomen of the male is greatly contracted so that it is impossible to see whether median spots are present or not.

Mr. F. W. Edwards has kindly examined the type ♀ and tells me that though it seems near the var. *mediopunctata* of *cumminsi*, there are differences, and that in certain aspects it approaches *A.* (*Aedimorphus*) *caliginosus*.

Culex decens, var. *invidiosus*, Theo.

Daru : tree-hole, 1 ♂, 1 ♀ ; ' Swamp A,' 28 ♂♂, 42 ♀♀ ; ' Swamp B,' 1 ♀ ; ' hospital drain area,' 30 ♂♂, 49 ♀♀. Cape Lighthouse Peninsula, Freetown : rock-pool, 1 ♀.

Culex annulioris, Theo.

Daru : ' Swamp A,' 1 ♂ ; latrine washing-bucket, 1 ♂.

Culex thallassius, Theo.

Rock-pool, Cape Lighthouse Peninsula, Freetown, 16 ♂♂, 17 ♀♀.

Culex (*Culiciomyia*) *nebulosus*, Theo.

Daru: banana tree, Mailemma, 19 ♂♂, 14 ♀♀; old mortar, near river, 4 ♂♂; tree-holes, 8 ♂♂, 5 ♀♀; kerosene tin, 1 ♂; stream, 4 ♂♂, 1 ♀; stream near river, 1 ♂.

Lutzia tigripes, var. *fusca*, Theo.

Daru: swamps 'A' and 'B', 5 ♂♂, 5 ♀♀; 'hospital drain area,' 1 ♂, 8 ♀♀.

Toxorhynchites brevipalpis, Theo.

Daru: fork in orange tree, Mailemma, 1 ♀; 'hospital drain area,' 1 ♀.

Eretmapodites chrysogaster, Graham.

Daru: banana tree, Mailemma, 3 ♂♂, 6 ♀♀.

REFERENCES

Edwards, F. W. (1925). Mosquito Notes, V. *Bull. Ent. Res.*, Vol. XV, p. 257.

Macfie, J. W. S., and Ingram, A. (1923). Certain Nurseries of Insect Life in West Africa. *Bull. Ent. Res.*, Vol. XIII, p. 291.

EXPLANATION OF PLATE III.

Fig. 1. *Aedes* (*Stegomyia*) *blacklocki*, sp.n. ♀. × 50 about.

Fig. 2. *Aedes* (*Stegomyia*) *blacklocki*, sp.n. Male hypopygium. *l.*—lobe of side piece; *p.*—phallosome; *pl.*—lateral plate of anal lobe.

Fig. 3. *Aedes* (*Aedimorphus*) *cumminsi* var. *daruensis* var. n. Side-piece of male hypopygium.

FIG. 1

FIG. 2

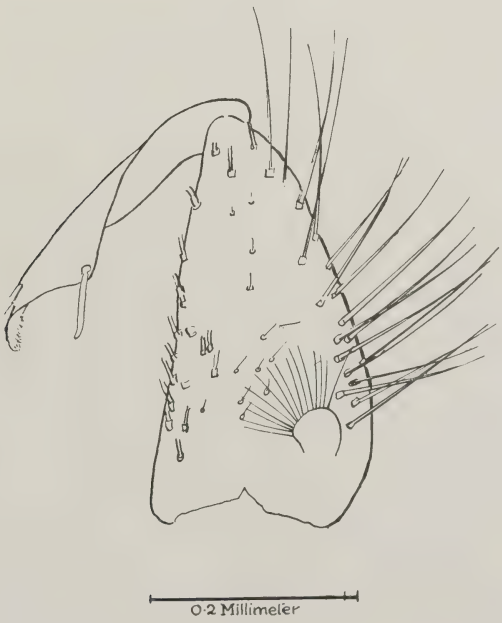


FIG. 3

C. Tinling & Co., Ltd., Imp.

A DISEASE OF FOWLS IN PALESTINE CHARACTERISED BY LEUCOCYTE INCLUSIONS

BY

S. ADLER

(*Microbiological Institute, Jerusalem*)

(*Received for publication* 13 *February*, 1925)

Mr. D. Ury, the director of an experimental poultry farm at Ben-Shemen, Palestine, called my attention to a disease of fowls on his farm which, although in its later stages it resembled spirochaetosis, was not amenable to treatment with atoxyl or neo-salvarsan. The disease is of considerable economic importance, as it attacks Rhode Island and Leghorns and hybrids of the above varieties with native fowls. Native fowls were not observed to be attacked. The first symptoms to be observed were depression and a refusal to take food ; later a tendency to stand still, and fever up to 110° F. ; finally the infected bird was unable to stand, diarrhoea with greenish stools developed and death took place from seven to fourteen days after the commencement of the first symptoms.

Examination of the blood revealed chromatic inclusions in the protoplasm of the leucocytes. The inclusions were of the following varieties :

(1) Minute granules of chromatin surrounded by a vacuole. The protoplasm of cells containing even a few of these forms was often markedly vacuolated.

(2) Small regular rings of chromatin.

(3) Spherical solid masses of chromatin.

(4) Irregular bacilliform masses of chromatin.

(5) Clusters of minute granules of chromatin not lying in vacuoles.

The above kinds of inclusions were also noted inside the nuclei of infected cells.

The normal polymorphs of fowls contain three types of granules.

(1) Spherical granules staining pale red with Romanowsky stains.

(2) Elongated fusiform granules usually staining like eosinophil granules with Romanowsky.

(3) Spherical granules staining deep blue with Romanowsky stains.

From the above types of granules the chromatic inclusions were readily distinguished, being stained with Giemsa like the nuclei of malaria parasites but more brilliantly.

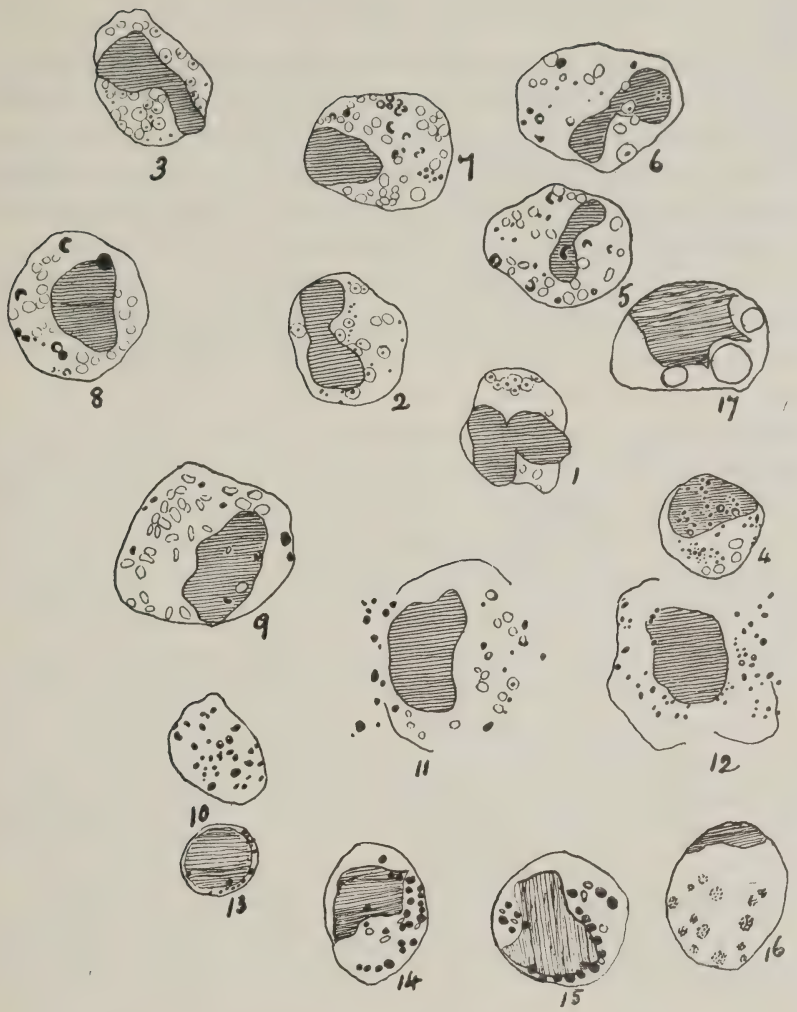
The number of inclusions in a leucocyte varied from one or two to very many ; in some instances the inclusions filled almost the whole cytoplasm of the leucocyte and appeared to escape into the general circulation by bursting the infected cell.

In addition to the inclusion in the leucocytes, small masses of protoplasm containing chromatic rings and solid spheres of chromatin were found in blood smears ; these masses are probably fragments of the cytoplasm of infected leucocytes.

All varieties of leucocytes except eosinophils and mast-cells contained the above-described inclusions, as many as 18 per cent. of the total leucocytes being infected. The nuclei of highly infected cells particularly of lymphocytes, tended to become degenerated and stained feebly with Giemsa in marked contrast to the brilliant staining of the inclusions. In highly infected lymphocytes the nucleus disappeared almost entirely and the cell stuffed with chromatic inclusions had a superficial resemblance to a Koch's blue body ; this form was found particularly in tissue smears. All stages between a slightly infected lymphocyte and the forms resembling Koch's blue bodies were found in tissue smears. A considerable number of the erythrocytes showed basophil staining of the protoplasm and rarely stippling resembling large Schüffner's dots.

Post-mortem the most striking changes were found in the liver and kidneys. The liver was enlarged and soft and studded with white patches ; on section, this organ showed fatty degeneration and infiltration ; the white patches were parts where fatty degeneration was most marked. In the kidneys, patches of necrosis were found.

Smears of the liver, lung, kidneys and spleen showed numerous leucocytes containing inclusions. Auto-erythro-phagocytosis, a phenomenon noted by Levaditi (1914) and by Macfie and Johnston



1 to 3.—Leucocyte with vacuoles some of which contain minute chromatic granules.

4 to 9.—Leucocytes with vacuoles and various forms of inclusions some of them apparently in the nucleus.

10.—Protoplasmic mass containing chromatic inclusions. From a lung smear.

11 to 12.—Leucocytes from which chromatic inclusions are escaping. Figure 11 from a case of *Leukaemia gallinarum*.

13.—An infected Lymphocyte.

14 to 15.—Leucocytes containing solid chromatic inclusions.

16.—A Leucocyte containing clumps of chromatic granules.

17.—An endothelial cell with three fragments of phagocyted erythrocytes.

× 1400.

(1914) in spirochaetosis of fowls was observed in smears of the organs.

The above described leucocytic inclusions were constantly found in this disease and it therefore appears that they are either casually related to the disease or are the effect of the disease on the leucocytes.

Since the disease was not observed in native fowls at Ben-Shemen it seemed probable that these acted as carriers and an examination of apparently healthy native fowls proved this to be the case. Twenty-five apparently healthy native fowls from the Jerusalem market were examined and leucocytic inclusions indistinguishable from those found in the sick fowls at Ben-Shemen were found in three (i.e., 12 per cent.).

Macfie (1914) described an acute disease of fowls in Eket, Nigeria, generally fatal in two days; the disease was characterised in the first stage by a tendency to stand stock-still with head and tail drooping, later the shoulders were hunched up, the head sunk, the tail feathers depressed, the feathers ruffled and the eyelids closed; finally the birds were unable to stand and lay on the ground without attempting to move. Diarrhoea was a marked symptom.

Macfie found in the leucocytes of infected fowls chromatic granules and rings of a type not occurring in healthy fowls. The inclusions appeared in the blood of a healthy native fowl five days after inoculation with the blood of a diseased fowl. The inoculated native fowl, however, showed no ill-effects as a result of the inoculation. In 1915, Macfie found inclusions in the leucocytes of a sick turkey in Accra. Blood from the turkey proved infective to a cock which succumbed ten days after the infection. Inclusions were found in the leucocytes of the cock on the fourth day after the infection.

The leucocytic inclusions described and figured by Macfie appeared to the writer identical with those found at Ben-Shemen. Blood smears from a healthy native fowl and from a sick fowl in Ben-Shemen were sent to Dr. W. Scott Macfie, of the Liverpool School of Tropical Medicine. Dr. Macfie kindly examined the slides and agreed that they contain inclusions indistinguishable from those he found in sick fowls in Eket. He further added that the disease in which he found the leucocytic inclusions was common in fowls in the Gold Coast and Nigeria.

In view of the similarity of the leucocytic inclusions and the pathology of the disease of fowls as found in Nigeria and the disease as found in Palestine, we consider the two diseases to be identical. The fact that the disease as it occurs in Ben-Shemen is of longer duration than the disease described by Macfie in Nigeria, is no evidence against the identity of the two diseases, for in spirochaetosis of fowls there is also an acute form of the disease lasting three to five days and a chronic form lasting about a fortnight after the appearance of spirochaetes in the blood. In Palestine both the acute and chronic form of spirochaetosis of fowls is present, but the chronic form lasting about a fortnight is much the commoner.

The following experiments were carried out :

(1) 4.11.24, blood (2 c.c.) from the wing vein of a healthy native fowl, No. 3, was injected intramuscularly into a healthy native fowl, No. 11. Leucocytic inclusions were found daily in No. 3, since 26.10.24. No. 11 had been examined daily, from 2.11.24 to 4.11.24, and no leucocytic inclusions were found. The total number of leucocytes in No. 11, at the time of the injection, was 8,500 per cmm. Leucocytic inclusions were found in the blood of No. 11, on 9.11.24. The first forms to appear were minute granules lying in vacuoles ; two days later chromatic rings and other forms appeared. On the day the inclusions appeared a leucocytosis of 20,000 per cmm. was observed ; the leucocytosis persisted for several days. Leucocytic inclusions persisted in the blood till 5.12.24. The fowl appeared healthy throughout an observation period of two months.

This experiment was repeated on healthy native fowls, No. 6, No. 7, No. 12, No. 13, with similar results. Chromatic inclusions in the leucocytes appeared five to six days after the injection, the first appearance of the inclusions being accompanied by a leucocytosis in one case, No. 12, up to 34,000 per cmm. Of the five healthy native fowls thus infected, one, No. 7, died ten days after the injection but the others appeared none the worse for the infection.

(2) Blood (2 c.c.) from the wing vein of No. 12 was injected into two healthy native fowls, No. 4 and No. 5, on 13.11.24. No. 4 and No. 5 had been under observation since 2.11.24 and no chromatic inclusions were found in their leucocytes. Chromatic inclusions were found in No. 4 on 19.11.24, and in No. 5 on 20.11.24. Neither of the two injected birds were affected by the injection.

(3) Blood (4 c.c.) from No. 3 was defibrinated and filtered through a Berkfeld filter. The filtrate was injected intramuscularly, on 13.11.24, into healthy native fowls, No. 8 and No. 9. These had been under observation since 2.11.24 and their leucocytes appeared free from the above described chromatic inclusions ; inclusions appeared in the leucocytes of No. 8 on 19.11.24. The bird died on 30.11.24 and smears of the blood and organs showed numerous leucocytic inclusions. Inclusions appeared in the leucocytes of No. 9 on 18.11.24, but no pathological results were noted as a result of the injection. It appears that the minute granules lying in vacuoles are infective, for the other forms of inclusions are too large to pass through a Berkfeld filter.

(4) Blood (2 c.c.) from No. 4 was injected into two healthy Leghorn cocks, No. 26 and No. 27, on 19.12.24. The leucocyte count of No. 26, at the time of the

experiment, was 6,400 per cmm. Chromatic inclusions were found in the leucocytes on 24.12.24. A leucocytosis of 18,000 per cmm. was noticed on the previous day, 23.12.24. Till 26.12.24 the only forms of inclusions noted in the leucocytes were minute granules lying in vacuoles ; on 28.12.24 the other forms appeared. The bird was noticed to be ill on 24.12.24. It refused food and stood perfectly still ; the temperature rose to 110° F. On 30.12.24 diarrhoea was noticed, the stools being greenish and on microscopical examination being found to contain numerous fat globules. Death took place on 31.12.24. Post mortem : the liver was found to be soft and fatty. Blood smears and organ smears showed numerous chromatic inclusions in the leucocytes.

In No. 27, leucocytic inclusions appeared in small numbers on 25.12.24. The leucocyte count on 19.12.24, the day of the injection, was 8,510 per cmm., and it rose to 14,500 per cm. on 25.12.24. The temperature rose to 110° F. on 24.12.24. The bird appeared ill from 24.12.24 till 30.12.24, and then recovered. Chromatic inclusions were present in small numbers in the leucocytes till 18.1.25.

(5) Highly infected blood (1 c.c.) from No. 12 was injected intramuscularly into three pigeons on 13.11.24. Chromatic inclusions such as described above were never found in the leucocytes of the pigeons during an observation period of six weeks.

Observations on healthy native fowls whose leucocytes contained chromatic inclusions showed that the inclusions persisted in the blood during a period varying from one to seven weeks. During this period crises of leucocytosis lasting one to two days were noted at irregular intervals. The leucocyte count rose to 34,000 per cmm. in one case. In this connection it is interesting to note that a blood smear from a sick fowl which died at Ben-Shemen was sent by Mr. D. Ury to the laboratory and a diagnosis of leukaemia was established ; chromatic inclusions were found in the leucocytes, but whether the inclusions were aetiologically related to the leukaemia or whether, as is more probable, the case was a mixed infection of leukaemia and the disease described by Macfie, it is impossible to say, as the author could not find any other cases of *Leukaemia gallinarum* in Ben-Shemen or in Jerusalem. It seems unlikely that the leucocytic inclusions are related to *Leukaemia gallinarum* since, according to Ellerman and Bang (1908), the latter disease has an incubation period of one to two months.

THE RELATIONSHIP OF THE LEUCOCYTIC INCLUSIONS TO THE DISEASE

The constancy with which the leucocytic inclusions are found in the disease and the fact that the inclusions appear regularly in the blood of inoculated fowls, led Macfie to conclude that the inclusions are true parasites belonging probably to the Chlamydozoa and are

causally related to the disease. The above observations support Macfie's view.

That the leucocytic inclusions are not products of cell degeneration is proved by the fact that in highly-infected cells, particularly in lymphocytes, they may be so numerous as to exceed in volume the nucleus of the host cell. Moreover, with Giemsa they stain more brightly than the nucleus of the host cell.

DISTRIBUTION OF THE DISEASE

Since the disease is common in Nigeria and the Gold Coast and is present in Palestine, it seems probable that it is also present throughout the whole of North-west Africa and throughout the whole of North Africa and Egypt, but has hitherto escaped attention owing to its clinical resemblance to spirochaetosis.

TREATMENT

Atoxyl by mouth and neo-salvarsan intramuscularly, did not cause the disappearance of the leucocytic inclusions in healthy native fowls or in sick fowls from Ben-Shemen, and produced no effect on the course of the disease in the latter.

Injections of Bismuth Sodium tartrate (to which fowls are remarkably tolerant, 0·8 gms. per kilo-body-weight producing no ill-effects) also proved useless. The above therapeutic tests suffice to differentiate the disease from spirochaetosis, for spirochaetosis of fowls in Palestine as elsewhere yields readily to treatment with atoxyl or neo-salvarsan and 0·03 gms. per kilo-body-weight of bismuth sodium tartrate was found to be sufficient to cure fowls of spirochaetosis in Jerusalem.

TRANSMISSION

Native fowls from the Jerusalem market and diseased fowls from Ben-Shemen were examined for ectoparasites ; *Mallophaga* sp. were observed and *Argas persicus* was found to be very common ; the experimental farm at Ben-Shemen was found to be heavily infested with *Argas persicus*. It was expected that *Argas persicus* would

prove to be the carrier and the following experiments were carried out :—

(6) Fifty specimens of *Argas persicus*, taken from the farm at Ben-Shemen, were macerated in 10 c.c. saline. After maceration the resulting brown fluid was injected intramuscularly into four native fowls. The injected fluid acted as a strong local irritant and also produced general toxic results and a marked leucocytosis. The fowls appeared depressed for several days after the injection and one died three days later. The other three recovered and none showed the typical chromatic inclusions in their leucocytes during an observation period of two weeks and none developed spirochaetosis.

(7) Three batches, each of ten specimens of *Argas persicus*, which had been kept in the laboratory several months without a feed, were allowed to bite but not to complete a feed on an infected fowl, No. 4, whose blood contained all the above described varieties of leucocytic inclusions ; the ticks were then allowed to bite three native fowls, five, twelve, and twenty days later. In no case did the chromatic inclusions appear in the leucocytes during an observation period of three weeks.

(8) A native fowl whose blood showed a natural infection of the above-described leucocytic inclusion was placed in a cage with nine other native fowls whose blood, at the time (24.10.24), appeared free from the inclusions. The birds were not allowed out of the cage. A month later all were examined and the leucocytic inclusions were found in six.

The above experiments are, however, not sufficient to exclude the probability of *Argas persicus* being the carrier of the disease. Even Experiment No. 8 cannot be regarded as conclusive, for although care was taken to exclude *Argas persicus*, yet this parasite is so common in Palestine that its absence from the cage for a whole month in Experiment No. 8 cannot be guaranteed.

I have to thank Mr. D. Ury, of Ben-Shemen, for supplying me with material ; Dr. A. Felix, Pathologist to the Rothschild Hospital, Jerusalem, for kindly allowing me the use of his laboratory ; and Dr. W. Scott Macfie, for kindly examining blood smears.

SUMMARY AND CONCLUSIONS

A disease of fowls in Palestine characterised by various forms of chromatic inclusions in the leucocytes is described.

The inclusions appear to be identical with the leucocytic inclusions described and figured by Macfie from Eket, Nigeria.

The disease in Palestine is considered to be the chronic form of the disease described by Macfie.

The disease can be transferred to healthy fowls by blood inoculation.

The inclusions appear five to six days after the inoculation of infected blood.

The inclusions are considered to be true parasites belonging to the Chlamydozoa.

Transmission experiments with *Argas persicus* were unsuccessful, but the experiments are not conclusive.

REFERENCES

Ellerman, V., and Bank, O. (1908). Experimentelle Leukämie bei Hühnern. *Centralb. f. Bakt., etc.* 1. Abt. Original, Vol. XLVI, No. 7, pp. 595-609.

Hutyra, F., and Marek, J. (1920). *Spezielle Pathologie und Therapie der Haustiere*, Vol. I, pp. 903-911, 953-958.

Macfie, J. W. S. (1914). Notes on some blood parasites collected in Nigeria. *Annals of Trop. Med. and Parasitol.*, Vol. VIII, No. 3, pp. 439-465.

——— (1916). A further note on a disease of fowls characterised by inclusions in the leucocytes. *Report of the Accra Laboratory for the year* 1915, pp. 68-70. London.

Macfie, J. W. S., and Johnston, J. E. L. (1914). A note on the occurrence of Spirochaetosis of fowls in southern Nigeria. *Annals of Trop. Med. & Parasitol.*, Vol. VIII, No. 1, pp. 41-47.

MISCELLANEA

CEYLON: PARASITE AND SPLEEN RATES. PARASITE RATIOS

The following data have been constructed from tables kindly sent to me by Mr. H. F. Carter, Malariologist, Ceylon :—

CEYLON (9 Provinces).

Spleen Rate

	Children (56372) %
Average	14
Maximum	56
Minimum	1

Parasite Rate

	Children (4647) %
Average	13
Maximum	29
Minimum	2

Parasite Ratios (1206)

	Average %	Maximum %	Minimum %
Malignant tertian	11	17	3
Simple tertian	61	85	57
Quartan	28	43	11
	100		

ANURADHAPURA LOCAL BOARD AREA (Ceylon).

Spleen Rate

	Children (661) %	Adults (1135) %
Average	50	30
Maximum	67	45
Minimum	31	21

Parasite Rate

	Children (300) %	Adults (410) %
Average	41	16
Maximum	85	47
Minimum	11	4

Parasite Ratios (209)

	%
Malignant tertian	10
Simple tertian	44
Quartan	46

J. W. W. STEPHENS

THE HOOKWORMS OF MAN IN SIERRA LEONE

4,305 hookworms were obtained from thirty-eight prisoners, treated by Dr. J. Wood, W.A.M.S., in the Freetown Jail, and from nine post-mortems. All the cases were natives of Sierra Leone.

Only two species, *Necator americanus* and *Ancylostomum duodenale* were found.

Of the total number of hookworms examined 3,929, i.e., 91·3 per cent., were *N. americanus* and 376, i.e., 8·7 per cent., were *A. duodenale*. The largest number of hookworms found in a single case was 483.

	N. Americanus.	*A. duodenale.*
Average number of hookworms per case.....	83·6	8
Highest number found in one case	483	196
Number of females in total number examined	2931	192
Number of males in total number examined	998	174

In *N. americanus* females were about three times as numerous as males, while in *A. duodenale* the sexes were about equal in number. Of the 376 specimens of *A. duodenale*, 302 were recovered from two post-mortems on natives from Rotifunk, in the interior. Excluding these two cases *A. duodenale* formed less than 2 per cent. of the total number of hookworms examined.

The presence of *A. duodenale* in Sierra Leone, both in man and in the civet cat, is of great interest, for according to Darling (1920) this parasite has not been recorded from man in Equatorial Africa. Darling states that *A. duodenale* is the only hookworm recorded from man in North Africa and *N. americanus* the only one recorded from man in Equatorial and South Africa. Sierra Leone is, evidently, intermediate between the zones of distribution of *A. duodenale* and *N. Americanus*, the latter hookworm largely predominating.

S. ADLER

REFERENCES

Adler, S. (1922). Ancylostomes of animals in Freetown. *Ann. Trop. Med. and Parasitol.*, Vol. XVI, No. 3, pp. 293-294.

——— (1924). Ancylostomes in a Leopard. *Ann. Trop. Med. and Parasitol.*, Vol. XVIII, No. 1, p. 128.

Darling, S. T. (1920). Observations on the geographical and ethnological Distribution of hookworms. *Parasitology*, Vol. XII, No. 3, pp. 217-233.

Yorke, W., and Blacklock, B. (1915). Ancylostomiasis in dogs in Sierra Leone. *Ann. Trop. Med. and Parasitol.*, Vol. IX, No. 3, pp. 425-427.

CESTODES FROM EAST AFRICA

The following from a guinea fowl in Kenya Colony, East Africa, and sent by Mr. Brassey Edwards, M.R.C.V.S., were identified :—

Cotugnia digonophora (Pasq., 1890).
Metroliasthes lucida (Ransom, 1900).

M. J. W. Walker

FASCIOLA HEPATICA

'7. *What causes* flounders, real little *flat fish*, brown on one side, white on the other, mouth side-ways, with tail, fins, and all, *leaping alive*, in the *inside* of a rotten sheep's, and every rotten sheep's *liver*?' ('Rural Rides,' William Cobbett, 1853, p. 281.)

J. W. W. Stephens

ON A COLLECTION OF ACANTHOCEPHALA IN THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

BY

T. SOUTHWELL

AND

J. W. S. MACFIE

(*Received for publication* 11 *March*, 1925)

TABLE OF CONTENTS

The examination of this small collection of Acanthocephala has led us to attempt a tentative classification of the numerous genera hitherto described. The classification is based largely on the published descriptions of various authors which, unfortunately, are sometimes incomplete in details, a knowledge of which would

have been of great assistance, since the simplified morphology of the Acanthocephala offers at best but few characters on which to base a classification, and even these are liable to variation. Our work has therefore been one of great difficulty, and the result leaves much to be desired. To classify the group satisfactorily it will be necessary to obtain a much larger collection of species than we have had at our disposal, and a more extensive knowledge of the life history of the various worms.

Amongst the somewhat unsatisfactory characters upon which it has been necessary to base classification, mention should be made of the following :—

(1) *Lemnisci.* Even in mature worms, the length of the lemnisci appears (at least in certain genera) to vary within rather wide limits ; the length also varies, of course, with age ; and, moreover, the length relative to the total length of the body varies somewhat with the state of contraction or relaxation of the worm. From a systematic point of view, therefore, account must be taken of the age of the specimen and of the degree to which it is contracted.

(2) *Testes.* In young worms the shape, size, and relative position of the testes may be quite different from what they are in the adult. Reference has been made to this fact in the description of *M. moniliformis.* The degree of contraction of the body may also alter to some extent the position of the testes in the body and their relationship to each other, and this should be taken into account in those cases in which the position of the testes is of systematic importance.

(3) *Prostatic glands.* No reliance can be placed on the appearance of the prostatic glands of young worms. In mature worms it is frequently extremely difficult to determine the number of prostatic glands, but as some authors attach great importance to it, we have been unable to avoid employing it as a diagnostic character (see ECHINORHYNCHIDAE). Moreover, our experience has convinced us that the shape and arrangement of the prostatic glands are by no means constant, and as diagnostic characters must not be pressed too far, only differences of considerable degree being significant.

(4) *Eggs.* Eggs taken from the body cavity may or may not be fully developed and therefore it is clearly unwise to describe the eggs from specimens obtained in this manner. We have frequently

observed notable differences to exist between the more and the less mature eggs in a single worm. As the characters of the eggs are occasionally of importance, however, and as usually the only eggs available for examination are those taken from the body of the worm, it is important to select for description none excepting those which appear to be mature, namely, those in which the three concentric membranes are clearly defined, and the ring of hooks on the embryo developed. As an aid to the recognition of mature eggs we may say that, so far as our experience goes, the embryos in them are of a brownish colour.

With reference to the retractibility of the proboscis, a distinction must be drawn between a retraction of the entire proboscis or ' proboscis-like structure ' within the anterior part of the body, and a retraction (invagination) of the proboscis within its sheath. In this paper a reference to the proboscis as being retractile means that it is capable of being invaginated into its sheath.

As we employ certain terms in a sense in which they are not used uniformly by other authors the following definitions must be given :—

(1) *Proboscis.* The proboscis, as usually understood, signifies the process at the anterior extremity of the body which is used as an organ of fixation, and which (excepting in *Apororhynchus hemignathi*) is armed with hooks. We consider that this structure is not always morphologically identical, and therefore we propose to limit the term ' proboscis ' to that part of the process at the anterior extremity of the body which lies anterior to the insertion of the proboscis-sheath, and to use the term ' proboscis-like structure ' when referring to the proboscis as understood colloquially. We cannot agree with Lühe and Van Cleave (1916) in considering this unreasonable because it involves the admission that in the genus *Gigantorhynchus* there is little or no true proboscis. On the contrary, we regard it as characteristic of the genus *Gigantorhynchus* that the proboscis is reduced, and maintain that the morphology of the ' proboscis-like structure ' of *G. echinodiscus*, and the forms of the hooks with which that structure is armed, afford strong support to the view that in this species the true proboscis is represented by only the one or two circles of large hooks at the anterior extremity.

(2) *Body.* We define the anterior limit of the body as being situated at the level of the insertion of the lemnisci. This is, of course,

a purely arbitrary definition which, however, we consider necessary for systematic purposes.

(3) *Neck.* Considerable importance is attached to the presence or absence of a neck in the Acanthocephala, and to the presence or absence of hooks on this neck, but there does not appear to us to be any general agreement as to what constitutes a neck, some authors using the term to indicate a zone, often devoid of hooks, at the base of the 'proboscis-like structure,' and others using it in a more restricted sense. We therefore propose to define the neck as being that part of the worm which lies between the base of the proboscis and the anterior extremity of the body, that is, between the level of the insertion of the proboscis-sheath and the level of the insertion of the lemnisci. Thus in the genus *Echinorhynchus* the proboscis, using the term in its colloquial sense, is entirely or almost entirely the true proboscis, in the genus *Gigantorhynchus* it is largely neck, whilst in the genus *Centrorhynchus* it is approximately half true proboscis and half neck.

Classification.—Westrumb, in 1821, briefly reviewed the earliest observations made on the Acanthocephala. The order **Acanthocephala** was established by Rudolphi, in 1809, the following being the characteristics assigned to it by him in 1819:—'Corpus teretiusculum, utriculare, elasticum. Proboscis seriatum uncinata retractilis. Individua alia mascula, alia feminea.' Rudolphi recognised one genus only, namely, *Echinorhynchus*, with the characters of the order. Diesing, in 1851, accepted Rudolphi's classification, recognising only the single genus *Echinorhynchus*, but considered the order **Acanthocephala** to be a tribe which he included in the sub-order **Aprocta.**

Cobbold, in 1879, erected the family ECHINORHYNCHIDAE to accommodate the single genus *Echinorhynchus*, but did not define its characters; and Leuckart, in 1886, used the family name ACANTHOCEPHALIDAE without stating either the characters of the family or the genera he proposed should be included in it, but apparently for the reception of the single genus *Echinorhynchus*.

The first important attempt to split up the Acanthocephala was made by Hamann, who, in 1892 and 1895, divided them into three families as follows:—

(1) ECHINORHYNCHIDAE. Body elongated, smooth. Proboscis-

sheath with double walls ; the proboscis-sheath receives the proboscis. Nerve ganglion in the proboscis-sheath, generally in its depth, centrally placed. Hooks chitinised only at their tips, and with a root-like process below.

Genus *Echinorhynchus ;* with the characters of the family.

(2) GIGANTORHYNCHIDAE. Large species with a segmented, flat, taenia-like body when alive. Hooks like those of *Taenia,* being entirely covered with chitin, and with two root-like processes. Proboscis-sheath muscular, inserted into the proboscis, and into which the proboscis cannot be retracted. Nerve ganglion situated behind the middle of the proboscis-sheath, lying laterally and eccentrically. The body cavity lined by a structureless membrane and traversed by oblique membranes. Lemnisci long coiled tubes with a central canal.

Genus *Gigantorhynchus ;* with the characters of the family.

(3) NEORHYNCHIDAE. Species which become sexually mature in the larval state. Proboscis-sheath a tube with a simple wall. In the skin, and in the lemnisci, are a few giant nuclei. Circular muscles very simply developed ; and the longitudinal muscles only present here and there.

Genus *Neorhynchus ;* with the characters of the family.

Since the publication of Hamann's classical work numerous authors have contributed to our knowledge of this interesting group of parasitic worms, amongst whom especial mention should be made of Lühe, Porta, Van Cleave, and Travassos.

The tentative classification which we propose is as follows. The species which we have had at our disposal are indicated in the body of the paper.

Phylum	NEMATHELMINTHES.
Order	*ACANTHOCEPHALA.*
Sub-order (1)	**Neoechinorhynchidea,** nom. nov.
Family (1)	NEOECHINORHYNCHIDAE Van Cleave, 1919.
Genera	*Neoechinorhynchus* Stiles and Hassall, 1905.
	Tanaorhamphus Ward, 1918.
	Octospinifer Van Cleave, 1919.
	Gracilisentis Van Cleave, 1919.
	Pandosentis Van Cleave, 1920.

Family (2)	QUADRIGYRIDAE Van Cleave, 1920.
Genus	*Quadrigyrus* Van Cleave, 1920.
Family (3)	APORORHYNCHIDAE Shipley, 1900.
Genus	*Apororhynchus* Shipley, 1900.
Sub-order (2)	**Gigantorhynchidea,** nom. nov.
Family (1)	GIGANTORHYNCHIDAE Hamann, 1892.
Genus	*Gigantorhynchus* Hamann, 1892.
Family (2)	OLIGACANTHORHYNCHIDAE, nom. nov.
Genera	*Macracanthorhynchus* Travassos, 1917.
	Oligacanthorhynchus Travassos, 1915.
	Prosthenorchis Travassos, 1915.
Sub-order (3)	**Echinorhynchidea,** nom. nov.
Family (1)	RHADINORHYNCHIDAE Travassos, 1923.
Genera	*Rhadinorhynchus* Lühe, 1911.
	Leptorhynchoides Kostylev, 1924.
	Arhythmorhynchus Lühe, 1911.
	Serrasentis Van Cleave, 1923.
	Telosentis Van Cleave, 1923.
Family (2)	CENTRORHYNCHIDAE Van Cleave, 1916.
Genera	*Centrorhynchus* Lühe, 1911.
	Mediorhynchus Van Cleave, 1916.
	Empodius Travassos, 1916.
Family (3)	CORYNOSOMIDAE, nom. nov.
Genera	*Corynosoma* Lühe, 1904.
	Bolbosoma Porta, 1908.
	Polymorphus Lühe, 1911.
	Filicollis Lühe, 1911.
	Tegorhynchus Van Cleave, 1920.
Family (4)	MONILIFORMIDAE Van Cleave, 1924.
Genus	*Moniliformis* Travassos, 1915.
Family (5)	ECHINORHYNCHIDAE Cobbold, 1879.
Genera	*Prosthorhynchus* Kostylev, 1916.
	Oligoterorhynchus Monticelli, 1914.
	Pomphorhynchus Monticelli, 1905.
	Acanthocephalus Koelreuter, 1771.
	Echinorhynchus Zoega, 1776.

PHYLUM NEMATHELMINTHES.

Order *ACANTHOCEPHALA*.

Nemathelminthes without a gut, and with a proboscis-like structure which is usually armed with hooks.

With three sub-orders.

Key to the Sub-orders of the Order Acanthocephala.

1. Prostatic glands a single syncytial mass..... *Neoechinorhynchidea* (1)
   Prostatic glands not a single syncytial mass.....2
2. Proboscis reduced, not capable of being withdrawn into the proboscis-sheath..... *Gigantorhynchidea* (2)
   Proboscis well developed and capable of being withdrawn into the proboscis-sheath..... *Echinorhynchidea* (3)

Sub-order I. NEOECHINORHYNCHIDEA, nom. nov.

Proboscis usually short and sub-spherical. Proboscis-sheath (when present) a tube with a simple wall. Prostatic gland a single syncytial mass. Nuclei of sub-cuticle and lemnisci few and very large.

The order is divided into three families.

Key to the Families of the Order Neoechinorhynchidea.

1. With a proboscis armed with hooks.....2
   Without such a proboscis..... *Apororhynchidae* (3)
2. Body bearing spines on the anterior region..... *Quadrigyridae* (2)
   Body devoid of spines..... *Neoechinorhynchidae* (1)

Family (1) Neoechinorhynchidae Van Cleave, 1919

Neoechinorhynchidea of small to medium size. Wall of proboscis-sheath a single layer of muscle. Central nervous system near base of proboscis-sheath. Body devoid of spines; spines or hooks on proboscis only. Nuclei of sub-cuticle and lemnisci extremely large, normally of fixed number and definite arrangement, the sub-cuticle with five in the mid-dorsal line of the body and one in the mid-ventral line near the anterior end, and the lemnisci with two in one lemniscus and a single one in the other. Testes elliptical, usually contiguous. Prostatic gland a single syncytial mass containing relatively few

giant nuclei. Eggs where known with three membranes, and without polar capsules. Parasitic* in fish and reptiles (turtles).

The family contains five genera.

Key to the Genera of the Family Neoechinorhynchidae.

1. Proboscis armed with 3 circles of hooks.....2
   Proboscis armed with more than 3 circles of hooks.....3

2. Proboscis armed with 3 circles of 6 hooks each..... *Neoechinorhynchus* (1)
   Proboscis armed with 3 circles of 8 hooks each..... *Octospinifer* (3)
   Proboscis armed with 3 circles of 12 hooks each..... *Gracilisentis* (4)

3. Proboscis several times longer than wide, armed with about 16 to 20 longitudinal rows each composed of about 10 hooks..... *Tanaorhamphus* (2)

   Proboscis short, cylindrical, armed with about 22 longitudinal rows each composed of about 4 hooks... *Pandosentis* (5)

With regard to the last two genera, Van Cleave (1923) states in his key to the genera of Acanthocephala that in the genus *Tanaorhamphus* the proboscis bears 'twenty or more circles of hooks,' and in *Pandosentis* 'eight circles of hooks.' We are unable to harmonise these statements with his earlier generic definitions which we give below.

Genus (1) *Neoechinorhynchus* Stiles and Hassall, 1905.

Synonyms :—*Echinorhynchus* Zoega, in Müller, 1776, in part.
Neorhynchus Hamann, 1892, preoccupied.
Eorhynchus Van Cleave, 1914.

Diagnosis.—Neoechinorhynchidae with short, globose proboscis armed with three circles of six hooks each. Terminal hooks conspicuously larger and heavier than those of remaining rows, and the only ones which bear conspicuous reflexed root-like processes. Each root a broad, flattened disc pyriform in surface view, usually approximately parallel to surface of proboscis wall. The thorn or hook proper attached at the apical or anterior end of the root, and appreciably longer than the root. Parasitic in fish and turtles.

Type species : *N. rutili* (Müller, 1780).

A single species belonging to this genus was found in the collection. This appeared to be a new species and is briefly described below.

* Unless otherwise stated the hosts given in this paper are those in whose alimentary canal the adult worms are found.

Neoechinorhynchus magnus, sp.n.

One immature female specimen only ; host unknown. Townsville, Queensland, Northern Australia. (Dr. P. A. Maplestone).

The specimen measured 90 mm. in length, and the maximum breadth was about 1·5 mm. The body is flattened and tape-like, the anterior extremity being much narrower than the posterior extremity ; the skin is slightly corrugated.

Proboscis. The proboscis is small, sub-globular, and armed, as is usual in the genus, with eighteen hooks in three rows, the anterior six being larger than the rest. The hooks of the terminal circle measure in length from 60μ to 71μ, those of the middle circle 30μ to 37μ, and those of the basal circle about 18μ.

Proboscis-sheath. This measures 0·5 mm. in length and the greatest breadth is 0·2 mm.

Lemnisci. These are slightly unequal in length and measure from five to six times the length of the proboscis-sheath.

The species differs from all other species of the genus in being very much longer.

Genus (2) *Tanaorhamphus* Ward, 1918.

Synonym :—*Neoechinorhynchus* Stiles and Hassall, 1905, in part.

Diagnosis.—Neoechinorhynchidae of small to medium size, with cylindrical proboscis several times longer than wide. Proboscis armed with about sixteen longitudinal rows of hooks. Rows frequently incomplete and imperfect. Prostatic gland of the type characteristic of the family. Parasitic in fish.

Type species : *T. longirostris* (Van Cleave, 1913).

Genus (3) *Octospinifer* Van Cleave, 1919.

Diagnosis.—Proboscis short, globose, usually slightly broader than long ; provided with three circles of eight hooks each. Hooks of terminal circle not much larger or stronger than hooks of middle circle and but little longer than the root-process. Testes elliptical, in contact with each other but not joined by a broad contact-surface. Prostatic gland not in direct contact with posterior testis. The two lemnisci dissimilar in nuclear content, one possessing two giant nuclei and the other a single one. Central nervous-system located at one side of the proboscis-sheath, near its base. Parasitic in fish.

Type species : *O. macilentus* Van Cleave, 1919.

Genus (4) *Gracilisentis* Van Cleave, 1919.

Synonym :—*Neoechinorhynchus* Stiles and Hassall, 1905, in part.

Diagnosis.—Neoechinorhynchidae of small size. Body proper unarmed. Proboscis provided with three circles of twelve hooks each. Each hook ensheathed in a prominent cuticular collar which permits only a small portion of it to protrude from the surface of the proboscis. Each hook of the terminal circle provided with a conspicuous root-process several times longer than the exposed portion of the spine. Root composed of a broad flat basal area which, by gradual diminution in size anteriorly, makes an ill-defined transition from thorn to root. Basal region of terminal roots frequently slightly indented. Hooks of middle circle similar in general form to those of terminal circle, except that root-processes are shorter and less easily observed. Basal hooks without recurved roots. Parasitic in fish.

Type species : *G. gracilisentis* (Van Cleave, 1913).

Genus (5) *Pandosentis* Van Cleave, 1920.

Diagnosis.—Neoechinorhynchidae, with the characters of the family, except for the variation in arrangement of giant nuclei within the sub-cuticle. These do not always lie in the sagittal plane, as in representatives of all the other genera previously included in this family, but are frequently lateral in distribution. Body proper small, devoid of spines. Proboscis short, cylindrical, provided with more than three circles of hooks. Boundary between root and thorn usually not sharply marked. Arrangement of male genital organs as in members of the genus *Gracilisentis*. Testes elliptical, contiguous. Prostatic gland a rounded syncytial mass immediately following the posterior testis, with its posterior boundary indented for the reception of the reservoir of the prostatic gland. Prostatic gland in the only known species contains sixteen giant nuclei. Central nervous system at base of proboscis-sheath. Retractors of sheath emerge from the sheath at its posterior extremity on dorsal and ventral surfaces. Lemnisci not as long as the proboscis-sheath. Parasitic in fish.

Type species : *P. iracundus* Van Cleave, 1920.

Family (2). Quadrigyridae Van Cleave, 1920.

Neoechinorhynchidea of medium size. Anterior body region provided with cuticular spines. Proboscis-sheath enclosed by a single muscular wall. Central nervous system located near the base of the proboscis-sheath. Subcuticular nuclei in anterior region elliptical, in sagittal plane ; in remainder of body a few large, branched nuclei laterally arranged. Parasitic in fish.

The family contains only a single genus.

Genus *Quadrigyrus* Van Cleave, 1920.

Diagnosis.—Quadrigyridae of medium size. Proboscis armed with four circles of hooks. Anterior surface of body usually provided with four circles of cuticular spines. Subcuticular nuclei of two types ; those of anterior part of body ovoid giant nuclei, dorsal and ventral in location ; those in remainder of body a large, central elongated mass, from which heavy lateral projections are given off, usually lateral in distribution. Proboscis-sheath provided with a single, heavy muscular wall. Central nervous system located near posterior extremity of proboscis-sheath. Parasitic in fish.

Type species : *Q. torquatus* Van Cleave, 1920.

Family (3) Apororhynchidae Shipley, 1900.

Neoechinorhynchidea of short form with the body divided into three well-marked regions. The head (proboscis) is pitted but not armed with hooks. There is no eversible introvert, no proboscis-sheath and no armature of hooks. The sub-cuticle and lemnisci have a few giant nuclei, and the lemnisci are long and coiled. Parasitic in birds.

The family contains only a single genus.

Genus *Apororhynchus* Shipley, 1900.

Synonym :—*Arhynchus* Shipley, 1896.

With the characters of the family.

Type species : *A. hemignathi* Shipley, 1896.

With regard to this species, Marval (1905) writes : ' Nous nous permettrons donc, maintenant, de considérer *Arhynchus hemignathi*

comme un *Neorhynchus*, endoparasite comme tous les Acanthocéphales, sans exception, et privé de rostre soit accidentellement ce qui est probable, soit à la suite de longues modifications telles que celles qui se produisent chez l'*Echinorhynchus filicollis* et *sphaerocephalus*, lors de la transformation du rostre en bulle.'

Sub-order II. GIGANTORHYNCHIDEA

Proboscis reduced, often composing only a small part of the proboscis-like structure ; proboscis-sheath with a thick muscular wall into which the proboscis (when present) cannot be retracted, the proboscis-sheath being inserted near the anterior extremity. Neck present. Nuclei of the sub-cuticle and lemnisci relatively small and numerous. Prostatic glands not a single syncytial mass. Parasitic in mammals and birds.

The order is divided into two families.

Key to the Families of the Sub-order Gigantorhynchidea.

Proboscis greatly reduced, represented by only one or two transverse rows of large hooks at the anterior extremity of the proboscis-like structure. Neck armed with numerous small hooks..... *Gigantorhynchidae* (1)

Proboscis sub-spherical, armed with 5 or 6 transverse rows of hooks. Neck unarmed..... *Oligacanthorhynchidae* (2)

Family (1) Gigantorhynchidae Hamann, 1892.

Gigantorhynchidea of large size. Body apparently segmented. Proboscis rudimentary, represented by one or two transverse rows of hooks. Hooks with double roots. Neck armed with numerous small hooks. Lemnisci filiform, with numerous nuclei. Testes ellipsoidal, elongated, situated posteriorly. Prostatic glands subspherical. Parasitic in mammals.

The family contains only a single genus.

Genus *Gigantorhynchus* Hamann, 1892.

Synonym :—*Echinorhynchus* Zoega, 1776, in part.

With the characters of the family.

Type species : *G. echinodiscus* (Diesing, 1851).

Family (2) Oligacanthorhynchidae, nom. nov.

Gigantorhynchidea of small to large size. Body more or less rugose. Proboscis sub-spherical or nail-like, armed with five or six transverse rows of hooks. Hooks (excepting those at the base) with double roots. Neck short, unarmed. Testes ellipsoidal or cylindrical. Prostatic glands eight, ellipsoidal or nail-like. Parasitic in mammals and birds.

The family contains three genera.

Key to the Genera of the Family Oligacanthorhynchidae.

1. Sexual dimorphism well marked; females very large and spirally coiled, males small, comma-shaped. Lemnisci relatively short and flat. Testes situated some distance anterior to the prostatic glands. Genital organs of the male occupying two-thirds of the body cavity..... *Macracanthorhynchus* (1)

   Sexual dimorphism not well-marked. Lemnisci narrow and cylindrical.....2

2. Genital organs of the male situated posteriorly and occupying about a quarter of the body cavity..... *Oligacanthorhynchus* (2)

   Genital organs of the male occupying two-thirds or more of the body cavity..... *Prosthenorchis* (3)

Genus (1) *Macracanthorhynchus* Travassos, 1917.

Synonyms :—*Echinorhynchus* Zoega, 1776, in part.
Gigantorhynchus Hamann, 1892, in part.

Diagnosis.—Sexual dimorphism well-marked ; females very large and spirally coiled, males small, comma-shaped. Proboscis very large. Lemnisci rather short and flat, extending backwards to the anterior testis. Genital organs of the male occupying two-thirds of the body cavity. Testes long, cylindrical. Parasitic in mammals.

Type species : *M. hirudinaceus* (Pallas, 1781).

A single species belonging to this genus was found in the collection, namely :—

Macracanthorhynchus hirudinaceus (Pallas, 1781).

Synonyms :—*Taenia haeruca* Pallas, 1766, preoccupied, in part.
Taenia hirudinacea Pallas, 1781.
Echinorhynchus gigas Bloch, 1782.
Gigantorhynchus gigas of Hamann, 1892.
Gigantorhynchus hirudinaceus of Porta, 1908.

Six females and five males ; host unknown. Hong Kong, January, 1914 (Dr. Bell). Also one male and one female ; host unknown. Kindly lent by A. W. Noel Pillers, F.R.C.V.S., D.V.S.M.

The largest female measured 532 mm. in length, and 9 mm. in greatest breadth. The largest male measured 80 mm. in length, and 6 mm. in greatest breadth.

Genus (2) *Oligacanthorhynchus* Travassos, 1915.

Synonyms :—*Echinorhynchus* Zoega, 1776, in part.
Gigantorhynchus Hamann, 1892, in part.
Hamania Travassos, 1915.
Hamanniella Travassos, 1915.

Diagnosis.—Sexual dimorphism not well-marked. Lemnisci filiform or cylindrical, long, with numerous nuclei. Genital organs of the male situated posteriorly and occupying about a quarter of the body cavity. Testes ellipsoidal. Parasitic in mammals (marsupials and edentates) and birds.

Type species : *O. spira* (Diesing, 1851).

In place of the genus *Oligacanthorhynchus,* Travassos recognises two genera, namely, *Oligacanthorhynchus* and *Hamanniella,* which are very closely allied but, according to Travassos, may be distinguished as follows :—

Prostatic glands ellipsoidal, in pairs. Parasitic in birds..... *Oligacanthorhynchus*
Prostatic glands nail-like, condensed. Parasitic in marsupials and edentates..... *Hamanniella*

The distinction based on the shape of the prostatic glands appears to us to be difficult to make out, and is not clearly shown in at any rate one of Travassos' figures, and therefore we have included both genera in a single genus for which the name *Oligacanthorhynchus* appears to have priority.

A single species belonging to this genus was found in the collection, namely :—

Oligacanthorhynchus microcephalus (Rud., 1819).

Synonyms :—*Echinorhynchus microcephalus* Rud., 1819.
Hamania microcephala Travassos, 1915.
Hamanniella microcephala Travassos, 1915.

One male specimen from the intestine of *Didelphis marsupialis.* British Guiana, 1912 (Dr. Minett).

Unfortunately the proboscis is missing, and consequently a definite identification is not possible. The incomplete worm measured about 35 mm. in length. Our specimen agrees in general with Travassos' figure of the male of this species, excepting that the lemnisci are, relatively, extremely long (about 17 mm.) and extend to the testes. Our specimen is young and consequently much shorter than the fully-developed specimen figured by Travassos, the length of which is given as 150 mm. to 200 mm. ; this fact probably accounts for the apparent difference in the length of the lemnisci in the two specimens.

Genus (3) *Prosthenorchis* Travassos, 1915.

Synonyms :—*Oncicola* Travassos, 1916.
Pardalis Travassos, 1917, preoccupied.
Echinopardalis Travassos, 1918.

Diagnosis.—Oligacanthorhynchidae of small to medium size. Sexual dimorphism not well-marked. Body rugose. Proboscis sub-spherical, armed with five or six transverse rows of hooks. Testes situated in the middle third of the body or more anteriorly ; genital organs of the male occupying two-thirds or more of the body cavity. Ejaculatory canal very long. Parasitic in mammals and birds.

Type species : *P. spirula* (Olfers, in Rudolphi, 1819).

In place of the genus *Prosthenorchis*, Travassos recognises three genera, namely *Oncicola*, *Echinopardalis*, and *Prosthenorchis*, which are very closely allied but, according to Travassos, may be distinguished as follows :—

1. Testes small, round. Prostatic glands large, condensed, situated just behind the testes..... *Oncicola*
   Testes larger, ellipsoidal. Prostatic glands not unusually large.....2
2. Prostatic glands ovoid, in pairs..... *Echinopardalis*
   Prostatic glands ellipsoidal, not in pairs..... *Prosthenorchis*

Some of Travassos' figures, however, do not fully support these distinctions, and therefore we have included all three in a single genus for which the name *Prosthenorchis* has priority.

Two species belonging to this genus were found in the collection, namely :—

Prosthenorchis spirula (Olfers, in Rud., 1819).

Synonyms :—*Echinorhynchus spirula* Olfers, in Rud., 1819.
Echinorhynchus elegans Diesing, 1851.
Prosthenorchis elegans Travassos, 1915.

Nine specimens, including two males, from the intestine of monkeys ; species and locality unknown.

In Travassos' figure of the male of *P. elegans*, the worm is shown to be short and broad, the lemnisci overlap the anterior testis, the two testes strongly overlap, and the prostatic glands are compacted into a single oval mass immediately behind them. In his figure of *P. spirula*, the entire worm is shown elongated, the lemnisci, although long, extend only half-way to the anterior testis, the two testes do not overlap but are situated one behind the other in the middle third of the body, and the prostatic glands are placed single file one behind the other, forming a long cylindrical mass.

Our male specimens show characters intermediate between the two above species ; thus, the body is relatively long, the lemnisci overlap the anterior testis, the testes are slightly separated, lying one in front of the other, and the prostatic glands in one male form a compact mass as in *P. elegans*, but in the other, the anterior glands are drawn out as in *P. spirula*, whilst the posterior glands are compacted as in *P. elegans*.

For the above reasons we consider *P. elegans* is indistinguishable from *P. spirula*.

It may be noted that in our specimens the eggs were similar in shape and size to those figured by Travassos for both the above species, and that the average measurements of ten eggs were 78μ by 47μ.

Prosthenorchis pardalis (Westrumb, 1821).

Synonyms :—*Echinorhynchus pardalis* Westrumb, 1821.
Echinorhynchus ovatus Leidy, 1850.
Echinorhynchus campanulatus Diesing, 1851.
Echinorhynchus oncicola v. Ihering, 1902.
Oncicola oncicola Travassos, 1916.
Pardalis pardalis Travassos, 1917.
Echinopardalis pardalis Travassos, 1918.

Numerous specimens, males and females, from the intestine of *Felis pardus*. Freetown, Sierra Leone, 10.III.1923 (Professor B. Blacklock and Dr. S. Adler).

Size. The females measured from 7 mm. to 14 mm. in length, and from 1·2 mm. to 1·8 mm. in breadth ; only one of the females, viz., the longest, was gravid. The males varied in length from 8 mm. to 15 mm., and in breadth from 1·3 mm. to 1·8 mm. ; only the larger males were mature. Travassos states that *Echinopardalis pardalis* has the following measurements : female, 30 mm. to 40 mm. by 1 mm. to 2·5 mm. ; male, 30 mm. by 1 mm. to 1·5 mm. Our specimens are therefore much smaller than Travassos' specimens of *E. pardalis* and correspond more closely in length to his *Oncicola oncicola* which measures as follows :—female, 10 mm. to 13 mm. by 3 mm. to 4 mm. ; male, 9 mm. to 11 mm. by 2·5 mm. to 3 mm.

Diesing's specimens of *E. campanulatus* measured 6 mm. to 35 mm. in length, and from 2 mm. to 6 mm. in breadth.

Shape of body. Our specimens varied within fairly wide limits ; all were slightly curved, some being cylindrical and tapering at each end, whilst others were more club-shaped, the broad end being anterior. The latter included specimens which were obviously shrunken. The skin, in the majority of the specimens, was smooth and ringed, but in others it was definitely wrinkled or rugose. Our specimens possess a peculiar collar-like structure identical with that figured by Diesing for his *E. campanulatus.* Travassos states that one of the differences between *O. oncicola* and *E. pardalis* is that the former possesses a ' neck ' and the latter does not ; but at the same time he gives Diesing's *E. campanulatus* as a synonym of *E. pardalis.*

Proboscis-sheath. The muscular wall of the proboscis-sheath is very thick and, when viewed in certain positions, resembles the letter ' J.' The central nervous system is situated eccentrically, slightly posterior to the middle, and close to the end of the short limb of the muscular ' J.' The anterior ends of the muscular portion of the proboscis-sheath are connected with the proboscis by non-muscular strands.

Lemnisci. The lemnisci are very long, extending to the posterior third of the body, and often reaching nearly to the posterior extremity. In this character the specimens resemble *O. oncicola.*

Testes. These lie near the middle of the body excepting in one or two specimens in which they are situated immediately behind the proboscis-sheath. The relative position of the testes is perhaps

to some extent dependent, firstly on the body contraction, and secondly on the contraction of the muscles attached to the proboscis-sheath, which tends to move the sheath posteriorly. The testes lie one in front of the other and are about twice as long as broad ; the largest testis measured 0·97 mm. by 0·46 mm. In *E. pardalis* the testes measure 2 mm. to 3 mm. in length by 0·5 mm. in breadth, whilst in *O. oncicola* they measure 0·8 mm. to 1 mm. in diameter.

The testes in our specimens thus resemble those of *O. oncicola* in length, they are intermediate between those of *E. pardalis* and *O. oncicola* in shape and appearance, whilst they resemble those of both species as regards their position.

Eggs. The eggs in our specimens averaged about 65μ by 45μ. Travassos gives the size of the egg of *E. pardalis* as 53μ to 63μ by 38μ to 42μ, and that of *O. oncicola* as 99μ by 71μ to 75μ. The eggs of our specimens thus resemble more closely those of *E. pardalis*.

It will be clear then that our specimens resemble *Oncicola oncicola* in some characters and *Echinopardalis pardalis* in others, and the facts cited above lead us to the conclusion that the two forms are identical, *Oncicola oncicola* being merely the contracted form of *Echinopardalis pardalis*.

Sub-order III. ECHINORHYNCHIDEA

Proboscis well-developed ; proboscis-sheath with double walls (except in the genus *Mediorhynchus*) into which the proboscis can be retracted. Nuclei of the sub-cuticle and lemnisci relatively small and numerous, or with few large, finely dendritic nuclei. Prostatic glands not a single syncytial mass.

The order is divided into four families.

Key to the Families of the Sub-order Echinorhynchidea.

1. Proboscis long, armed with numerous hooks which are stronger on the ventral than on the dorsal aspect..... *Rhadinorhynchidae* (1)
   Proboscis armed with hooks arranged radially and symmetrically.....2
2. Proboscis sheath inserted near the middle of the proboscis-like structure, that is, the neck is armed with spines..... *Centrorhynchidae* (2)
   Proboscis sheath inserted at the base of the proboscis ; neck absent or unarmed.....3
3. Anterior region of body, in males at least, clothed with cuticular spines..... *Corynosomidae* (3)
   Anterior region of body without spines.....4
4. Body moniliform..... *Moniliformidae* (4)
   Body not moniliform..... *Echinorhynchidae* (5)

Family (1) Rhadinorhynchidae Travassos, 1923.

Echinorhynchidea of small to medium size. The anterior region of the body armed with scattered cuticular spines (except in the genus *Leptorhynchoides*). Proboscis long (at least twice as long as broad, and often much longer), usually bent ventrally, and armed with numerous hooks which are stronger on the ventral than on the dorsal aspect. Basal portion of proboscis often without hooks. Neck absent. Proboscis-sheath long. Central nervous system near the middle of the proboscis-sheath. Eggs with or without polar capsules. Parasitic in fish, reptiles and birds.

The family contains five genera.

Key to the Genera of the Family Rhadinorhynchidae.

1. Body not armed with spines..... *Leptorhynchoides* (2)
   Body armed with spines.....2
2. Body with ventral transverse rows of spines..... *Serrasentis* (4)
   Body without ventral transverse rows of spines.....3
3. Posterior extremity of the body in both sexes armed with a few scattered cuticular spines..... *Telosentis* (5)
   Posterior extremity unarmed.....4
4. Body covered anteriorly with scattered, powerful spines; not differentiated into two structurally distinct portions. Proboscis sub-cylindrical, hooks on dorsal and ventral aspects not differing notably in size, but more in a varied formation of their roots..... *Rhadinorhynchus* (1)

   Body with anterior region sharply differentiated structurally. Proboscis spindle-shaped, hooks on dorsal and ventral aspects differing distinctly in size..... *Arhythmorhynchus* (3)

Genus (1). *Rhadinorhynchus* Lühe, 1911.

Synonyms :—*Polyacanthorhynchus* Travassos, 1918.
Echinosoma Porta, 1907, preoccupied, in part.

Diagnosis.—Rhadinorhynchidae with very long, cylindrical proboscis, and very long lemnisci. Anterior portion of body not structurally differentiated from the rest. Body armed at the anterior end with large, scattered, cuticular spines, but without ventral transverse rows of body spines. Parasitic in fish and reptiles.

Type species : *R. pristis* (Rudolphi, 1802).

A single species belonging to this genus was found in the collection, namely :—

Rhadinorhynchus pristis (Rudolphi, 1802).

Synonym :—*Echinorhynchus pristis* Rudolphi, 1802.

Four males from the intestine of *Thynnus vulgaris*. Locality unknown.

Genus (2). *Leptorhynchoides* Kostylev, 1924.

Diagnosis.—Rhadinorhynchidae with very long, slightly club-shaped proboscis, and very long lemnisci. Body not armed with spines ; nuclei dendritic. Parasitic in fish.

Type species : *L. plagicephalus* (Westrumb, 1821).

Genus (3). *Arhythmorhynchus* Lühe, 1911.

Diagnosis.—Rhadinorhynchidae with a long spindle-shaped proboscis ; without ventral transverse rows of body spines. Lemnisci slightly longer than proboscis-sheath. Anterior region of body sharply differentiated from posterior region in structure of body wall ; nuclei present in the sub-cuticle of anterior region only. Parasitic in birds.

Type species : *A. frassoni* (Molin, 1858).

Genus (4). *Serrasentis* Van Cleave, 1923.

Synonyms :—*Echinogaster* Monticelli, 1905, preoccupied.
Echinosoma Porta, 1907, preoccupied.
Lepidosoma Porta, 1907, preoccupied.

Diagnosis.—Rhadinorhynchidae with ventral transverse rows of body spines. Lemnisci very long. Parasitic in fish.

Type species : *S. socialis* (Leidy, 1851).

A single species belonging to this genus was found in the collection, namely :—

Serrasentis socialis (Leidy, 1851).

Synonyms :—*Echinorhynchus socialis* Leidy, 1851, not Leidy, 1856.
Echinorhynchus sagittifer Linton, 1889.
Echinogaster (species not stated) Monticelli, 1905.
Echinosoma sagittifer of Porta, 1907.
Echinogaster sagittifer of Lühe, 1912.

Thirty-five specimens found encysted in the body cavity of *Platycephalus fuscus* (' Flathead '). Townsville, Queensland, Australia, 12.1.1921 (Dr. P. A. Maplestone).

All the specimens were adult but immature; they varied in length from about 3 mm. to 8 mm., and the maximum breadth was about 0·6 mm. The specimens agreed in general with Linton's description of *E. sagittifer*, but the following points of difference were noted:—(1) the number of hooks on the proboscis, counted antero-posteriorly, varied from about sixteen to eighteen, and there were about twenty-four such rows; (2) the number of ventral transverse rows of body spines varied from about fourteen to sixteen. There is no neck. The lemnisci arise at the base of the proboscis, and are very long, extending a little beyond the middle of the body. Central nervous system situated about the middle of the proboscis-sheath.

Van Cleave (1918) re-described the species and later (1923) erected the genus. In his description he states that the number of spines in the ventral transverse rows varied from six to twenty-four; this presumably means on each side as stated by Linton. In our specimens the first row contained about forty-five, the number decreasing in posterior rows.

Genus (5). *Telosentis* Van Cleave, 1923.

Diagnosis.—Rhadinorhynchidae with the posterior extremity of the body adjacent to the genital orifice armed in both sexes with a few scattered cuticular spines. Genital orifice sub-terminal. Parasitic in fish.

Type species: *T. molini* Van Cleave, 1923.

Family (2). Centrorhynchidae Van Cleave, 1916.

Echinorhynchidea of small to medium size. Proboscis-sheath inserted near the middle of the proboscis-like structure; that is to say, the neck is armed with spines. Hooks on the proboscis distinct in type from, and usually larger than, those on the neck. Central nervous system situated near the middle of the proboscis-sheath. Eggs where known without polar capsules. Parasitic in birds.

The family contains three genera.

Key to the Genera of the Family Centrorhynchidae.

1. With three prostatic glands..... *Centrorhynchus* (1)
   With eight prostatic glands.....2
2. Proboscis-sheath with a single wall..... *Mediorhynchus* (2)
   Proboscis-sheath with a double wall..... *Empodius* (3)

Van Cleave (1924) states 'that the names *Heteroplus* and *Mediorhynchus* have been applied to the identical generic concept,' and that, moreover, the generic name *Empodius* is a synonym of *Mediorhynchus*, and has been recognised as such by its author Travassos. As the prior name *Heteroplus* is preoccupied, the valid name for the genus becomes *Mediorhynchus*.

In suggesting this synonymy, Van Cleave has apparently disregarded one of the characteristics of his genus *Mediorhynchus*, namely, that 'the wall of the proboscis receptacle is composed of a single muscular layer instead of two layers' (a feature which is well shown in his figure accompanying his description of the type species *M. papillosus*), for in the genera *Heteroplus* and *Empodius* the proboscis-sheath has a double wall. Again, on comparing the figures given by Van Cleave of the proboscis-like structure of *M. papillosus*, the type species of the genus *Mediorhynchus*, and of *M. grandis*, which was subsequently placed by him in the genus *Heteroplus*, there is seen to be an important difference, the number of longitudinal rows of hooks on the neck being in *M. papillosus* about the same as on the proboscis proper (in this respect resembling species of the genus *Centrorhynchus*), whereas in *M. grandis* they are much more numerous.

Having regard to these two important differences we are unable to accept without further explanation Van Cleave's suggested synonymy, and we therefore recognise in this paper two genera in place of his *Mediorhynchus*.

With regard to the genus *Micracanthorhynchus* Travassos, 1917, Van Cleave maintains that it is a synonym of his *Mediorhynchus*. He bases this conclusion on a re-examination of Rudolphi's type of *E. micracanthus*, a species which Travassos states is closely related to *M. emberizae*, the type species of the genus *Micracanthorhynchus*. Van Cleave has figured the anterior extremity of *E. micracanthus*, and from this figure it appears probable that the species should be referred to the genus *Empodius*.*

Genus (1). *Centrorhynchus* Lühe, 1911.

Synonyms :—*Paradoxites* Lindemann, 1865.
Chentrosoma Monticelli, 1905, in part.

Diagnosis.—Centrorhynchidae having a proboscis-sheath with double walls. Proboscis and neck bearing approximately equal

* See Addendum to this paper, p. 182.

numbers of longitudinal rows of hooks. Prostatic glands three (Van Cleave), long and tubular.

Type species : *C. aluconis* (Müller, 1780 or 1784).

A single species belonging to this genus was found in the collection, namely :—

Centrorhynchus asturinus (Johnston, 1913).

Synonym :—*Gigantorhynchus asturinus* Johnston, 1913.

One male and one female from the intestine of the sparrow-hawk (*Accipiter cirrocephalus*). Townsville, Queensland, Northern Australia (Dr. P. A. Maplestone).

The male measured 18 mm. in length, and the maximum breadth was 0·6 mm. The female measured 25 mm. in length, and the maximum breadth was 0·8 mm. The body is slightly curved and cylindrical ; in both specimens there was a small constriction which, in the male, was situated immediately behind the testes, and in the female a little way behind the ends of the lemnisci. The cuticle is smooth. The proboscis-like structure measures 0·85 mm. by 0·25 mm. ; it is armed with numerous hooks radially arranged in about forty antero-posterior rows of about thirty hooks each. The hooks on that portion of the proboscis anterior to the insertion of the sheath are larger than the rest, and have long rectangular roots. The neck is marked off from the commencement of the body proper by a slight constriction.

Proboscis-sheath. The sheath arises a little anterior to the middle of the proboscis-like structure ; it measures about 1·3 mm. in length, and the maximum breadth is about 0·25 mm. The central nervous system lies a little posterior to the middle of the sheath.

Lemnisci. These organs extend backwards to a level a little posterior to the proboscis-sheath.

Testes. The testes are oval, lie one in front of the other, and are in apposition. They lie immediately behind the proboscis-sheath. Each testis measures about 0·9 mm. in length and 0·28 mm. in breadth.

Prostatic glands. These commence immediately behind the testes and are cylindrical and extremely long.

Female. The posterior extremity of the female is produced into a short, blunt, conical protuberance.

Eggs. These measure about 55μ by 22μ, and are without polar capsules.

Johnston's original description (1913) was based on the examination of a few specimens from *Astur novae-hollandiae* obtained in the neighbourhood of Townsville. He pointed out that the specimens were very much coiled, and we presume he had difficulty in examining them fully because he subsequently published an emended description (1918).

In addition to the two well-preserved specimens described above, we have at our disposal a few other specimens of this species from the same locality, obtained from the intestine of a white goshawk (*Astur novae-hollandiae*). These specimens were very much coiled, as were Johnston's. An examination of these coiled specimens showed clearly that they were morphologically identical with the specimens from *Accipiter cirrocephalus*, but were a little longer and narrower.

We have also examined three male and seven female specimens of the same species from the intestine of a grey goshawk (*Astur clarus*) obtained in the neighbourhood of Townsville, 3.6.1912 (Dr. P. A. Maplestone). The largest female specimen measures 60 mm. in length and 1 mm. in breadth. The terminal papilla noted above is absent, the specimen being distended with eggs.

Also one male and two females, all immature, from the intestine of a brown hawk (*Hieracidea orientalis*) obtained in the neighbourhood of Townsville, 19.6.1913 (Dr. P. A. Maplestone). The only point in these specimens calling for comment is the relative position of the testes which are situated a little in front of the middle of the worm. The prostatic glands are rudimentary. These differences are probably due to the worm being immature.

There seems to be little doubt but that all the forms examined by us are specimens of *Centrorhynchus asturinus* (Johnston, 1913). If this surmise is correct, then Johnston's description can be somewhat amplified by details observed in the better preserved specimens, especially with regard to the number and character of the hooks on the proboscis.

Genus (2). *Mediorhynchus* Van Cleave, 1916.

Diagnosis.—Centrorhynchidae having a proboscis-sheath with a single wall. Longitudinal rows of hooks on the proboscis and neck similar in number. Prostatic glands eight, rounded or pear-shaped.

Type species : *M. papillosus* Van Cleave, 1916.

Genus (3). *Empodius* Travassos, 1916.

Synonyms :—*Heteroplus* Kostylev, 1914, preoccupied.
Micracanthorhynchus Travassos, 1917.

Diagnosis.—Centrorhynchidae having a proboscis-sheath with a double wall. Proboscis and neck bearing different numbers of longitudinal rows of hooks, those on the neck being the more numerous. Prostatic glands eight, rounded or pear-shaped.

Type species : *E. empodius* Skrjabin, 1913.

A single species belonging to this genus was found in the collection, namely :—

Empodius segmentatus (Marval, 1902).

Four females from the intestine of a guinea fowl (*Numida ptilorhynchus*). Transvaal, 1907 (G. Arnold). Also three males and five females from the intestine of a guinea fowl (*Numida ptilorhynchus*). Upper Shire, Nyasaland, 1911 (Professor R. Newstead and Dr. Davey).

The males measured from 62 mm. to 74 mm. in length, and the greatest breadth was 2·3 mm. ; the number of pseudo-segments varied from fifty-eight to seventy-three. The females measured from 65 mm. to 90 mm. in length, and the greatest breadth was 2·3 mm. ; the number of pseudo-segments varied from sixty-three to eighty-eight. The body is tape-like and flattened laterally, and the pseudo-segments extend practically to both extremities ; the body is broadest anteriorly and tapers gradually and continuously towards the posterior extremity. In the female the posterior extremity is bluntly rounded, but in the male, when the bursa is retracted, there are at the posterior extremity two conspicuous lateral folds.

Proboscis. In the majority of our specimens the proboscis is retracted and the anterior extremity of the worm is quite rounded.

In some specimens the proboscis lies slightly ventrally, whilst in others it is median. When the proboscis is completely protruded it is continuous with the anterior part of the body, from which it can only be distinguished by the presence of large hooks. In this condition it is evident that two distinct types of hooks are present on the anterior part of the worm, namely, a few large hooks situated anteriorly, on the proboscis, and a large number of small hooks situated more posteriorly, on the neck. When the proboscis is retracted, as it is in most of our specimens, the cuticle at the anterior extremity is invaginated and consequently the small hooks are more or less hidden, the number visible depending on the degree of retraction.

The proboscis is small and bluntly conical; it measures about 0·25 mm. in length, and its diameter across the base is about 0·4 mm. It is armed with about twenty antero-posterior rows each composed of four hooks; the hooks measure about 45μ to 55μ, and have large root-like processes. On the neck are at least forty antero-posterior rows each composed of about four hooks; the hooks are very delicate, slender, and decrease in size posteriorly, the anterior hooks measuring about 26μ to 40μ in length. These small hooks have no root-like processes.

Proboscis-sheath. The proboscis-sheath has double walls and arises at the base of the proboscis proper; it is slightly curved and tapers a little posteriorly. It measures about 1·2 mm. in length, and its greatest breadth is 0·4 mm. The central nervous system is situated about the middle of the sheath.

Lemnisci. These measure about 3 mm. to 4 mm. by 0·3 mm.

Testes. The position of the testes varies slightly, but in all our specimens they lie in the posterior quarter of the worm. They are separated from each other by a short interval. Each testis is an elongated oval body measuring from 2·7 mm. to 3·8 mm. in length and in greatest breadth from 0·9 to 1·1 mm.

Prostatic glands. These lie a little distance behind the testes, and consist of eight more or less elongated bodies, loosely compacted together. In one male they extended over 7 mm. of the body length, but in another over only 3·9 mm.

Eggs. The average size of ten eggs was 87μ by 50μ.

In 1902, Marval described a worm from *Numida ptilorhynchus*

to which he gave the name *Echinorhynchus segmentatus*. Of this worm he had only a single specimen, the sex of which was not determined, and the proboscis of which was missing. His description, therefore, was necessarily incomplete, but considering the facts that the worm came from the same host as our specimens, that its body was divided into a similar number of pseudo-segments, and that the eggs were alike, we have little hesitation in concluding that it was probably of the same species as our specimens, and accordingly we have adopted Marval's specific name. From our more abundant and complete specimens we have been able to supplement Marval's earlier description.

Family (3). Corynosomidae nom. nov.

Echinorhynchidea of small to rather large size. Anterior region of the body in the males, and (except perhaps in some species of *Filicollis*) in the females also, clothed with closely-set cuticular spines which extend backwards as a mantle for a variable distance. Proboscis armed with hooks arranged radially and symmetrically, i.e., without any distinction in size between those situated dorsally and those situated ventrally. Neck, when present, without spines. Eggs either with or without polar capsules. Parasitic in cetacea, birds and fish.

The family contains five genera.

Key to the Genera of the Family Corynosomidae.

1. Proboscis covered by a thick hyaline membrane beyond which the hooks protrude only a short distance. Central nervous system at anterior end of proboscis-sheath..... *Tegorhynchus* (5)

   Proboscis not covered by such a membrane. Central nervous system at, or posterior to, middle of proboscis-sheath.....2

2. Body proper dilated into a bulb anteriorly..... *Bolbosoma* (2)

   Body proper not so dilated.....3

3. Proboscis bent ventrally at an angle with the axis of the body. Spines on the anterior part of the body extending backwards much further ventrally than dorsally..... *Corynosoma* (1)

   Proboscis not bent ventrally. Spines on the anterior part of the body not extending backwards much further ventrally than dorsally.....4

4. Body sac-like, not notably thickened anteriorly. Prostatic glands irregularly egg-shaped..... *Filicollis* (4)

   Anterior region of body thickened. Prostatic glands tubular... *Polymorphus* (3)

Genus (1). *Corynosoma* Lühe, 1904.

Diagnosis.—Corynosomidae of small to medium size. Body club-shaped, the anterior end thickened but not separated from the posterior part. Spines on anterior part of body extending much further backwards ventrally than dorsally. Genital opening in the male armed with hooks. Proboscis bent ventrally, often spindle-shaped and unarmed at the base. Central nervous system near the middle of the proboscis-sheath. Lemnisci short. Eggs with polar capsules. Parasitic in birds.

Type species: *C. strumosum* (Rudolphi, 1802).

Genus (2). *Bolbosoma* Porta, 1908.

Synonym:—*Bolborhynchus* Porta, 1906, preoccupied.

Diagnosis.—Corynosomidae of rather large size. Body proper dilated anteriorly, a little behind the proboscis, into a bulb. Spines on the anterior part of the body not extending posterior to the dilation. Proboscis short, sub-cylindrical, unarmed at its base. Neck absent. Central nervous system near the middle of the proboscis-sheath. Eggs long and narrow, with polar capsules. Parasitic in cetacea.

Type species: *B. capitatus* (v. Linstow, 1880).

Genus (3). *Polymorphus* Lühe, 1911.

Diagnosis.—Corynosomidae of small size. Body thickened anteriorly, sometimes narrowed immediately behind the spine-bearing region. Spines on the anterior part of the body not extending backwards much further ventrally than dorsally. Genital opening in the male unarmed. Proboscis sub-cylindrical, often unarmed at the base. Central nervous system in the posterior third of the proboscis-sheath. Lemnisci of moderate length. Prostatic glands tubular. Eggs spindle-shaped, with polar capsules. Parasitic in birds.

Type species: *P. minutus* (Zed., 1800).

A single species belonging to this genus was found in the collection, namely:—

Polymorphus minutus (Zed., 1800).

Four females from the intestine of *Anas* sp., Egypt.

The specimens measured about 4 mm. to 6 mm. in length, and the greatest breadth was 1·3 mm.

The body is short and broad, tapering towards the posterior extremity, which is bluntly rounded. In one female the diameter of the anterior part was 1·3 mm., and that of the posterior part was 0·66 mm. Van Cleave states that in the genus *Polymorphus* the 'anterior end of the body is swollen and separated from the more attenuated posterior region by a constriction.' In our specimens this constriction, although clearly present in one specimen, was not obvious in the others, but neither is it shown in Van Cleave's figure of the male *P. obtusus*. The cuticle is smooth, but at the extreme anterior end it is closely beset with minute spines about 18 μ long; those on the ventral surface extend rather further back than those on the dorsal surface, but they do not extend beyond the anterior fifth of the worm, whilst the dorsal spines cover only about 0·4 mm. of the anterior dorsal surface.

Proboscis. The proboscis is situated somewhat ventrally and although bent slightly ventrally it is almost in line with the body. It is sub-cylindrical, slightly narrowed both anteriorly and posteriorly, and unarmed at the base. There is no neck. The length of the proboscis is about 0·5 to 0·6 mm., and its greatest breadth 0·3 mm. The proboscis is armed with numerous large hooks radially arranged and distributed in about sixteen antero-posterior rows each composed of about nine or ten hooks. The largest hooks are situated in the middle of the proboscis, measure about 70μ in length, and have long rectangular root-like processes.

Proboscis-sheath. The sheath is curved in the form of an arc; it measures about 1·3 mm. in length and its greatest breadth is about 0·2 mm. The central nervous system lies a little posterior to the middle of the proboscis-sheath.

Lemnisci. The lemnisci are slightly longer than the proboscis-sheath, and arise at the base of the proboscis.

Eggs. The eggs in the body cavity are long and narrow and vary in size and appearance according to the degree of maturity. The fully mature egg measured about 120μ by 30μ (average of 10) and polar capsules were not seen. The embryo within it is cylindrical,

about 60 μ by 17 μ, with rounded ends ; it is of a light brown colour and its surface presents a pitted or shagreen appearance. The eggs, immediately before becoming mature, show one or two polar capsules at each end, and the contained embryo is transparent. Some eggs thus resemble Lühe's figure of the egg of *P. minutus*, whilst others resemble Marval's figure of the egg of *E. anatis*.

Genus (4). *Filicollis* Lühe, 1911.

Diagnosis.—Corynosomidae of medium size. Body sac-like, anterior part not notably thickened, armed with spines anteriorly which do not extend much further backwards ventrally than dorsally. In the male the spines are well-developed, but in the gravid female they may be very small and well-nigh unrecognisable. Genital opening unarmed. Proboscis spherical or ovate ; in the female the proboscis may be bulbular and bear hooks only on its anterior extremity. Neck long and unarmed. Central nervous system in the posterior third of the proboscis-sheath. Prostatic glands irregularly egg-shaped. Eggs with or without polar capsules. Parasitic in birds.

Type species : *F. anatis* (Schrank, 1788).

Genus (5). *Tegorhynchus* Van Cleave, 1920.

Diagnosis.—Corynosomidae of small size. Posterior extremity of body unarmed ; in the female, terminating in two short, blunt papillae. Proboscis covered by a thick hyaline membrane beyond which the hooks protrude only a short distance. Central nervous system at the anterior extremity of the proboscis-sheath. Lemnisci long, about half the length of the body. Prostatic glands elongated. Parasitic in fish.

Type species : *T. brevis* Van Cleave, 1920.

Family (4). Moniliformidae Van Cleave, 1924.

Echinorhynchidea of medium to large size. Body without spines, and divided into a large number of pseudo-segments. Neck absent. Proboscis well developed, sub-cylindrical, armed with numerous hooks which are small and have only a single, posteriorly directed root. Lemnisci filiform, long, with numerous nuclei. Testes ellip-

soidal, situated quite posteriorly. Prostatic glands eight, almost spherical, compressed. Parasitic in rodents and insectivores.

The family contains only a single genus.

Genus *Moniliformis* Travassos, 1915.

Synonyms :—*Echinorhynchus* Zoega, 1776, in part.
Gigantorhynchus Hamann, 1892, in part.
Hormorhynchus Ward, 1917.

With the characters of the family.

Type species : *M. moniliformis* (Bremser, 1811).

Two species belonging to this genus were found in the collection, namely :—

Moniliformis moniliformis (Bremser, 1811).

Synonyms :—*Echinorhynchus moniliformis* Bremser, 1811.
Gigantorhynchus moniliformis of Railliet *et al.*
Hormorhynchus moniliformis of Ward, 1917.
Echinorhynchus cestodiformis von Linstow, 1904.
Gigantorhynchus cestodiformis of Porta.
Moniliformis cestodiformis of Travassos.

A very large number of specimens were examined, from rats (*Rattus rattus* and *Rattus norvegicus*), collected in Liverpool, West Africa (Freetown and Accra), South America (Manaos), and Australia (Townsville). Also one specimen from man (British Honduras), and numerous specimens from *Cricetomys gambianus* (Accra). An examination of the above specimens led us to the conclusion that they were all of the same species and we give below a general account of its characters.

The males varied in length from 5·5 mm. to 86 mm., and the females from 7 mm. to 239 mm. In worms, from a single host, the size often varied within very wide limits, some being large and fully mature, whilst others were small and incompletely developed.

Shape. In very small and immature worms the body is sub-cylindrical and decidedly broader at the anterior extremity than it is posteriorly. In fully-developed worms, however, the body is flat, tape-like and, excepting at the two extremities, marked out into a large but variable number of pseudo-segments ; the posterior end is somewhat broader than the anterior end.

Proboscis. Relatively short, cylindrical, with a broadly rounded end. Length, 0·5 mm. to 0·67 mm., greatest breadth about 0·2 mm.

Armed with twelve to sixteen (usually twelve) antero-posterior rows each composed of ten to twelve (usually eleven) hooks. The arrangement of the hooks is not quite regular. Hooks in the middle third of the proboscis about 25μ to 30μ in length ; with a single root. The size, shape, and armature of the proboscis are similar in the worms regardless of their size. The proboscis was occasionally found retracted within the sheath, and frequently the entire proboscis was invaginated into the anterior extremity of the worm.

There is no neck, but the proximal end of the proboscis is devoid of hooks.

Proboscis-sheath. Large, with a double muscular wall, arising at the base of the proboscis. Length, 0·5 mm. to 1·3 mm. ; greatest breadth, 0·22 mm. to 0·42 mm.

Lemnisci. Length, 2·4 mm. to 8·76 mm. Narrow, with a few large nuclei. Often very unequal. Lemnisci largest in the largest specimens.

Testes. Situated in the posterior part of the worm where they sometimes cause a slight swelling of the body ; placed close together, the one anterior to the other. In the smallest immature worm examined by us they were sub-spherical and measured respectively 44μ and 63μ in diameter. In all the other specimens they were oval and usually elongated, in length varying from 201μ to 4 mm., and in greatest breadth from 120μ to 0·96 mm. ; as a rule, in fully-developed worms they measured about 2 mm. to 2·5 mm. by 0·4 mm. In one specimen only a single testis was present.

Prostatic glands. Situated a little behind the posterior testis. There are, apparently, eight glands which are compacted together into a single oval mass and are usually individually indistinguishable. The mass of prostatic glands in mature worms varied in length from 0·45 mm. to 3·6 mm., and in greatest breadth from 0·25 mm. to 1·1 mm. In the smallest, immature specimens, the prostatic glands were almost unrecognisable.

Eggs. Rather variable in size and appearance. When fully mature the outer shell is slightly wrinkled and the enclosed embryo brown or dark-coloured. There are no polar capsules. In thirty eggs (ten from each of three females) measured by us, the length varied from 109μ to 137μ, average 123μ, and the greatest breadth from

57μ to 63μ, average 60·5μ. Both larger and smaller eggs were, however, seen in other worms examined. It may be noted here that the smallest female examined by us in which there were eggs of the mature form (but not fully developed), measured only 13 mm. in length. In the same host we observed very much larger females (some 47 mm. long) which were without eggs.

The species is extremely variable in size, both in a single host and in different hosts. The proboscis is, however, remarkably constant in size. There seems to be no justification for dividing up the species on account of the variations in size which it exhibits.

Van Cleave (1924) states that he has examined von Linstow's type specimen of *M. cestodiformis* and that he has discovered no points of difference between this species and *M. moniliformis*. On account of the small size of the proboscis in *Hormorhynchus clarki* Ward, 1917, we are of opinion, however, that this species is probably distinct.

Moniliformis erinacei, sp.n.

One male and one female from the small intestine of a hedgehog (*Erinaceus europaeus*). Accra, Gold Coast, West Africa (Dr. J. W. S. Macfie).

The male measured about 85 mm. in length by 1·6 mm. in maximum breadth ; the female measured about 110 mm. in length by 1·5 mm. in maximum breadth. The entire body, with the exception of the anterior and posterior extremities, is divided up into about 100 obvious pseudo-segments.

Proboscis. The proboscis measured about 0·4 to 0·5 mm. in length by about 0·2 mm. in maximum breadth. It is armed with numerous hooks arranged radially and distributed in eighteen antero-posterior rows each composed of seven to eight hooks, decreasing in size posteriorly. Each hook is short and stout, the largest measuring about 30μ in length.

A pseudo-neck is present, which, when the proboscis is partly retracted, forms a prominent ruff or frill.

Proboscis-sheath. Its length is about 0·8 mm., and its greatest breadth 0·3 mm.

Lemnisci. The lemnisci are long, cylindrical, and relatively

narrow; they measure 7 mm. to 8 mm. in length, and in greatest breadth 0·2 mm.

Testes. The testes are situated quite at the posterior extremity of the body; they are large oval bodies measuring about 5 mm. in length and 1·3 mm. in breadth.

Prostatic glands. These form a somewhat compact mass immediately posterior to the testes.

Eggs. These resemble those figured by von Linstow, and measure (average of 10) 92μ by 51μ.

This worm agrees closely with von Linstow's description of *Echinorhynchus cestodiformis*, excepting that in the type specimens, which were from Nigerian hedgehogs, the lemnisci measured, in length, 1·7 mm. only, whilst in the specimens from the Gold Coast they measured from 7 mm. to 8 mm. Van Cleave (1924), however, has recently examined the type specimen of *M. cestodiformis* and has stated that it does not differ in any respect from *M. moniliformis*, and, therefore, the species described above, which differs from *M. moniliformis* in several respects, such as the size and armature of the proboscis and the dimensions of the eggs, must be regarded as a new species.

Family (5). Echinorhynchidae Cobbold, 1879.

Echinorhynchidea of small to medium size. Body and neck (when present) without spines. Proboscis armed with hooks arranged radially and symmetrically. Eggs with or without polar capsules. Parasitic in mammals, birds, amphibians, and fish.

This family contains a heterogeneous group of species from which, during recent years, a number have been separated as distinct genera, leaving, however, a residue of species, not yet susceptible of more exact classification, in the original genus *Echinorhynchus*.

The genus *Plagiorhynchus* we regard as only another name for *Echinorhynchus*, the species referred to it appearing to be distinct only in the length of the lemnisci, and in that they occur in birds, characters which we do not consider to be of generic importance.

The family contains five genera.

KEY TO THE GENERA OF THE FAMILY ECHINORHYNCHIDAE.

1. With three prostatic glands..... *Prosthorhynchus* (1)
   With four prostatic glands..... *Oligoterorhynchus* (2)
   With six prostatic glands.....2
2. Neck very long, expanded at its anterior extremity into a sub-spherical bulla..... *Pomphorhynchus* (3)
   Neck short or absent, without a bulla.....3
3. Central nervous system at the posterior extremity of the proboscis-sheath..... *Acanthocephalus* (4)
   Central nervous system near the middle of the proboscis-sheath..... *Echinorhynchus* (5)

Genus (1). *Prosthorhynchus* Kostylev, 1916.

Diagnosis.—We have not been able to consult Kostylev's description of this genus, but according to Van Cleave (1923) the following appear to be its chief characteristics. Body without spines. Without giant nuclei. Proboscis very long, cylindrical or clavate, armed with hooks which are arranged radially and symmetrically. Neck short, unarmed. Proboscis-sheath sac-like, with double walls. Prostatic glands three, long and tubular. Parasitic in birds.

Type species: ?

Genus (2). *Oligoterorhynchus* Monticelli, 1914.

Diagnosis.—Echinorhynchidae of medium size. Proboscis sub-cylindrical, small, armed with numerous hooks. Base of proboscis unarmed. Neck absent. Lemnisci a little longer than the proboscis-sheath. Testes oval, situated in the middle third of the body. Prostatic glands four, long, sac-like, narrow. Parasitic in birds.

Type species: *O. campylurus* (Nitzsch, 1866).

Genus (3). *Pomphorhynchus* Monticelli, 1905.

Diagnosis.—Echinorhynchidae of small to medium size. Proboscis sub-cylindrical. Neck very long, cylindrical, excepting at its anterior extremity where in some species it expands into a sub-spherical bulla. Central nervous system at the posterior end of the proboscis-sheath. Parasitic in fish.

Type species: *P. laevis* (Zoega, 1776).

Genus (4). *Acanthocephalus* Koelreuter, 1771.

Synonym :—*Echinorhynchus* Zoega, 1776, in part.

Diagnosis.—Echinorhynchidae of small to large size. Proboscis short, ovate or cylindrical. Neck very short. Central nervous system at the posterior extremity of the proboscis-sheath. Parasitic in amphibians and fish.

Type species : *A. anguillae* (Müller, 1780).

A single species belonging to this genus was found in the collection, namely :—

Acanthocephalus bufonis (Shipley, 1903).

Synonym :—*Echinorhynchus bufonis* Shipley, 1903.

Three females and one male from the intestine of a toad. Hong Kong (Dr. Bell).

The male specimen measured 9 mm. in length, and the maximum breadth was 1·5 mm. ; the females measured from 20 mm. to 25 mm. in length, and the maximum breadth (near the anterior end) was 1·5 mm. to 1·8 mm.

Body cylindrical, slightly thickened anteriorly and tapering a little posteriorly, the posterior extremity being bluntly rounded. The body is curved, especially in the female, and the skin is smooth.

Proboscis. This organ is cylindrical and is situated asymmetrically as pointed out by Shipley ; length 0·5 mm. to 0·6 mm. ; breadth 0·3 mm. It is armed with eighteen to twenty antero-posterior rows each composed of six to eight hooks. The hooks are strongly geniculated at their base and in the middle of the proboscis measure 80μ to 90μ in length. The roots resemble those described by Lühe as present in *E. ranae*, i.e., they have no lateral wing-like expansions.

Proboscis-sheath. This measures about 1 mm. in length and 0·4 mm. in breadth, and is inserted at the base of the proboscis. Neck absent, or extremely short. The central nervous system lies at the posterior extremity of the proboscis-sheath.

Lemnisci. These are about twice as long as the proboscis-sheath and are rather broad.

Testes. These are situated at the beginning of the posterior half of the body ; they measure 0·6 mm. in length by 0·5 mm. in

breadth, and, in the single specimen examined, they lie one in front of the other and are in apposition.

Prostatic glands. These glands are elongated and extend to the posterior margin of the posterior testis.

Eggs. The eggs in the body cavity measured about 75μ by 26μ.

The specimens, therefore, agree with Shipley's description excepting as regards size. They differ from *E. ranae* in (1) the greater length of the lemnisci, and (2) the greater breadth of the eggs.

Genus (5). *Echinorhynchus* Zoega, 1776.

Synonym :—*Plagiorhynchus* Lühe, 1911.

Diagnosis :—Echinorhynchidae of small to large size. Proboscis long, sub-cylindrical, armed with numerous circles of alternating hooks. Hooks of almost uniform size excepting those of the few basal rows which are much reduced. Neck very short or absent. Central nervous system near the middle of the proboscis-sheath. Parasitic in mammals, birds, and fish.

Type species : *E. gadi* Zoega, 1776.

The following species found in the collection are referred to this genus :—

Echinorhynchus bazae, sp.n.

One male and two females from the intestine of a crested hawk (*Baza subcristata*). Townsville, Queensland, Northern Australia, 8.12.1913 (Dr. P. A. Maplestone).

The male measured 33 mm. in length, and the greatest breadth was 2 mm. Both females were incomplete, the fragments measuring 45 mm. and 50 mm. in length respectively, and about 2 mm. in breadth. Body rugose, without spines.

Proboscis. The proboscis is short and broad, slightly constricted about the middle, broadest in the basal half, with a rounded anterior extremity. In the male it measured 0·9 mm. by 0·64 mm., and in the females 1·2 mm. by 0·7 mm. The hooks, which extend to the base of the proboscis, are arranged radially in about thirty-eight to forty-one antero-posterior rows each composed of twelve or thirteen hooks. The hooks on the distal two-thirds are larger than the rest and have long rectangular root-like processes ; the larger (anterior) hooks measure about 90μ in length.

Proboscis-sheath. The proboscis-sheath is inserted at the base of the proboscis. There is no neck. In the male the sheath measured 1·4 mm. by 0·76 mm., and in the females 1·78 mm. by 0·7 mm. The central nervous system lies about the middle of the sheath.

Lemnisci. These are slightly more than twice the length of the proboscis-sheath.

Testes. The testes are situated just posterior to the proboscis-sheath, lie obliquely one in front of the other, and measure about 1·5 mm. by 0·66 mm.

Prostatic glands. There are, apparently, six very long cylindrical prostatic glands terminating immediately behind the posterior testis.

Eggs. These measure 78μ by 41μ ; they have no polar capsules.

Echinorhynchus bulbocaudatus, sp.n.

Very numerous specimens from a bush pheasant (*Centropus phasianus*). Townsville, Queensland, Northern Australia.

The females measured about 58 mm. in length, and in greatest breadth about 1·1 mm. The male (we had only one adult at our disposal) measured about 26 mm. in length, and the greatest breadth was 0·9 mm. The cuticle is smooth. The worms are long and cylindrical. In the female the terminal 3 mm. is oval and dilated, and the body ends in a point.

Proboscis. The shape of the proboscis varies from oval to subspherical. It is small and arises somewhat obliquely. The proboscis is separated from the body by a short neck (about 0·2 mm. long), which is devoid of hooks. When the proboscis is partly retracted, as it is in most of our specimens, the anterior portion of the body overhangs its base and the cuticle is folded so as to resemble a frill or ruff. The proboscis measures about 0·5 mm. to 0·7 mm. in length, and 0·4 mm. to 0·5 mm. in greatest breadth. It is armed with numerous hooks, arranged radially and distributed in about twenty-eight antero-posterior rows, each composed of about nine hooks. The hooks in the fourth and fifth rows are the largest and measure about 45μ in length. Each hook is provided with a conspicuous, long, rectangular root, slightly hollowed out at its posterior margin.

Proboscis-sheath. This arises a little anterior to the base of the proboscis-like structure, that is, there is a short neck which is unarmed. In the male the proboscis-sheath measured 1·46 mm.

by 0·24 mm., and in the female 1·5 mm. to 1·6 mm. by 0·27 mm. The central nervous system lies in the anterior half of the sheath.

Lemnisci. These are rather more than twice the length of the proboscis-sheath and are massive ; in the male they overlap the anterior testis.

Testes. These are situated obliquely one behind the other and they overlap ; they lie about 0·7 mm. behind the proboscis-sheath. Each testis measures about 1 mm. by 0·6 mm. In an immature specimen, however, the testes were well separated from each other, and were situated more posteriorly.

Prostatic glands. These are, apparently, six in number, long and tubular, extending to the posterior testis.

Eggs. These measure about 60μ by 30μ and are without polar capsules.

Echinorhynchus clavula Dujardin, 1845, *nec* Hamann.

Three females and one male from the body cavity of a sea bream (*Sparus berda*). Townsville, Queensland, Northern Australia, 8.11.1920 (Dr. P. A. Maplestone). Also two males and one female from the intestine of a ' yellow tail ' (*Trachurus declivis*), Australia, 8.11.1920 (Dr. P. A. Maplestone).

Echinorhynchus gadi Zoega, 1776.

Synonym :—*Echinorhynchus acus* Rud., 1802 (according to Lühe, 1911).

Four females and one male from the intestine of a haddock. Townsville, Queensland, Northern Australia (Dr. P. A. Maplestone). Also one gravid female from the intestine of a pollack (*Gadus pollachius*). Port Erin, Isle of Man (Dr. Annett). Also very numerous males and females from a codling, North Sea, October, 1922 (Professor James Johnstone).

Lühe gives the size of the eggs as 76μ by 13μ, but in our specimens they were larger and measured 107μ by 24μ (average of 10).

Echinorhynchus truttae Schrank, 1788.

Synonym :—*Echinorhynchus fusaeformis* Zeder, 1803.

Six females and six males from a trout, 11.1.1923 (A. W. Noel Pillers, F.R.C.V.S.). The females varied in length from about 11 mm. to 19 mm. They are broadest near the anterior extremity,

the maximum breadth being 1·2 mm. The largest male measured 10 mm. in length, and had a maximum breadth of 0·86 mm.

Lühe gives the size of the egg as 100μ to 110μ in length by 23μ to 24μ in breadth; v. Linstow states that they measure 136μ to 140μ by 23μ to 26μ. The average of ten eggs in the body cavity of one of our females was 137μ by 26μ.

Three females and four males from the body cavity of a sea bream (*Sparus berda*), 20.9.1920.

Three females and one male from the intestine of a fish ('grunter'). Townsville, Queensland, Northern Australia, 3.10.1920 (Dr. P. A. Maplestone). In these specimens the lemnisci extended slightly beyond the extremity of the proboscis-sheath.

Genus. *Lueheia* Trav., 1919 (?).

Travassos recently (1923) described under the name *Lueheia lueheia* a species of *Acanthocephala* obtained from *Thamnophilus severus* and *T. guttatus* with the following characters:—'Body broad, thick, fusiform, having large folds, milky-white in colour, measuring about 7 mm. in the case of the male and 12 mm. in the female in length, by 1·2 to 1·8 mm. in greatest breadth; proboscis slightly globose, not invaginable in the adult, but retractile into the extremity of the body, measuring about 0·43 to 0·52 mm. in length by 0·38 to 0·46 in greatest breadth, furnished with 22 to 24 longitudinal rows of eight or nine hooks each; the hooks increase in size from the head to the more enlarged part; from thence, as far as the base, they grow progressively smaller; these hooks are comparatively strong, and of three chief types, the anterior hooks are delicate, those in the middle are very strong and **U**-shaped, and finally, those at the base are falcated.

Measurement of hooks:—

Specimen.	Base.	Lamina.
1	0·037 mm.	0·034 mm.
2	0·037 mm.	0·042 mm.
3	0·054 mm.	0·045 mm.
4	0·068 mm.	0·059 mm.
5	0·071 mm.	0·059 mm.
6	0·048 mm.	0·054 mm.
7	—	0·048 mm.
8	—	0·048 mm.

Neck absent; sheath of proboscis club-shaped, measuring about 0·78 to 1 mm. in length by 0·26 to 0·27 mm. in greatest breadth; lemnisci six in number, cylindrical, straight in the female, curved in the male, measuring more or less 1·9 to 2·8 mm. in length; testes ellipsoid, situated some distance from the sheath but in contact with, or partly over-lapping, the lemnisci, measuring about 0·7 by 0·3 mm.; prostatic glands in contact with the nearest testis, elongated, voluminous, measuring about 1·3 mm. in length; deferent canals showing symmetrical extensions to the level of the nearest third of the prostatic glands, and joining up at the level of the most remote third, to form a voluminous seminal vesicle, in the shape of a very thick **Y** which straightens out to form the ejaculatory canal. The ejaculatory canal and the ducts of the prostatic glands measure about 0·7 mm. in length; the copulative pouch is comparatively small; eggs bearing bacilliform nuclei and without polar capsules, measuring, in the median plane, 0·078 to 0·075 mm. in length by 0·028 to 0·31 mm. in greatest breadth; small egg-ejector 1 to 1·5 mm. long.

Habitat. Small intestine of *Thamnophilus severus* and *Th. guttatus.*

Specimens in the Oswaldo Cruz Institute, No. 1888, Angra dos Reis, Rio.

This species is very closely related to *L. inscripta* W., from which it is distinguished by a proboscis with a greater number of longitudinal rows of hooks, and a greater number of hooks in each row, the hooks themselves being also larger.

'The walls of the body appear less rugose, and in this species there is a difference in the structure of the peripheral stratum near the middle of the walls of the body, where it is clear in comparison with Westrumb's species, a difference appreciable even in specimens prepared whole. The differences in the male genital organs, which are not placed within strictly defined limits, can scarcely be observed in the very young male specimen of *L. inscripta.* It is interesting to note that while the two species exist side-by-side in the neighbourhood of Angra dos Reis, yet *inscripta* is rare and generally found as isolated specimens; the other is common and found in large numbers in every carrier.'

We have, unfortunately, been unable to obtain Travassos's

description of the genus *Lueheia* and no figures of *Lueheia lueheia* are given. Travassos apparently places the genus in the sub-family CENTRORHYNCHIDAE.

The somewhat reduced proboscis, which in the adult is not retractile within its sheath, are characters which ally the species to the OLIGACANTHORHYNCHIDAE, and especially to the genus *Oligacanthorhynchus* or the genus *Prosthenorchis*, but on the other hand the body is small, the proboscis bears numerous hooks and the worm is found in birds, characters which suggest affinities with the *Echinorhynchidea*.

The species *L. lueheia* is, however, unique in possessing six lemnisci instead of two. Until we know whether the proboscis sheath has a single or a double wall, where the sheath arises and how many prostatic glands are present, it is impossible to classify the genus satisfactorily, but in any case the presence of six lemnisci is a character sufficiently striking to identify the species ; although there is, of course, the possibility (amounting, in this case, to probability) that the number ' 6 ' occurring in the description of the lemnisci is really a misprint for ' 2.'

ADDENDUM

Whilst this paper was in page proof Travassos' supplement to the Revision of the Family Gigantorhynchidae (1924) has come to hand.

In this paper he includes the genera *Micracanthorhynchus* Travassos, 1916 ; *Empodius* Travassos, 1916 = *Heteroplus* Kostylev, 1914, n.p. ; *Mediorhynchus* Van Cleave, 1916 = *Micracanthorhynchus* Travassos, 1917, in the family *Gigantorhynchidae*, and he defines the genera *Empodius* and *Mediorhynchus* as follows :—

Empodius. Proboscis armed with four transverse series of relatively large hooks and about fourteen longitudinal series of hooks with two hooks in each series. Neck sharply differentiated and armed with hooks having simple roots. Sheath of the proboscis *not* invaginable. Eggs with concentric membranes. Intestine of birds.

Mediorhynchus. Proboscis armed with from ten to twelve

transverse series of relatively small hooks and with about twenty longitudinal series of hooks, with five or six hooks in each series. Neck well differentiated and armed with small simple hooks. Hooks of the proboscis and neck situated in the centres of papilliform projections. Sheath of proboscis slightly developed. Proboscis not invaginable. Eggs with concentric membranes. Intestine of birds.

We agree that the two genera are distinguishable, but as we have found the proboscis invaginable in *E. segmentatus* we refer them to the Sub-order **Echinorhynchidea.**

REFERENCES

(To recent literature only.)

Chandler, Asa C. (1921). Notes on the Occurrence of *Moniliformis* sp. in Rats in Texas. *Jour. Parasitol.*, Vol. VII, pp. 179-183.

Johnston, T. Harvey (1911). *Echinorhynchus pomatostomi* (n. sp.), a subcutaneous parasite of Australian Birds. *Proc. Roy. Soc. of N.S. Wales*, Vol. XLV, pp. 111-114.

——— (1914). Some New Queensland Endoparasites. *Proc. Roy. Soc. of Queensland*, Vol. XXVI, pp. 76-84.

——— (1918). Notes on Miscellaneous Endoparasites. *Proc. Roy. Soc. of Queensland*, Vol. XXX, pp. 209-218.

Kostylev, N. (1924). La genre *Leptorhynchoides*, nouveau genre d'Acanthocephale parasite des poissons. *Annales de Parasitol. Humaine et Comparée*, Vol. II, pp. 214-223.

v. Linstow (1904). Neue Helminthen aus Westafrika. *Centralblatt f. Bakt., Parasit., u Infekt.*, Vol. XXXVI, Orig., pp. 379-383.

Linton, E. (1889). IV. Notes on Entozoa of Marine Fishes of New England, with descriptions of several new species. *U.S. Fish Commission Report.*

Lühe, M. (1911). Die Süsswasserfauna Deutschlands, Heft 16, Acanthocephalen. Jena.

——— (1912). Zur Kenntnis der Acanthocephalen. *Zool. Jahrb., Suppl.*, Vol. XV. Festschrift, J. W. Spengel, Vol. I, pp. 271-306.

de Marval, L. (1905). Monographie des Acanthocéphales d'Oiseaux. *Revue Suisse de Zoologie*, Vol. XIII, pp. 195-386 (and long list of references to papers published previous to 1905).

Monticelli, F. S. (1914). Sull' *Echinorhynchus campyluris* Nitzsch. *Bol. della Soc. di Nat. in Napoli*, Vol. XXVII, pp. 112-128.

Porta, A. (1906). Ricerche anatomiche sull' *Echinorhynchus capitatus* v. Linst., e note sulla sistematica degli echinorinchi du cetacei. *Zool. Anzeig.*, Vol. XXX, pp. 235-271.

——— (1908). Gli Acantocefali dei Mammiferi. *Arch. de Parasitol.*, Vol. XII, pp. 268-282.

Shipley, A. E. (1900). Entozoa. *Fauna Hawaiiensis*, Vol. II, pt. 4, pp. 427-441.

——— (1903). On the Ento-Parasites collected by the 'Skeat Expedition' to Lower Siam and the Malay Peninsula, in the years 1899-1900. *Proc. Zool. Soc.*, 1903, Vol. II, pp. 145-156.

Skrjabin, K. I. (1913). Zur Acanthocephalen—Fauna Russisch Turkestans. *Zool. Jahrb.*, Vol. XXXV, pp. 403-413.

Travassos, L. (1915). Revisao dos *Acantocephalos brazileiros*, I. Familia *Gigantorhynchidae*, Hamann, 1892. *Brazil Medico*, 8 May, 1915, p. 137.

——— (1915). Revisao dos *Acantocephalos brazileiros*, II. Familia *Echinorhynchidae*, Hamann, 1892. *Brazil Medico*, 18 Dec., 1915, p. 377.

——— (1917). Revisao dos *Acantocefalos brazileiros*. *Memor. do Inst. Oswaldo Cruz.*, Vol. IX, pp. 5-62.

——— (1923). Informaçoes sobre a fauna helminthologica de Matto Grosso., II. Acantocephalos. *Folh. Med. Ann.*, Vol. IV, p. 12.

——— (1923). Contribuicao para o conhecimento dos Acantocefalos da sub-familia *Centrorhynchidae*. *A Folha Medica*. Anno IV, N. 4, pag. 29 de 15 de Fevereiro.

——— (1924). Contributions à l'étude de la helminthologique du Brésil. XVII. Revision des *Acanthocéphales brésiliens*, I. La famille *Gigantorhynchidae* Hamann, 1892, Supplément. *Memorias do Instituto Oswaldo Cruz*. Tomo XVII. Fascicule II, pp. 377-287.

Van Cleave, H. J. (1915). Acanthocephala in North American Amphibia. *Jour. Parasitol.*, Vol. I, pp. 175-178.

——— (1916). *Filicollis botulus*, n.sp., with notes on the characteristics of the genus. *Trans. Amer. Micros. Soc.*, Vol. XXXV, pp. 131-134.

——— (1916). A Revision of the Genus *Arhythmorhynchus*, with descriptions of two new species from North American Birds. *Jour. Parasitol.*, Vol. II, pp. 167-174.

——— (1916). Acanthocephala of the Genera *Centrorhynchus* and *Mediorhynchus* (new genus) from North American Birds. *Trans. Amer. Microscop. Soc.*, Vol. XXXV, pp. 221-232.

——— (1918). The Acanthocephala of North American Birds. *Trans. Amer. Microscop. Soc.*, Vol. XXXVII, pp. 19-48.

——— (1918). *Centrorhynchus pinguis*, n.sp., from China. *Jour. Parasitol.*, Vol. IV, pp. 164-167.

——— (1918). Acanthocephala of the Sub-family *Rhadinorhynchidae*, from American fish. *Jour. Parasitol.*, Vol. V, pp. 17-24.

——— (1919). Acanthocephala from the Illinois River, with descriptions of species and a synopsis of the Family *Neoechinorhynchidae*. *Bull. Illinois Nat. Hist. Survey*, Vol. XIII, pp. 225-257.

——— (1920). Acanthocephala collected by the Swedish Expedition to the Juan Fernandez Islands (1916-1917). *The Nat. Hist. of Juan Fernandez and Easter Island*, Vol. III, pp. 75-80.

——— (1920). Acanthocephala of the Canadian Arctic Expedition, 1913-1918. *Report of the Canadian Arctic Expedition*, 1913-18, Vol. IX, Part E, pp. 3-11.

——— (1920). Notes on the Life Cycle of Two Species of Acanthocephala from Freshwater Fishes. *Jour. Parasitol.*, Vol. VI, pp. 167-172.

——— (1920). Two New Genera and Species of Acanthocephalous Worms from Venezuelan Fishes. *Proc. U.S. Nat. Mus.*, Vol. LVIII, pp. 455-466.

——— (1921). Acanthocephala parasitic in the dog. *Jour. Parasitol.*, Vol. VII, pp. 91-94.

——— (1923). Telosentis, a New Genus of Acanthocephala from Southern Europe. *Jour. Parasitol.*, Vol. IX, pp. 174-175.

——— (1923). A Key to Genera of Acanthocephala. *Trans. Amer. Microsc. Soc.*, 42, pp. 184-191.

——— (1924). A Critical Study of the Acanthocephala described and identified by Joseph Leidy. *Proc. Acad. Nat. Sci. Philadelphia*, Vol. LXXVI, pp. 279-334 (and other references to recent literature).

Ward, H. B. (1917). "Echinorhynchus moniformis" in North America. *Jour. Parasitol.*, Vol. III, p. 141.

——— (1917). On the structure and classification of North American parasitic worms. *Jour. Parasitol.*, Vol. IV, pp. 1-11.

Zschokke, F., and Heitz, A. (1914). Entoparasiten aus Salmoniden von Kamtschatka. *Revue Suisse de Zoologie*, Vol. XXII, pp. 197-256.

TETRACAMPOS Wedl 1861 AS A GENUS OF THE BOTHRIOCEPHALIDAE

BY

W. N. F. WOODLAND

(*Wellcome Bureau of Scientific Research, Endsleigh Gardens, London, N.W.*1)

(*Received for publication* 15 *April*, 1925)

In 1913, Southwell very briefly described from the Indian Siluroids *Ophiocephalus striatus*, *Labeo rohita* and *Wallago attu* a Cestode which, from the characters of the scolex, he identified as *Ophryocotyle bengalensis*, i.e., as one of the Davaineidae. In 1924, I described in some detail the anatomy of two species of Proteocephalids also from Indian Siluroids, viz., *Wallago attu* and *Macrones seenghala*, which I provisionally named *Gangesia wallago* and *G. macrones*, and I contended that the former species was almost certainly identical with Southwell's *'Ophryocotyle' bengalensis*. Southwell (1925) admits that my contention was correct, whence it follows that the specific name of my first species, assuming the retention of the genus *Gangesia*, should read *Gangesia bengalensis* (Southwell 1913).

This brief resumé of the history of this species serves to show that external characters, and especially scolex characters, cannot always be depended upon as a guide for the correct allocation of a new species in any modern system of classification. Southwell, however, has apparently not taken this view of the matter since in the communication referred to (1925) he revives an ancient undefined and inadequately-described genus first created by Wedl in 1861, viz., *Tetracampos*, and argues, once more chiefly on the basis of scolex characters, that *Gangesia bengalensis* is a second species of this genus, and that the name *Gangesia* must, therefore, lapse. This assertion that Wedl's species *Tetracampos ciliotheca* from the Nile Siluroid *'Heterobranchus' anguillaris* (= *Clarias lazera* according to Boulenger) was a Proteocephalid is very questionable. Wedl's other species (and new genus) *Marsypocephalus rectangulus* was

undoubtedly a Proteocephalid, as I have shown in a forthcoming paper (Woodland 1925), but there is every reason to believe, with La Rue* (1914), that *Tetracampos ciliotheca* was a Bothriocephalid, and I propose to give the reasons for that belief, but before doing so, it will be as well to state the evidence offered by Southwell in favour of *Tetracampos* belonging to the Proteocephalidae. This evidence, when examined, appears to consist solely of the general statement that ' the adult cestode parasites most common in fresh-water fishes belong to the genus *Proteocephalus*,' and the very superficial resemblance of Wedl's drawing of the scolex of *Tetracampos ciliotheca* to the scolex of *Gangesia bengalensis* (!). As regards the general statement, this is of course true enough, but Southwell omits to mention the fact that Bothriocephalids are also sometimes to be found in fresh-water fishes, and that at least one, and a very well-known one, viz., *Polyonchobothrium polypteri*, is to be found in a fresh-water fish from the Nile, viz., *Polypterus bichir*. I have also recently described (Woodland 1925) a new species of *Clestobothrium*—*C. clarias*—from a Nile Siluroid, *Clarias anguillaris*. As regards Southwell's comparison of Wedl's drawing of the scolex of *Tetracampos ciliotheca* with the scolex of *Gangesia bengalensis*, I may point out that the hooks of the two scolices are very different in form, and that whereas those of *Tetracampos* are in four groups and vary in size, those of *Gangesia* form a single complete circle and are of the same size, and that Southwell's remark that ' it is impossible to decide from Wedl's figure and descriptions,' whether Wedl's four ' Lappen ' (' Jeder Lappen besteht aus einem dünnwandigen, contractilen Parenchym und ragt an der Aussenseite des Kopfes als eine platte Scheibe hervor . . . Nach vorne sind diese Hautlappen (Bothridien van Beneden) näher an einander gerückt und umkreisen eine kuppelförmig hervorragende, bewaffnete Papille.') are ' really outgrowths from the head or whether they are true acetabula ' is certainly no justification for his implied assumption that they *are* outgrowths which bear acetabula, such as exist in Proteocephalids. The foregoing constitutes the whole of the actual evidence offered by Southwell in support of his contention, though in further support of his view he has gone so far as to conclude that Wedl erred in

* *Tetracampos ciliotheca*, ' because of its ventral genital pore, ciliated embryo and two bothria, evidently belongs to the order Pseudophyllidea.' (La Rue.)

describing the genital openings as being situated on the ventral surface.

A careful examination of Wedl's figures and description affords, I think, decisive evidence that *Tetracampos ciliotheca* was a Bothrio-

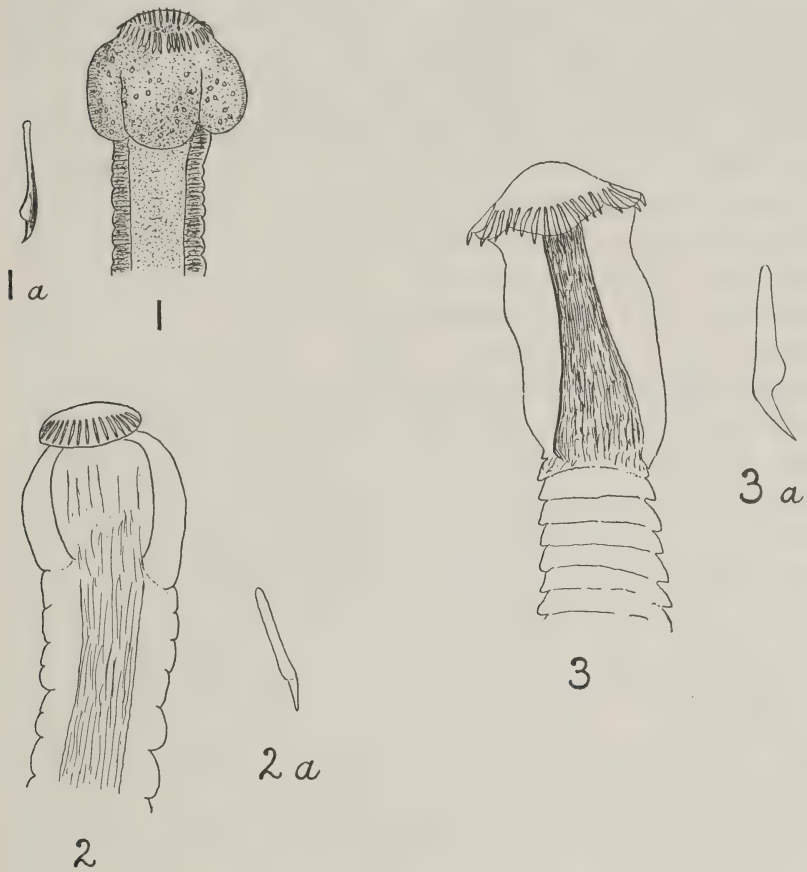


Fig. 1. Approximate copy of Wedl's figure of *Tetracampos ciliotheca*. Magnification about 100.
Fig. 1a. Approximate copy of Wedl's figure of a hook on the scolex of *T. ciliotheca*. Magnification unknown.
Fig. 2. Contracted scolex of *Clestobothrium clarias* Woodland. × 87.5.
Fig. 2a. Hook from scolex of *C. clarias*. × 395.
Fig. 3. Scolex of *Polyonchobothrium polypteri* Leydig. × 56.
Fig. 3a. Hook of scolex of *P. polypteri*. × 180.

cephalid. The hooks are very similar in form, number and arrangement to the hooks found on the crown of *Polyonchobothrium polypteri* (cf. figs. 1 and 3). In this latter species (fig. 3), as in *Tetracampos ciliotheca*, the hooks are arranged in four groups. In each group

in *T. ciliotheca* the number of hooks is usually nine (' of which the longest odd one is in the middle and the shortest pair on the outer side of each group '), while in *P. polypteri* the number varies between six and eight (Klaptocz 1906), and the hooks vary in size and in the position of the longer and shorter in each group, as in *Tetracampos*. In both species the general shape of the scolex is similar save that in *T. ciliotheca* the part below the crown of hooks is much shorter. This shortness is either natural and peculiar to the species or the drawing represents an unusually contracted specimen, similar to that which I have figured (fig. 2) for *Clestobothrium clarias*. This fig. 2 is a true representation* of a contracted scolex of *Clestobothrium clarias* (though the normal scolex is much more elongated—Woodland 1925†), and the general similarity between this representation and Wedl's figure of *T. ciliotheca* affords an explanation of all the general features shown in the latter. Wedl's species cannot be *Clestobothrium clarias* because in this latter the hooks are arranged in a complete circle, and are all of the same size, so markedly differing from the hooks of Wedl's species; neither can Wedl's species be identical with *Polyonchobothrium polypteri* because of the different sizes of the worms, among other reasons, but there is every reason to believe that Wedl's *T. ciliotheca* is a Bothriocephalid of about the same size as *Clestobothrium clarias* (my largest specimen of which measures 14·5 mm.; *T. ciliotheca* measured 10-15 mm. in length), but with the hooks similar to those of P. *polypteri* and possibly a shorter scolex. I have already quoted Wedl's description of the four scolex ' Lappen,' which are evidently the four walls bordering the bothrial or sucking grooves. Other typically Bothriocephalid features of *T. ciliotheca* are the shape of the anterior proglottids, the ventral position of the genital apertures (so conspicuous in these forms, even with an imperfect technique) and the ciliated embryophores enclosing the hexacanth embryos.

I conclude, therefore, that Southwell is mistaken in supposing that Wedl's genus *Tetracampos* has any connection with *Gangesia bengalensis* and *G. macrones*.

As regards Southwell's remarks on the systematic position of the Proteocephalidae this, of course, is a disputed subject, but I may

* As Dr. C. M. Wenyon can testify.

† This paper will provide my reason for including this species in the genus *Clestobothrium*.

say that for me the possession of lateral vitelline strands and of ventral uterine pores affords two very good reasons for relegating the family to the Tetraphyllidea, and that, with me, scolex characters count for very little, though even in this connection, Southwell appears to ignore the lobes upon which the suckers in this family are usually borne (*vide* Beddard 1913, pp. 8, 11, 12 e.g.).

I wish to acknowledge my indebtedness to Dr. H. A. Baylis for the kind gift of a number of specimens of *Polyonchobothrium polypteri*, and to Miss I. M. Bellis for assistance in connection with the literature.

LITERATURE

Beddard, F. E. (1913). Contributions to the Anatomy and Systematic Arrangement of the Cestoidea, VII. On Six Species of Tapeworms from Reptiles belonging to the Genus *Ichthyotaenia* (s.l.). *Pro. Zool. Soc.* London. Vol. I, p. 4.

Klaptocz, B. (1906). *Polyonchobothrium polypteri* (Leydig). *Centrbl. Bakt. Parasit. Infekt.*, Bd. XLI, 1 Abt., Orig., p. 527.

La Rue, G. R. (1914). A Revision of the Cestode Family Proteocephalidae. *Illinois Biological Monographs*, Univ. of Illinois, Vol. I, Urbana, Illinois, Nos. 1 and 2.

Southwell, T. (1913). Notes from the Bengal Fisheries Laboratory, Indian Museum. No. 1. *Rec. Ind. Mus.*, Vol. IX, p. 79, and On Some Indian Cestoda, Part I. *Ibid*, IX, p. 279.

——— (1925). On the Genus *Tetracampos* Wedl 1861. *Ann. Trop. Med. & Parasit.*, Vol. XIX, p. 71.

Wedl, K. (1862). Zur Helminthenfauna Ägyptens. *Sitzungsb. Math.-Nat. Classe k. Akad. Wissen.* Bd. XLIV, 1 Abth., Jahrg., 1861, p. 463.

Woodland, W. N. F. (1924). On a new *Bothriocephalus* and a new Genus of *Proteocephalidae* from Indian Fresh-water Fishes. *Parasitology*, Vol. XVI, p. 441.

——— (1925). On some remarkable new *Monticellia*-like and other Cestodes from Sudanese Siluroids. A paper which will probably appear in the June number of the *Quart. Jour. Micros. Science.*

THE MEASURE OF HOOKWORM INFECTION IN COMMUNITIES

BY

ASA C. CHANDLER, Ph.D.

(*From the Hookworm Research Laboratory, endowed by Indian Jute Mills Association, Calcutta School of Tropical Medicine*)

(*Received for publication 22 March*, 1925)

INTRODUCTION

In recent years the necessity for some measure of the degree, as well as the extent of hookworm infection in localities and communities, has been realised by a number of investigators, e.g., Darling (1922) and Cort (1924). It is an obvious but, nevertheless, largely ignored fact, that the percentage of individuals in whose stools eggs can be found is far from giving a true index to the severity of the hookworm situation, and yet, until very recently, this percentage has been accepted as the standard of measurement. The fallacy of this standard is, perhaps, nowhere more evident than in Bengal where, in spite of the fact that 70 per cent. or more of the population are infected, the individual infections are, on the whole, so light as to make hookworm disease in this province a comparatively unimportant problem. A correct estimate of the need for hookworm work and the judicious allocation of funds available for hookworm campaigns, as well as the strategy of campaigns, should depend on the degree as well as the prevalence of the disease. The relative value of different control measures and of different methods of treatment, also, can be correctly judged only by a consideration of the reduction in amount as well as in the incidence of infection. Hill (1923), for instance, showed that in certain areas in Porto Rico an intensive campaign reduced the percentage of infection from 87·2 to 34·1, but it reduced the egg output, which was used as a measure of the amount of infection, by 92·4 per cent.

METHODS OF MEASURING INTENSITY OF INFECTION

They may be classed as follows: (1) effects on host, (2) worm counts after anthelminthic treatment, and (3) estimation of the egg ouptut in the faeces.

Clinical symptoms, haemoglobin percentage and eosinophilia are the principal factors used in measuring effects on the host. All workers agree that the estimation of the amount of hookworm disease on the basis of clinical symptoms is difficult and complicated by differences in individual resistance, age, conditions of life, and concurrent disease; by the personal element in the classification of symptoms and severity of cases; and by the difficulty in making anything more than a very rough classification into light, moderate and severe cases. Such a clinical classification is of value in giving supplementary data as to the effects of the disease under local conditions, and in demonstrating individual and racial resistance, but it is of very little value, *per se*, as an indication of the degree of hookworm infection in a community. One might as well attempt to determine elevation on a mountain by reference to the permanent snow line, without consideration of other circumstances.

The haemoglobin content of the blood, as a measure of the degree of hookworm infection, is of little or no value in individual cases, although some authors, e.g., Darling, Barber and Hacker (1920), maintain that when sufficiently large numbers are averaged the amount of anaemia is proportional to the number of worms. Darling (1922) and Sawyer and Sweet (1922) have suggested definite ratios between the number of worms harboured and the percentage loss of haemoglobin. The haemoglobin content, however, is affected by so many factors such as sex, work, age, malnutrition, and such blood diseases as malaria, kala-azar, etc., that it can be used as a measure of hookworm infection only within wide limits. The process of elimination of other causes of anaemia is long and tedious, and in light cases there is usually no measurable drop in haemoglobin content. In a study of 100 individuals in the Alipore Central Jail, Calcutta, 67 of whom were infected with hookworm, but only six of whom had more than 1,000 eggs per gram of faeces, no difference in haemoglobin percentage between the infected and non-infected individuals could be found. The average for the uninfected ones,

according to the Tallquist scale, was 82·3, and for the infected ones 83. Two uninfected and only one infected case fell to 60 per cent., whereas one infected and one uninfected case reached 95 per cent. Darling (1922) shows that, in especially selected homogeneous groups, it requires fewer worms to cause a given loss of haemoglobin in a woman than in a man, and still fewer in a child, and also that a given number of *Ancylostoma duodenale* produces more anaemia than a similar number of *Necator americanus*, a fact which is now quite generally recognised. It is very probable that, as the number of worms increases, the haemoglobin content decreases at an accelerating rate, since it would become increasingly difficult for the patient to make good the loss produced by the worms.

Eosinophilia, as an indication of hookworm disease, is open to much the same criticisms as is the estimation of haemoglobin, since hookworm is only one of many causes of this condition. Practically all helminth infections produce more or less eosinophilia. Moreover, McVail (1922) observes that the eosinophilia in ankylostomiasis is not proportional to the number of worms present, even in uncomplicated cases, and he shows that kala-azar, and to a less extent malaria, is a powerful factor in reducing the eosinophilia due to helminthiasis.

The counting of worms passed after anthelmintic treatment, as a method of estimating the degree of infection in a community, is of unquestionable value when it can be properly carried out, but the difficulties involved are in most instances practically insurmountable. Only a small number of persons, and these especially selected ones, who can be relied upon to save all stools, can be examined in this way at a reasonable cost. The method requires a trained personnel, and cannot be left to subordinates; carelessness on the part of patients or laboratory staff, the partial failure of anthelmintics, and the loss of worms by maceration are all factors which interfere with the accuracy of the results. Even with every precaution which we have found practicable in the case of our hospital patients, we have not infrequently found lightly-infected cases to become microscopically cured after treatment, without finding any worms in the stools. It has usually been assumed that the washing of stools for 48 to 72 hours after treatment is sufficient to recover all of the worms passed, but even when a saline purge is given we have found

worms in stools as late as the sixth day after treatment, and quite frequently on the fourth day. Occasionally the stools have been negative on the second or third day, and again contained worms on the third or fourth day. For these reasons it is obvious that, however desirable a method for estimating degree of infection the worm count may be from a theoretical standpoint, it is certainly not in most instances practicable, and is always expensive.

The estimation of degree of infection by the egg output in the stools has very distinct advantages in the way of simplicity and practicability, providing that the egg output actually indicates the amount of infection. Even if this should prove to be true only to a limited extent and only when considerable numbers of individuals are averaged together, the knowledge of the number of eggs being deposited on soil, as Payne, Cort and Riley (1923) and Hill (1923) have pointed out, is, in itself, an important bit of information from the point of view of the spread of the disease. It is the egg output, and not the number of worms harboured or the clinical symptoms, which measures the public health menace.

ESTIMATION OF EGG OUTPUT

A number of different methods of estimating the actual or relative numbers of eggs in faeces have been utilised by different workers. Most of the methods of microscopic diagnosis can be used to give rough quantitative as well as qualitative information, but few of them are well adapted to give accurate information on this point. One of the first exact methods was Lane's (1918) ' standardising count,' which is Howard's centrifugal concentration technique reduced to accuracy of measurement, but this was not used for determining intensity of infection in groups or communities of people. The method devised by Stoll (1923a) is the only one which has been utilised in this way on a large scale. It is very simple, consisting merely of accurate dilution of a weighed sample of faeces in a decinormal NaOH solution, to clarify the fatty constituents of the faeces, and the accurate counting of a carefully-measured sample of the dilution. Lane (1924) has suggested the use of his direct centrifugal flotation method for this purpose, but, excellent as it may be for diagnosis, if the necessary apparatus is available,

it does not seem to me to be well adapted for quantitative work since, except where less than 500 eggs per gram are involved, the difficulty and tediousness of counting the great number of eggs thrown on the slide would counterbalance the advantage in reduced area of examination. It would be necessary, if numerous eggs were found, to repeat the process, using a much smaller quantity of stool, which would involve both time and inaccuracy due to the difficulty of measuring, say, 0·1 c.c. of stool.

The favourable results obtained by the use of Stoll's egg-counting method in Porto Rico led to a trial of it, with a few modifications, in Bengal. We have modified Stoll's method (1) by diluting the 3-gram sample of faeces to 90 c.c. instead of 45 c.c. ; (2) by counting the eggs in a 0·3 c.c. sample of the dilution instead of 0·15 c.c. ; and (3) by examining the preparation uncovered. There are several advantages in these modifications. In searching for eggs on an area of about two square inches on an uncovered slide, marked off by means of a glass pencil, it was found that 0·3 c.c. of the faecal suspension was necessary, under tropical conditions, to prevent the preparation from partial drying before the examination was complete. In order to get a sufficiently clear field for examination of ordinary stools with this quantity of the suspension, a dilution of 1 to 30 instead of 1 to 15 was necessary. The greatest advantage of using the larger amount of fluid and examining the preparation uncovered lay in the ability lightly to blow aside the flocculent masses of débris which often tend to hide the eggs, by gently puffing on the slide while the examination is actually in progress. Camouflage of eggs by débris is the most important source of error in all the techniques in which accurate measurements of material are made. Lane (1923, 1924) gives convincing evidence of the loss of eggs by camouflage. Maplestone (1924), in testing Stoll's method, nearly always obtained higher counts per gram when the faeces were further diluted before the egg count was made, obviously due to overlooking of eggs as the result of concealment in the more concentrated samples. By using a suspension in decinormal NaOH on an open slide with 0·3 c.c. of fluid spread over an area of 2 square inches, it is ordinarily possible, by gently blowing on the slide while making the examination, to see practically 100 per cent. of the surface of the slide with sufficient clearness to render the eggs easily visible. The eggs are heavier than

the flocculent material which makes up the great bulk of the débris on the slide, and, therefore, rest on the slide and remain visible as the overlying material is puffed aside. Furthermore, one can almost instantaneously determine whether or not an object which resembles an egg is such, since its position can be slightly changed or it can be rolled over by the same gentle puffing process. In very concentrated formed stools, we sometimes find it necessary to divide the 0·3 c.c. sample on two slides and dilute them further.

There are a few possible sources of error in this method which may be briefly commented on. In the first place, the selection of a 3-gram sample of faeces should, when possible, be made from an entire stirred stool, since the number of eggs contained in different parts is not always the same. Making duplicate counts on two samples from different parts of a single stool, the widest differences we obtained were counts of 700 and 900 eggs per gram on one sample and 1,600 and 1,800 on the other. After stirring this stool, examination of a third sample gave two counts of 1,200. In field work it is usually not practicable to get entire stools, and counts must be made on the samples submitted. As will be subsequently shown, however, the error arising from this, in individual cases, is neutralised when 50 or 100 samples are averaged.

We have tested a number of diluting fluids but found that the decinormal NaOH solution gave clear fields and more readily visible eggs than any other fluid. Addition to the NaOH of 1·5 per cent. NaCl had the effect of causing the faecal débris to clump together into large light flocculent masses which could be blown about, leaving a beautifully clear background on which the eggs showed up with striking clearness, but the occasional entanglement of eggs in these masses reduced its accuracy.

The thorough mixing of the samples in homogeneous suspensions is sometimes slow and difficult, and it is easy to overlook small masses of faeces which have failed to disintegrate. Unless carefully watched, this is one of the most fruitful sources of error. When available, a mechanical shaker is of great advantage. Settling of eggs in the diluting fluid must also be carefully guarded against; the stopper of the flask should be removed and the sample withdrawn immediately after a thorough and vigorous shaking. Even a few seconds' delay entails inaccuracy. We have found that the samples

can be withdrawn more quickly and accurately into rubber-bulb pipettes of drawn glass tubing marked at the 0·3 c.c. level, than into bacteriological pipettes. Only the required amount of fluid is sucked into the pipette, and all of it expelled on to the slide.

The NaOH solution does not appreciably change the appearance of the eggs of hookworms, *Trichuris*, *Hymenolepis nana*, or *H. diminuta*, but Ascaris eggs have the rough albuminous coat more or less completely dissolved off and thus often look quite different from the normal eggs, especially in the case of unfertilised ones. Taenia eggs undergo a peculiar change in that the embryophore swells to a diameter of from 50 to 60μ, leaving a much-enlarged clear space between it and the embryo ; the latter shrinks somewhat and assumes a characteristic elongated form.

Recounts of the same slide, duplicate counts from the same suspension, and counts on higher dilutions have shown that camouflage of eggs is practically done away with by the method here described. Lane warns against the loss of eggs held on the surface of a film too deep to be in one optical plane and in which only the bottom is searched. Apparently this rarely happens in a decinormal NaOH solution since I have several times gone over the surface of a slide containing several hundred eggs without finding a single egg. The entanglement of eggs in flocculent masses occasionally occurs, though much more frequently with Ascaris than with hookworm eggs. It usually takes a little time for the débris on the slide to clump, a process which takes place much more extensively in some stools than in others ; consequently, it only rarely happens that the eggs do not have time to settle. The blowing process also aids in liberating them. There is no doubt but that some loss of eggs does occur in these ways, but even if there were a constant loss of, say, 10 per cent. of eggs, it would be of little consequence, since what is desired is not so much an absolute knowledge of the number of eggs as a comparative measurement of the egg output.

That the method here described gives a good comparative measurement of eggs per gram of faeces is shown by the uniformity of counts which are obtained from examinations of two different samples prepared from the same stool. Where the average count on the slide is 10 or less, in about 80 per cent. of several hundred duplicate examinations, the counts were identical or within one

of each other, and, therefore, as close as possible to the average. In another 16 per cent. the counts were two numbers apart, whereas in only about 4 per cent. were the counts three numbers apart. Where the average slide count is between 10 and 100, in 35 per cent. the two counts came as near as possible to the average, in another 44 per cent. they were not over 10 per cent. from the average, whereas in only 8 per cent. were they more than 15 per cent. from the average. Where the average count exceeded 100, 87 per cent. of the duplicate counts fell within 10 per cent. of the average and none over 15 per cent. from it.

In nearly every instance in which there was any considerable discrepancy in the two counts a clumping of the eggs was observed, evidently due to their being held together by strands of mucus which had not been broken up in the shaking, in spite of an apparently homogeneous suspension. This clumping was also observed by Davis (1924), but with our technique we have only rarely obtained as irregular duplicate counts as Davis records in many of his cases; undoubtedly he was dealing with mucous stools.

In a series of about 600 faecal samples received from the Alipore Central Jail, counts have been made on two different slides prepared from a single suspension made from samples collected in quarter-ounce faeces-tins. The results which have been obtained from these counts compare very closely with those obtained from examinations of two separately prepared suspensions. This indicates that the differences in the counts are due not to variations in different parts of a stirred stool, but to errors in the counting technique. It appears, therefore, that a single suspension made from a stirred stool gives a sufficiently fair sample of the entire stool.

RELIABILITY OF EGG COUNTS AS AN INDICATION OF DEGREE OF INFECTION

It is now important to know the amount of variation which occurs in the eggs per gram of faeces in individuals according to the consistency of the stool, and from day to day. To get some light on this we studied the egg content of the stools of 36 hospital patients on from 3 to 22 different days, and made duplicate egg counts on separately prepared suspensions from 194 stools. By classifying the

stools as liquid, mushy, semi-formed and formed, and comparing the egg counts of these several groups in the case of each individual, it soon became apparent that, roughly speaking, the formed stools contained twice as many eggs and the liquid stools half as many or less, as the mushy stools. This compares fairly closely with Stoll's findings in Porto Rico (1923b). It was evident, therefore, that if intensity of infection were to be measured by egg counts, the factor of consistency would have to be considered.

Since, in India, mushy stools are normal and formed ones are rare, we accept the count on mushy ones as normal and correct the counts on formed and liquid stools by dividing or multiplying by 2. Such counts we refer to as ' corrected counts.' In a paper which has recently come to hand, Stoll (1924) arrives at exactly similar conclusions, except that he accepts formed stools as normal and multiplies the counts on mushy and liquid stools by 2 and 4 respectively, to bring them to ' basis of formed stool.'

Our counts on these preliminary 36 patients showed, however, that even when the consistency of the stool does not vary, there is a surprising variation in the egg output per gram of faeces on different days. In case 22, for instance, considering only the mushy stools, there was a maximum variation from 250 to 1,100 eggs per gram, in case 29 from 500 to 1,250, in case 32 from 250 to 1,000, and in case 35 from 50 to 350. These are the most extreme cases ; in most instances, if the consistency of the stool is taken into consideration, the variation is much less. There appears to be a much more marked tendency to vary in some individuals than in others. Stoll (1924), in a study of the egg output of two individuals, for 15 and 40 days respectively, found a similar day-to-day variation. In one of his cases the mushy stools varied from 1,000 to 2,600 and in another from 430 to 800, whereas the formed stools in the latter case varied from 400 to 1,330.

An attempt was made to get 24-hour samples of stools and to calculate the total daily egg output for 24 hours by means of the egg count and stool weight, since it seemed probable that the amount of the stool would to some extent counterbalance the variations in eggs per gram. Our results, however, failed to show any such counterbalancing tendency, since it just as frequently happened that a low egg count was accompanied by a small 24-hour output of stool

as the reverse, thus giving a greater variation in the total egg output than had been found in the number of eggs per gram. Stoll's (1924) tables show a similar lack of correlation. The most obvious reason for this appears to be that the extent to which the bowels are emptied on each day varies, even if the habits are fairly regular. In most of my cases the hour at which the stools are passed each day varies considerably, so it occurred to me that better results might be obtained by weighing only a single stool each day and keeping a record of the time between the last previous stool and the one examined. In this way we should know the number of hours during which the faeces and the eggs contained in them had been accumulating and could calculate from this the number of eggs produced in 24 hours. 112 stools from 23 different cases were examined in this way, but practically the same amount of variation was found in daily output as when 24-hour outputs of faeces were weighed without reference to time of stools, undoubtedly due to the same factor of completeness of evacuation of the bowels.

As Stoll has pointed out, it is only when the total daily output of eggs, calculated from eggs per gram and weight of stool, is averaged for at least three days that the coefficient of variation is reduced to a low level. For one-day examinations the egg count by itself gives less variable results than the calculated total egg output. Since, under field conditions, the collection and weighing of stools for three days on any considerable number of individuals is out of the question, for the same reasons that worm counts are impracticable, reliance must be placed on the egg counts alone, even though some inaccuracy is involved. Stoll has shown that in the two cases he examined, which were of widely different types, the total egg outputs showed a relation of 5·4 : 1, whereas the average corrected egg counts per gram showed a relation of 3·3 : 1. The failure of the egg counts to show a correct relationship is, of course, due to differences in food habits and consequent daily amount of faeces in which the eggs are distributed. We believe, however, that in more or less homogeneous groups, such as tea garden coolies, mine labourers, etc., habits are sufficiently alike for the corrected egg count, if averaged for three days, to give a reasonably good index of the relative egg output of different individuals. Since the egg counts of individuals approach a level when averaged for three or four days, it is obvious that in

determining the degree of infection of a group or community by counts on 50 or 100 individuals, single egg counts are quite sufficient, since variations would automatically be blotted out in the consideration of such numbers.

To test this point a study was made of 100 prisoners in the Alipore Central Jail, with the kind co-operation of the Superintendent, Lt. H. A. Young, I.M.D. A double count was made from a single suspension on two separate occasions, about a week apart. Most of the infections found were extremely light, so that although 67 were shown to be positive for hookworm, by the Kofoid and Barber technique, only 45 positives were found by examination of two slides prepared for egg counts on the first examination, and 44 on the second. Eleven which were negative on the first examination were positive on the second, and 12 which were positive on the first were negative on the second. Of these 23 cases, 12 showed only a single egg on four slides, six more gave an average of one egg per slide on the positive examination, and the remaining five gave average counts of from 1·5 to 2·5 on the positive examination. In spite of the high percentage of these low counts, which would tend to increase the probable error in the two counts, the average number of eggs per gram of faeces on the first examination was 282 and on the second 257, a deviation of only 4·6 per cent. above and below the average of the two. This compares quite favourably with the deviations of 3·9 per cent. and 3·3 per cent. from the average, which were found in the first and second slides in the first and second examinations respectively. This justifies the conclusion that a single egg count on a fair number of individuals gives a reasonably good estimate of the average egg output of that group.

Owing to the fact that we have not found it practicable to control our hospital patients sufficiently so that the preservation of all stools passed after anthelmintic treatment could be depended upon, we can give no reliable statistics on the relationship between egg counts and worms harboured. In cases in which we have reason to believe that all the stools were saved, the number of eggs per gram per female worm usually falls between 8 and 20. In one instance, however, in which duplicate counts were made for three successive days, without finding any eggs at all, although the case was positive by flotation, four female Necators were passed. In another case which

passed 16 female Necators two eggs were found on each of two slides on one day and no eggs on duplicate examinations on two subsequent days ; in this case something must have inhibited oviposition on these two days. There is likely to be less variation of this kind in the field than in a hospital, where alterations in diet, drug treatments, and concurrent disease may influence both the quantity of the stool and the oviposition of the worms.

That the correlation between egg count and worms harboured is not close in individual cases is evident from the day to day variations in the count. Mhaskar (1923) gives a table of 30 cases which purports to show that there is no correlation at all. Darling (1922) on the other hand, gives a table in which a distinct correlation is shown. Smillie (1921) and Stoll (1923b) also find a correlation. On purely theoretical grounds one is forced to the conclusion that, other things being equal, there *must* be some relationship between egg output and number of worms harboured. For instance, if a patient harbouring 10 female worms produced, on successive days, 100, 500, and 200 eggs per gram of faeces, is there any reason to doubt that if he harboured 20 female worms, other conditions being the same, he would pass on each of these days approximately twice as many hookworm eggs ? It is reasonable to assume, then, that when the egg output of a large number of representative individuals is averaged together, this number gives a sufficiently accurate estimate of the degree of infection so that it can be used for comparison of different groups of individuals living under similar conditions and having similar food habits, or of the same groups before and after treatment, or for the establishment of control measures. The average eggs per gram is a less accurate guide in comparing groups living under quite different conditions and having widely different food habits, but even here, within wider limits, rough comparisons can be made. This is, however, of far less value and importance, for practical purposes, than the comparison of different groups of a single area by age, sex, occupation, etc., and the comparison of such groups at different times for the valuation of the effectiveness of control measures.

ESTIMATION OF INFECTION INDEX

Although Cort (1924) suggests the substitution, in surveys, of the egg counting method for the routine faecal examinations now generally used, and describes hypothetical cases which show its advantage, it seems to me that there is fallacy in accepting either the degree of infection as determined by worm or egg counts, or the mere percentage of incidence of infection, as an index of the amount of hookworm infection in a community, or of the benefits derived from treatment or control measures. For example, let us suppose that in two communities both living under climatic and soil conditions favourable for the propagation of hookworm, the number of eggs per gram of faeces averages exactly the same, but that the sanitary conditions and habits of the people differ. In one community the majority of the people are sanitary in habits and the hookworm infection is largely confined to a few families who are backward and careless in habits, while in the other community sanitary conditions throughout are not so good and the infection is more uniformly scattered through a high percentage of the people. In such a case it is clear that the two groups should not be placed on a par, as would be the case if only the degree of infection for the group, based on egg output, were considered ; nor should the condition of the first community be considered as far superior to that of the second as the difference in percentage of infection would probably place it. From the standpoint of the general effect on the community, the probable spread of the disease, and the sanitary conditions indicated, it is important to take into consideration the number of individuals among whom the egg output is divided. Certainly the higher the percentage of individuals who are scattering a given number of hookworm eggs daily, the greater the opportunity for the spread of the disease, and the more important it is that control measures should be inaugurated. One hundred individuals each with an output of 100 eggs per gram of faeces certainly constitute a greater menace to the community than ten individuals each with an output of 1,000 eggs per gram, or one individual with an output of 10,000 per gram, since, although the total number of eggs produced is the same in each instance, the extent to which they are scattered is largely proportional to the number of persons who are passing them, and the more they are

scattered the more opportunity there is likely to be for the larvae which develop from them to gain access to new hosts. The incidence of infection, then, rather than the degree of infection, is the correct measure of the extent to which the entire community has been, and is likely to be, exposed to the infection, whereas the degree of infection rather than the incidence of it is a rough measure of the extent to which individuals have been, and are likely to be, exposed, and of the facility with which infection can occur, under the climatic and soil conditions of the locality, when carelessness in habits permits it.

It seems to me, therefore, that both factors must be taken into consideration in order to arrive at a true hookworm infection index. To do this I have tried various ways of combining the incidence and degree of infection, as indicated by eggs per gram of faeces, to obtain a number which would give a true relative index in various actual and hypothetical cases, as judged by a common-sense consideration of all the facts involved Such an index number can, I think, be obtained by taking the square root of the product of the average eggs per gram, multiplied by the percentage of infection, or, alternatively, by taking the square root of the product of the egg counts, averaged for the infected individuals only, multiplied by the square of the percentage infected, i.e., by the equation :

$\sqrt{\frac{e.p.g.}{100} \times \%^2} = I$, where *e.p.g.* stands for average eggs per gram of the infected individuals ($\frac{e.p.g.}{100}$ being the average of the eggs counted on the slides), % the percentage infected, and *I* the resulting infection index. For example, if 50 of 100 individuals have an average of 400 eggs per gram by corrected counts, the other 50 having none, the equation would be : $\sqrt{\frac{400}{100} \times 50^2} = 100$, which is the infection index. The three hypothetical cases mentioned above of a 100 per cent. infection with 100 eggs per gram, a 10 per cent. infection with 1,000 eggs per gram, and a 1 per cent. infection with 10,000 eggs per gram, are all on a par on the basis of degree of infection for the group ; they stand in the ratio of 100 : 10 : 1 on the basis of incidence of infection ; while their infection indices work out at about 100 : 32 : 10, which seems to come much nearer their true relationships. It will be seen, however, that this method of calculation

gives correct results only if all the infections are uniform, since the average implies that the egg output is evenly divided among all the infected individuals, which is seldom the case. To get a correct estimate, therefore, the entire group of infected individuals should be broken arbitrarily into sub-groups according to the size of the egg counts, and the infection index for each sub-group separately figured and then all of them added together. For example, in a community with a 60 per cent. infection, 20 per cent. with egg counts of 100 to 500 (average 300), 20 per cent. with counts of 500 to 2,100 (average 1,000) and 20 per cent. with 2,100 to 5,100 (average 3,000), the infection index, if figured for the entire group, would be: $\sqrt{\frac{1433}{100} \times 60^2} = 228$, whereas if figured for each group separately, the infection index works out as follows: $\sqrt{\frac{300}{100} \times 20^2} + \sqrt{\frac{1000}{100} \times 20^2} + \sqrt{\frac{3000}{100} \times 20^2} = 209$. We consider as very satisfactory the grouping used by Payne, Cort and Riley (1923), according to the following numbers of eggs per gram: 1-599, 600-2,099, 2,100-5,099, 5,100-11,099, and 11,100 up.

Table I gives the infection index, as worked out on a number of actual cases, based on my own work in Bengal and on statistics given by Payne, Cort and Riley (1923), and Hill (1923), in Porto Rico. It should be noted, however, that the Jute Mill statistics are not entirely correct, since the entire percentage of infection was not determined by a concentrative method, and therefore, as the egg counts run very low, a considerable number of light infections would probably be passed over by the egg counting technique, as was shown by the Alipore Jail investigation mentioned above. It is necessary, therefore, that the egg-counting method be supplemented by a concentrative technique in order to discover the light infections which would otherwise be missed. In calculating the infection index those cases which are positive by the concentrative method only, and negative on two egg-count slides, can be calculated arbitrarily as having 25 eggs per gram.

The method we have adopted, therefore, as a routine for determining the infection index, and which we recommend for general use, is as follows :—

Table I.

	Number Examined	% with 1-599 e.p.g.	Average e.p.g.	% with 600-2099 e.p.g.	Average e.p.g.	% with 2100-5099 e.p.g.	Average e.p.g.	% with 5100-11099 e.p.g.	Average e.p.g.	% with 11100 or more e.p.g.	Average e.p.g.	Total % infected	Average e.p.g. for the entire group	Index of Infection
Coolies in Jute Mills and Coolie Lines	143	36†	260	18	1020	4	3100	1	5700	...	...	58†	420†	140†
Coolies in Jute Mills and Coolie Lines	48	19†	275	17	1160	2	3400	...	...	...	...	38†	310†	100†
Coolies living outside Jute Mill	98	44†	242	14	830	...	...	...	...	...	...	58†	220†	109†
Prisoners in Allipore Jail (1st exam.)	100	56**	133	9	810	1	3200	1	8500	...	...	67	272	106
Prisoners in Allipore Jail (7 days later)	100	57**	144	7	1040	2	3320	1	6450	...	...	67	286	110
Cases in Porto Rico, Area C (Payne, Cort and Riley)	92	17	300*	16	1350*	25	3600*	20	8100*	17	15000*	96	7740	630
Cases in Porto Rico Areas (before treatment) (Hill)	282	28	300*	27	1350*	17	3600*	9	8100*	6	15000*	88	2820	408
Cases in Porto Rico Areas (after treatment) (Hill)	282	25	300*	6	1350*	3	3600*	1	8100*	·35	15000*	35	215	92

* These are means instead of averages, data for the latter not being available.
† These figures are too low, since no concentration method was used to discover infections too light for detection by the egg-counting method.
** These include 23 cases detected by concentration method, but negative by egg-counting method. These infections were arbitrarily figured as having 25 e.p.g.
The figures in all cases are given to the nearest integer.

(1) Determination of the incidence of infection by a concentrative technique. In my experience the Kofoid and Barber method has given the most uniformly satisfactory results; according to tests we have made it is more accurate than the Willis method or any of the usual centrifuge methods. If the necessary equipment is at hand the published evidence in favour of Lane's direct centrifugal flotation method indicates it as the method of choice, but since our centrifuges are not adapted to this method I have not had an opportunity of trying it myself. Neither the Kofoid and Barber, nor the Willis methods are reliable for light Ascaris infections; to detect these we have found Lane's levitation method the most satisfactory.

(2) Determination of eggs per gram, by examination of all positives, by the modification of the Stoll egg-counting technique here described. By preference two slides should be examined and averaged, and the count corrected according to the consistency of the stool; if, however, 50 or 100 specimens are examined, the total averages, though not the individual counts, will be very nearly correct if only one slide is examined of specimens showing two or more eggs. Specimens found positive by the concentrative technique, but negative on two egg-count slides, may be arbitrarily calculated as having 25 eggs per gram.

(3) Determination of the infection index by the equation: $\sqrt{\frac{e.p.g.}{100} \times \%^2}$ = Infection Index, where *e.p.g.* stands for the average eggs per gram of the infected individuals, and where the equation is separately figured for different groups showing different degrees of infection, as suggested above.

In conclusion I take pleasure in acknowledging the painstaking and reliable help given by my assistant, Dr. A. K Mukerji.

SUMMARY

1. Recent investigations have shown the necessity for the measurement of the degree of hookworm infection as well as its incidence; such a measurement is of value from the point of view of the urgency, nature and valuation of methods of treatment and control.

2. Degree of infection may be measured by clinical symptoms, haemoglobin content, eosinophilia, worm counts after treatment, or by estimation of egg output in the faeces. The first three are not reliable, and the worm counts are too difficult, impracticable and expensive for use on a large scale.

3. Estimation of egg output is simple and practical, and is of value in itself as an accurate measure of potential soil pollution whether or not it indicates accurately the number of worms harboured. A modification of Stoll's egg-counting method is described and recommended for general use. Uniformity of duplicate counts indicates that it gives a good comparative measurement of egg output.

4. The consistency of the stool must be taken into consideration in estimating the egg output from the number of eggs per gram of faeces. Counts on formed or liquid stools, where mushy stools are normal, as in India, can be corrected by dividing or multiplying by 2. Day-to-day variations in eggs per gram are considerable, but the counts approach a level when averaged for three or more days. Consideration of the quantity of the stool does not lessen the variation unless averaged for at least three days, and is, therefore, not practicable in field work. The corrected egg count alone must be relied upon, and in more or less homogeneous groups we believe that this gives a reasonably good indication of the relative degree of infection in different individuals. When averages of large groups are being considered, single egg counts of individuals are sufficient.

5. In individual cases the correlation between egg counts and number of worms harboured is not very close, but when the egg counts for a group are averaged, a fair estimate of the relative numbers of worms harboured can be obtained, especially when homogeneous groups, or the same groups at different times, are compared.

6. It is not advisable to measure hookworm infection in a community by the degree of infection alone ; the incidence should also be considered, since the higher the percentage of individuals who are scattering a given number of eggs, the greater the danger to the community. The incidence of infection is a measure of the extent to which the entire community is exposed to infection ; in a general way it measures sanitary conditions. The degree of

infection, on the other hand, is a measure of the facility with which infection can occur under the climatic and soil conditions of the region when carelessness in habits permits it.

7. A good infection index can be obtained only by taking both factors into consideration. This can be done by means of the equation : $\sqrt{\frac{e.p.g.}{100}} \times \%^2 =$ Infection Index, where *e.p.g.* stands for average eggs per gram of infected individuals only. This equation should be separately figured for different groups falling into certain abritrary divisions according to number of eggs per gram, and all of them added together.

8. It is recommended that the infection index in survey work be determined as follows : (1) determination of incidence of infection by a concentrative technique ; (2) estimation of the degree of infection by means of egg-counts ; and (3) determination of the index of infection by the equation given above.

REFERENCES

Cort, William W. (1924). Investigations on the Control of Hookworm Disease. XXXII : Methods of Measuring Human Infestation. *Amer. Journ. Hyg.*, 4, No. 3, pp. 213-221.

Darling, Samuel T. (1922). The Hookworm Index and Mass Treatment. *Amer. Journ. Trop. Med.*, 2, No. 5, pp. 397-447.

Darling, S. T., Barber, M. A., and Hacker, H. P. (1920). Hookworm and Malaria Research in Malaya, Java and the Fiji Islands. *Rep. of Uncinariasis Comm. to the Orient*, 1915-1917. *Int. Health Bd., Publ.*, No. 9.

Davis, Nelson C. (1924). Experience with the Stoll Egg-Counting Method in an Area Lightly Infested with Hookworm. *Amer. Journ. Hyg.*, 4, No. 3, pp. 226-236.

Hill, Rolla B. (1923). Investigations on the Control of Hookworm Disease. XXV : The Use of the Egg-Counting Method in an Intensive Campaign. *Amer. Journ. Hyg.*, 3, July Suppl., pp. 37-60.

Lane, Clayton (1918). Methods, Old and New, for the Detection of Hookworm Infection. *Ind. Journ. Med. Res.*, 5, No. 2, pp. 350-385.

——— (1923). The Mass Diagnosis of Ankylostome Infestation. Part I. *Trans. Roy. Soc. Trop. Med. and Hyg.*, 16, pp. 274-313. Parts II to VII. *Ibid.*, 1924, 17, pp. 407-436.

Maplestone, P. A. (1924). A Critical Examination of Stoll's Method of Counting Hookworm Eggs in Faeces. *Ann. Trop. Med. and Parasitol.*, 18, No. 2, pp. 189-194.

McVail, J. Borden (1922). The Blood Count in Ankylostomiasis. A Warning. *Ind. Med. Gaz.*, 57, No. 10, pp. 366-367.

Mhaskar, K. S. (1923). The Diagnosis of Hookworm Infection. *Ind. Journ. Med. Res.*, 10, No. 3, pp. 665-686.

Payne, G. C., Cort, W. W., and Riley, W. A. (1923). Investigations on the Control of Hookworm Disease, XX. Human Infestation Studies in Porto Rico, by the Egg-Counting Method. *Amer. Journ. Hyg.*, 3, No. 3, pp. 315-338.

Sawyer, W. A., and Sweet, W. C. (1922). The Investigation and Control of Hookworm Disease at the Hospital for the Insane at Sandy Gallop, Queensland. Australian Hookworm Campaign, Survey Report, No. 30.

Smillie, W. G. (1921). A Comparison of the Number of Ova in the Stool, with the Actual Number of Hookworms Harbored by the Individual. *Amer. Journ. Trop. Med.*, 1, No. 6, pp. 389-396.

Stoll, Norman R. (1923). Investigations on the Control of Hookworm Disease, XV. An Effective Method of Counting Hookworm Eggs in Faeces. *Amer. Journ. Hyg.*, 3, No. 1, pp. 59-70. 1923b. *Ibid*, XVIII. On the Relation between the Number of Eggs Found in Human Faeces and the Number of Hookworms in the Host. *Amer. Journ. Hyg.*, 3, No. 2, pp. 156-179. 1924. *Ibid*, XXXIII. The Significance of Egg Count Data in *Necator americanus* Infestations. *Amer. Journ. Hyg.*, 4, No. 5, pp. 466-500.

A NEW VARIETY OF *ANOPHELES MARSHALLI* THEOBALD FROM THE BELGIAN CONGO

BY

A. M. EVANS, M.Sc.

(*Received for publication* 9 *May*, 1925)

Anopheles marshalli var *moucheti* var. n.

FEMALE. *Head* with upright forked scales pure white anteriorly, black posteriorly; forwardly projecting tuft of long white scales reaching well beyond the base of the clypeus. Palpi with three white bands, the proximal narrow, the two distal bands very wide, equal in length, and separated by a black ring one-quarter to one-half of their length. Antennae with white scales on the second segment,

FIG. 1. *Anopheles marshalli* var. *moucheti* var. n. ♀ wing.

hairs of whorls white. *Thorax:* prothoracic lobes with blackish bristles, mesonotum with long, white, narrow, curved scales. *Abdomen* with dark integument and light brown hairs. *Wings* with white and dark scales disposed as shown in the illustration (Fig. 1). Typical plume scales from distal dark area of upper

fork of second vein (Fig. 2, A) with five striae and greatest width from one-fourth to one-fifth of the total length,* lateral squames from distal dark area of third vein (Fig. 2, B) mostly with five widely-separated striae, and greatest width one-fourth of the length. Legs black scaled ; in all three pairs the tibiae and first three tarsal segments with narrow, but distinct, apical white rings. Hind legs with apical white ring also on fourth tarsal segment, mid legs with traces of pale scales apically on this segment. *Length* of white ring on hind metatarsus about equal to its greatest width, length of succeeding rings, progressively slightly shorter.

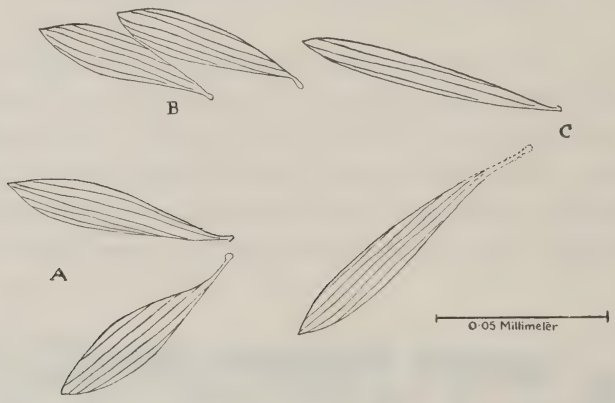


Fig. 2. *Anopheles marshalli* var. *moucheti* var. n. Wing scales. *A*—Plume scales from upper branch of second vein ; *B*—Lateral squames from distal dark area of third vein ; *C*—Plume scales from stem of second vein near fork.

Wing length : *c.* 3 mm.

Male. Palpi with long segment black-scaled with narrow, apical, pale ring ; last two segments white scaled with narrow basal black rings. Antennae with hairs of whorls whitish internally on proximal segments. Colouration as in the female.

The wing markings are subject to a certain amount of variation ; the third pale area involving the costa and first vein may be as short on both veins as that shown on the costa in the illustration (Fig. 1), or on both veins as long as, or slightly longer than, that shown

* This description refers to scales on a wing mounted with the dorsal surface uppermost, in canada balsaam, under slight pressure.

on the first vein. The fourth vein may have the dark area on the upper branch, or the second long pale area on the stem, interrupted.

Type ♂, Buta, November, 1922, Dr. R. Mouchet; co-type ♀ ♀ (3), one from Buta, one from Bambili, and one from Api, collected in November, 1922, by Dr. R. Mouchet. Other specimens from Bambili, 5 ♀ ♀, and Buta, 1 ♂, November, 1922, Dr. R. Mouchet; Basoko, Aruwimi, 18.2.1924, Service Médicale, 1 ♂, 2 ♀ ♀; districts de l'Equator et de l'Ubangui, 18.9.1924, Dr. Trolli, 13 specimens; Kinshasa, Dr. Duren, 1922, 3 specimens.

The specimens were submitted for identification by Dr. G. Severin, of the Musée Royal d'Histoire Naturelle de Belgique, and Dr. H. Schouteden, of the Musée du Congo Belge.

Type ♂ and one co-type ♀ in the collection of the Musée Royal d'Histoire Naturelle de Belgique, the other co-type ♀ ♀ in the collection of the Liverpool School of Tropical Medicine.

The variety is named in honour of Dr. Mouchet, who has made extensive and valuable collections of Culicidae in the Belgian Congo.

This variety differs most obviously from typical *A. marshalli* Theo. in the absence of the interruption on the third large dark area of the first vein, and in the great length of the two distal white bands of the female palpi. Mr. F. W. Edwards, who very kindly compared co-type females of this variety with typical *A. marshalli*, informed me that the wing scales were shorter as well as broader than in the type form, agreeing with *A. domicolus* as regards their length, but that they were broader and denser than in this latter species. Mr. Edwards stated further that the variety resembled typical *A. marshalli* in having narrow hind tarsal rings, and differed from *A. domicolus* in this character.

THE IDENTITY OF THE RARER SCHISTOSOMES OF MAN AND THEIR INTERMEDIATE HOSTS

BY

F. G. CAWSTON, M.D., M.Sc.

(*Received for publication* 10 *April*, 1925)

It is remarkable that at least four distinct types of Schistosome ova should occur in the urine of Natal patients, when only one type is known from the Far East and only two from Egypt. If the spindle-shaped ova were merely a variety of the ova of the *Schistosomum haematobium*, one would have expected them to occur in Egypt and if, as is thought, one type is that of *Schistosomum bovis*, one would have expected it to be more common in North Africa, in view of the heavy infestation of Sardinian cattle with this parasite and the relative immunity of South African cattle to Schistosome invasion.

In South Africa very little has been reported of the adult schistosomes and there is always an element of uncertainty where the diagnosis rests solely on the appearance of the ova that are detected in the urine of a patient.

A small ovum which is occasionally present in the urine of Natal patients has been regarded as that of *S. haematobium*, but its outline is identical with that of *S. bomfordi* Montgomery. There is certainly need for further research into the identity of those schistosomes which attack man in Africa. The subject is complicated by the difficulty that has been experienced in determining the usual intermediate host of the rarer schistosomes and because of the difficulty that therefore arises in rearing the adult parasites from the miracidia which escape from the ova in infested persons. Fig. 1 shows four different schistosome ova isolated from the urine of Natal schoolboys, as well as that of *Schistosomum japonicum* from the Far East and of two schistosomes from India.

In South Africa it is only rarely that schistosomes are found in fresh-water snails other than *Physopsis africana*. I have found schistosomes in both *Isidora globosa* Morelet and *Planorbis pfeifferi* Krauss at Laurenço Marques. Various *Isidorae* occur in South Africa ; they are very commonly infested with amphistome cercariae. *Limnaea natalensis* is the common host of *Fasciola gigantica*. *Melanoides tuberculata* Müller is the only species with an operculated shell that I have found infested with cercariae ; but J. D. F. Gilchrist has isolated cercariae from *Tomichia ventriculosa* (Sowerby) Reeve. *Ancylidae* harbour cercariae of various kinds and *Burnupia gordonensis* M. & P. is one of the commonest and largest species of this genus in South Africa.

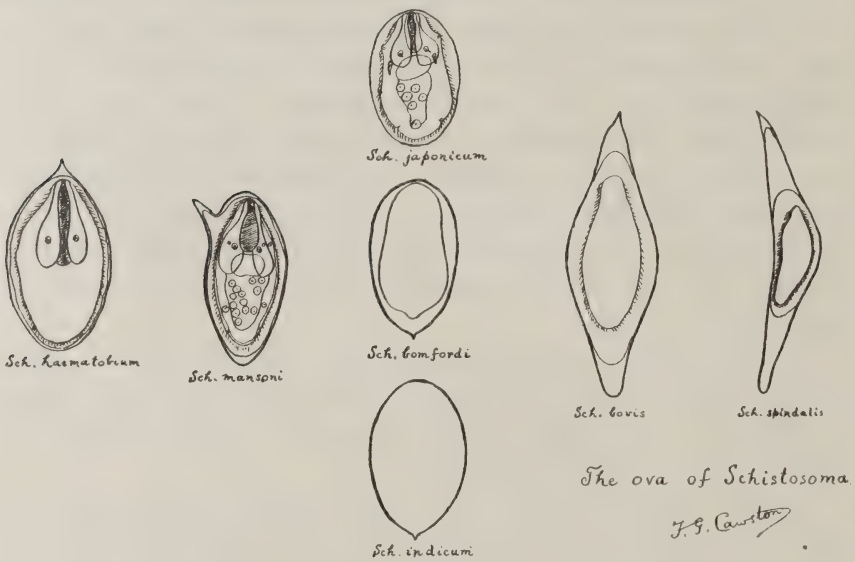


FIG. 1.

It is possible that a careful study of the radulae of intermediate hosts may assist in the determination of those species which resemble one another very closely. *Isidorae*, for instance, are notoriously variable and more than one species may occur in the same pool, each being represented by examples at various stages of growth. Although *Physae*, of which there are very few examples south of the Zambesi, might possibly be mistaken for *Isidorae* where the shell

Fig. 2. 1.—*Physopsis africana* Krauss; 2.—*Isidora globosa* Morelet; 3.—*Isidora craveni* Ancey; 4.—*Isidora tropica* Krauss; 5.—*Planorbis pfeieffri* Krauss; 6.—*Melanoides tuberculata* Müller; 7.—*Limnaea natalensis* Krauss; 8.—*Tiara coacta* Meusch; 9.—*Septaria tesselata* Lamarck; 10.—*Theodoxus natalensis* Reeve.

alone is set aside for study, there is little danger of this mistake being made where the individual teeth of the two genera are examined. Although there is a good deal of variation in the appearance of the teeth in individual examples of the same species, even when taken from the same locality and grown under apparently identical conditions, yet a careful study of the teeth reveals the fact that this variation is almost confined to the CONES which grow from the CROWN of the tooth, so that the variation is not so great as at first sight appears.

THE INCUBATION PERIOD OF BENIGN TERTIAN MALARIA

BY

G. DE M. RUDOLF

CLAYBURY MENTAL HOSPITAL

(*Received for publication* 14 *April*, 1925)

The incubation-period of naturally-acquired benign tertian malaria is usually given as being from two to three weeks. Thus James (1920) states that the period is usually from 14 to 18 days, Stitt (1922) from 14 days, Acton cited by Castellani and Chambers (1919) 6 to 21 days, and Castellani and Chambers (1919) from 9 to 12 days. These authorities add that there are great variations in the length of the period outside the limits of the above figures. In addition, the cases of latent infection must also be remembered. In these cases several months, or even a year, may elapse since possible inoculation before the primary malarial attack develops. Often the onset is delayed until the patient undergoes some severe strain of either a mental or physical nature (James, 1920). The incubation-period of the naturally-acquired infection is thus seen to be extremely variable.

ARTIFICIALLY-INOCULATED MALARIA

(i) Mosquito-inoculations. Sir Ronald Ross (1911) gives details of cases inoculated artificially by means of infected mosquitos. The length of the incubation-period as measured by the first rise in temperature is recorded in nine instances and was found to vary from 10 to 14 to 25 days. In seven of these cases *Plasmodium vivax* was first demonstrated in from 16 to 30 days after infection.

In five general paralytics inoculated from mosquitos by Lieut.-Col. S. P. James the incubation-period varied from 11 to 16 days as regards the first definite malarial rise of temperature. In four of these cases the parasites were first found on the 17th and 18th days, three on the 17th and one on the 18th. Davidson (1925) found in a series of 23 cases that the period varied from 7 to 20 days.

In, therefore, a total of 37 cases the incubation-period, as measured by the first rise of temperature, varied from 7 to 25 days, and in 34 cases from 7 to 30 days as regards the first appearance of parasites, Davidson measuring the period by both methods.

(ii) Subcutaneous-inoculation. Most authorities give approximately corresponding lengths of time for the duration of the incubation-period when inoculation is practised subcutaneously. Thus Gerstmann (1924) states the period varies from 4 to 28 days, Scripture (1923) from 6 to 31 days, Donner (1925) from 5 to 21 days, Yorke and Macfie (1924) usually from 8 to 15 days, but with considerable variations, Worster-Drought and Beccle (1923) from 9 to 24 days and Nonne (1922) from 10 to 24 days. The table given by Pijper and Russell (1924) shows an incubation-period of from 9 to 18 days and that of McAlister (1924) from 9 to 32 days. In a series of 43 benign tertian malaria inoculations given by Grant and Silverston (1924) the first rise of temperature occurred from 1 to 18 days after inoculation, whereas parasites were first found from 6 to 22 days after. Korteweg (1924), in a series of 52 cases, found that parasites were first demonstrated in thick-films in from 5 to 21 days after inoculation.

Unfortunately, not all of the above authors state whether they define the termination of the incubation-period by the date upon which parasites were first found or by the date upon which the first increase of temperature occurred. It is, however, clear that the incubation period may be of many days' duration.

The incubation-period of a disease is that period which elapses between the admission to the body of the infecting organism and the first onset of symptoms. The latter may be subjective or objective (Gould, 1915). As the first appearance of symptoms may be objective in nature, the first day on which parasites are found in the peripheral blood-stream might be taken as the termination of the incubation-period. The disadvantage of this method, however, lies in the fact that there are so many factors to be taken into consideration when comparing the lengths of the incubation-period in different patients. At the commencement of an attack of malaria the parasites are usually comparatively few in number. In this case the day upon which the plasmodia are first found will depend upon : (*a*) whether thick or thin blood-films are used, (*b*) whether the whole or only a part of the film is examined and, if a part, which part (see below) ; and (*c*) the length of time each film is studied. In certain instances a fourth factor must be added : (*d*) the previous experience of the observer. If it were possible to

adopt general standard conditions, the method of measuring the incubation-period by the first appearance of the parasites would be of value.

With regard to the subjective symptoms it is clear that the length of the incubation-period cannot be determined by observing the occurrence of the first rigor, for many general paralytics inoculated with malaria do not shiver at all during their febrile treatment. The same applies to a subjective sensation of coldness, to sweating and to general enlargement of the spleen. The temperature, however, is raised during the initial malarial paroxysms, although, of course, parasites may be present during a relapse without fever occurring. But when the length of the incubation-period is under discussion malarial relapses do not enter into the subject. It should, however, be added that occasionally patients who have suffered from malaria previously may not develop febrile paroxysms but may show parasites for a few days. One such instance has occurred at Claybury Mental Hospital. In these cases, which are very rare, the method of recording the incubation-period by the first rise in temperature is not suitable. Now, non-inoculated general paralytics are subject, from time to time, to variations in temperature (Rudolf, 1925), and therefore an isolated elevation, or a succession of elevations, of temperature not immediately followed by typical malarial paroxysms or other definite signs of active malarial infection must not be taken as the termination of the incubation-period. As described by Korteweg (1924) and Rudolf (1924), some patients commence the attack of malaria by showing an irregularly moderately high temperature sometimes persisting for days, others by showing a series of elevations becoming progressively higher, and still others by a sudden very high elevation following a low, perhaps subnormal, temperature. Clearly, an initial rise of temperature to perhaps 100° F. in one case cannot be taken as the equivalent of a primary elevation to 105° F. in another case. To obviate this difficulty it is suggested that two rises of temperature be recorded to show the commencement of the malarial fever,—(*a*) the first elevation to 101° F. or over, and (*b*) the first to 103° F. or over. By adopting this method it is possible to tell at a glance whether a patient commenced his paroxysms suddenly or gradually. It will be observed that in this method increases of temperature under 101° F.

are not included. Now it is, of course, possible for a malarial paroxysm to show a rise of temperature of less than 101° F., but such a small rise of temperature would be extremely difficult to differentiate from an elevation accompanying the general paralysis. Rises of temperature above 101° F. are less common in untreated general paralytics.

In this connection the method adopted for recording the patient's temperature is important. From the time of inoculation the temperature should be recorded at least every four hours. Whenever it rises above normal it should be taken at least every hour, or even every ten minutes. The temperature varies so considerably within a short time that unless it is recorded very frequently the height of the fever might be missed.

The following table shows the length of the incubation-period as measured by the onset of the fever in the first 50 cases of general paralysis inoculated with *Plasmodium vivax* at Claybury Mental Hospital.

Table I

First rise of temp. occurring	101° F. to 102.9° F.		103° F. or over	
	No. of cases	Per cent.	No. of cases	Per cent.
Up to 10 days	23	46	19	38
From 11 to 20 days	22	44	23	46
From 21 to 30 days	5	10	8	16

The above table shows that the greater number of cases show an incubation-period of less than twenty-one days.

With regard to the first appearance of parasites, Tables IV and V show the number of days after inoculation when parasites were first found. Table IV is adapted from Korteweg (1924). This observer used the thick-film method. Table V shows the first days on which parasites were found in cases treated at Claybury Mental Hospital. Korteweg does not state that he searched the thick-films for a definite length of time and similarly the thin-films of the Claybury series were not searched during a standard time.

TABLE II. Occurrence of first rise of temperature to 101° F.

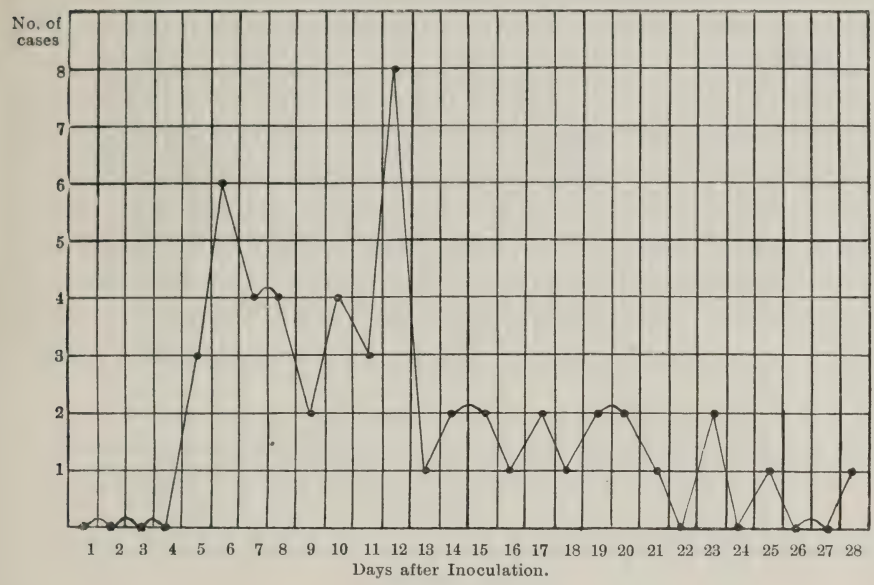


TABLE III. Occurrence of first rise of temperature to 103° F.

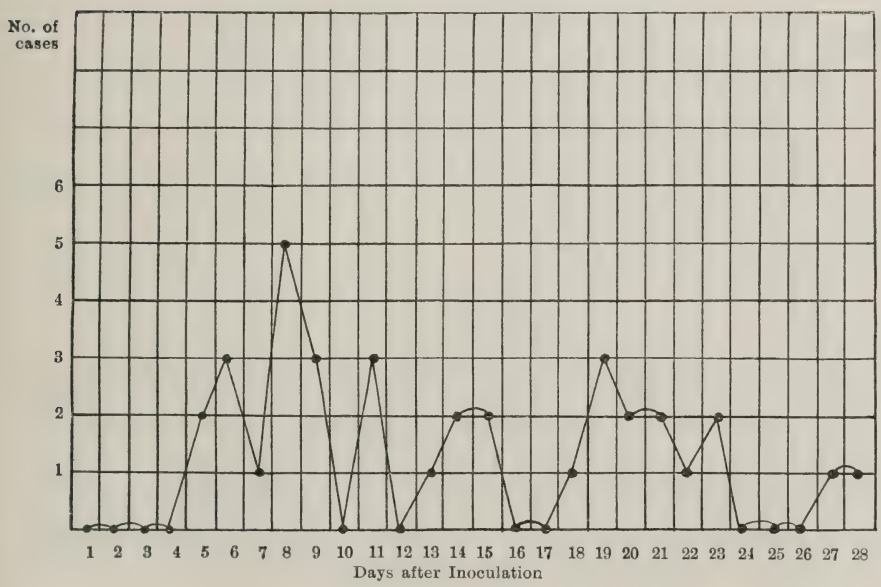


TABLE IV. First appearance of parasites in thick-films (adapted from Korteweg).

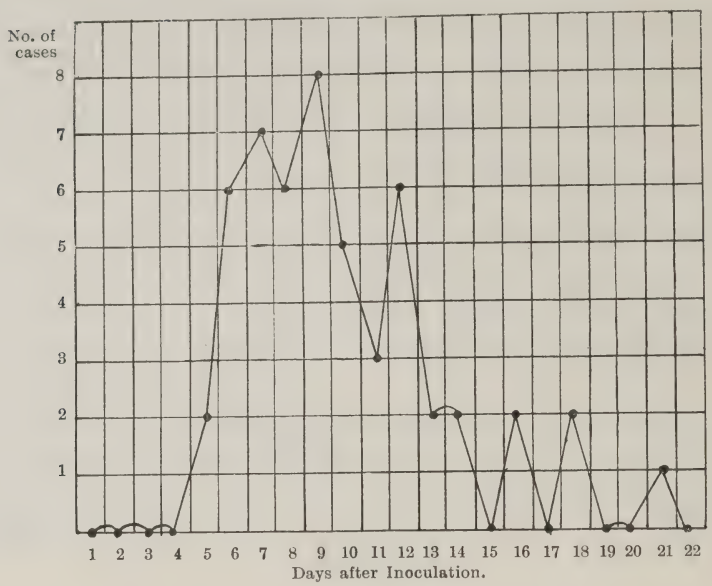
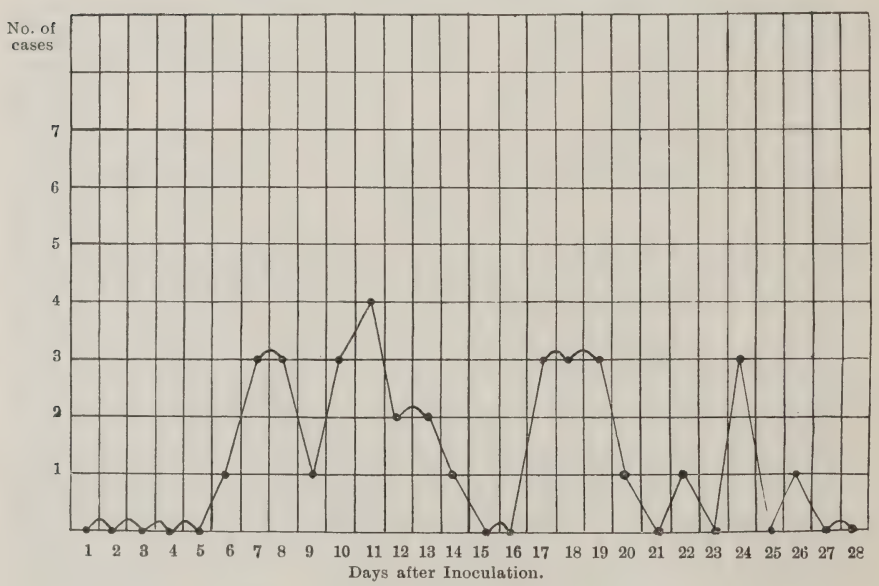


TABLE V. First appearance of parasites in thin-films.



On comparing Tables IV and V with Tables II and III it will be observed that the curves of the first rise of temperature to 101° F. and of the first time that parasites were found in thick-films are very similar. This is the more remarkable when it is remembered that the observations were made upon two series of cases treated with different strains of *P. vivax*. On comparing Table III with Table V a similarity between the curves will be again seen, both the curve of the first rise of temperature to 103° F. and the curve of the first time that parasites were found in thin-films being divided into three groups. The groups do not, however, correspond with regard to the periods in which they occur. The observations in Tables III and V were made on the same patients.

TABLES VI AND VII. Graphs of the lengths of the incubation-periods as measured by the first finding of parasites and by the first temperature-rises.

TABLE VI.

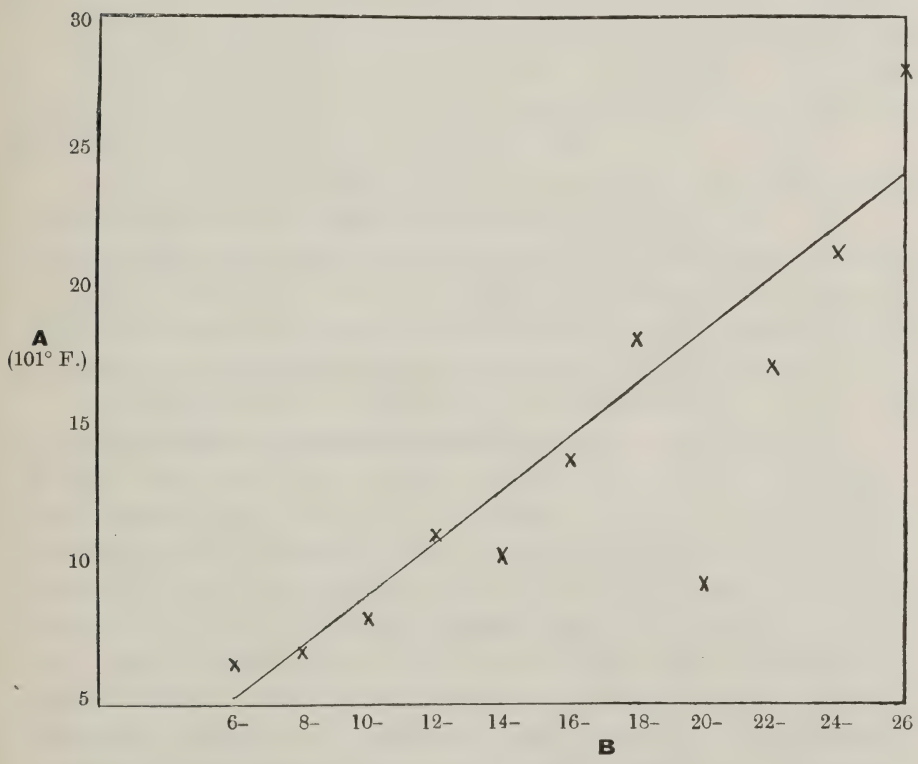
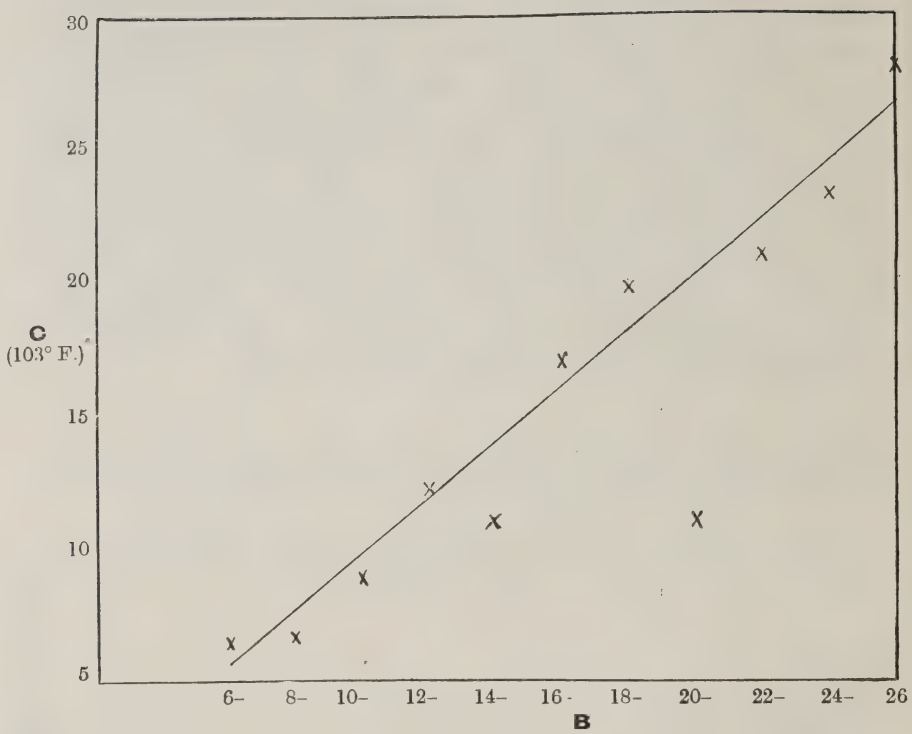


Table VII.



A. Number of days to the first rise of temperature to 101° F. expressed as averages of each group of *B*.

B. Number of days to the first finding of parasites. Cases arranged in two-day periods; e.g., patients in whom parasites were first found on the 6th and 7th days are grouped together in group 6–.

C. Number of days to the first rise of temperature to 103° F. expressed as averages of each group of *B*.

X = Average number of days before the first rise of temperature to 101° F. or 103° F., the diagonal lines are drawn to agree with the majority of the crosses.

If observations of the first time that parasites were found in thin-films, searched for varying periods, and of the first rises of temperature to 101° F. and to 103° F., in the same patients, are grouped, it will be seen that the means lie close to a straight line as shown in Tables VI and VII. These tables show that, in the same patients, there is a marked tendency for the parasites to be found for the first time when the first rise of temperature occurs. On working out the correlation-coefficients for the same variables a high correlation is observed. The coefficient for the first time that parasites were found and the first rise of temperature to 101° F. is + ·9032, and that for the same first variable but the rise of

temperature to 103° F. is + ·9091. There is, therefore, little difference between the correlation-coefficients when either the first rise to 101° F. or that to 103° F. is used.

The above confirms the observation that parasites are first found when the first rise of temperature occurs.

(iii) Intravenous inoculation. The duration of the incubation-period when this method is chosen would appear to be shorter than when the injection is made subcutaneously. Sir Ronald Ross (1911) gives details of six intravenous inoculations of benign tertian malaria. In these cases fever first appeared in from 3 to 12 days and parasites were first found in from 4 to 12 days after inoculation. Templeton (1924) states that in twenty cases of dementia praecox inoculated intravenously with 2 to 3 c.c. of malarial blood the temperature usually rose the day after inoculation. Macbride and Templeton (1924) found that pyrexia usually developed on the second or third day in a series of eighteen general paralytics. In both series of cases the temperature was, as a rule, irregular for a few days. Davidson (1925), in a series of sixteen general paralytics, found that the incubation-period varied from 4 to 19 days. The period was measured by the occurrence of fever and the first appearance of parasites.

Therefore, in 60 intravenous inoculations the incubation-period was found to vary from 1 to 19 days.

(iv) Intramuscular inoculation. Dr. D. R. Alexander has kindly supplied me with details of cases of general paralysis inoculated intramuscularly at Bexley Mental Hospital. The following table shows the length of the incubation-period as measured by the first rises of temperature. The strain of *P. vivax* utilised was the same as that used at Claybury Mental Hospital.

Table VIII

First rise of temp. occurring	101° F. to 102.9° F.		103° F. or over	
	No. of cases	Per cent.	No. of cases	Per cent.
Up to 10 days	9	60.0	8	53.3
From 11 to 20 days	6	40.0	6	40.0
From 21 to 30 days	0	0.0	1	6.7

On comparing the above table with Table I it will be observed that when the same strain of parasite is used there is a tendency for the incubation-period to be slightly shorter with intramuscular inoculation than with subcutaneous. On account of the relatively small number of cases, namely fifteen, in Table VIII it is probable that the differences between the lengths of the incubation-period with the two methods of inoculation are actually smaller than appears from the tables. This is in accordance with the findings of Davidson (1925). This observer noted that in a series of 13 cases the length of the incubation-period, as measured by the first fever and the first appearance of parasites, varied from 10 to 23 days, this approximating to the incubation-period when the subcutaneous route is adopted. In the two series of a total of 28 cases the incubation-period varied from 6 to 23 days as measured by the first occurrence of fever.

DOSAGE AND INCUBATION PERIOD

The usual dose of malaria-infected blood inoculated into general paralytics is from 1 to 5 c.c., although Pijper and Russell (1924) have used 10 c.c. There can, therefore, be great variations in the quantity injected. If one patient were inoculated with 2 c.c. of blood and a second with 4 c.c. it might be expected that the incubation-period of the former patient would be twice as long as that of the latter. But as the malarial parasite is the cause of the clinical signs of malaria it is clear that the volume of blood in itself can bear no relation to the incubation-period, but that the number of parasites present in the blood is the important factor. Therefore, in order to endeavour to determine whether there is any correlation between the number of parasites injected and the length of the incubation period it is necessary to know the actual, or the comparative, number of parasites in unit volume of blood. If the comparative number is chosen then the blood for inoculation must be drawn at one time from one patient, for the numbers of parasites vary in different cases, and also in the same case at different times.

The blood should then be divided and inoculated into the patients whose incubation-periods are to be compared. The blood must be well-shaken before each inoculation or the red cells containing the parasites will sink to the bottom and the patients will not receive the correct number of red cells according to the volume of blood injected. For the same reason no more than the exact quantity of blood required for each injection must be sucked into the syringe. If more than required is in the syringe, the first patient to be inoculated may receive too few or too many erythrocytes per cubic centimetre according to whether the needle of the syringe is held pointing upwards or downwards.

The above comparative method has been used in the study of a series of cases inoculated subcutaneously with benign tertian malaria at Claybury Mental Hospital. The following method was that adopted for determining the first appearance of the parasites. Thin-films were examined daily, commencing seven days after inoculation, except in certain cases in whom the rises of temperature started before that date. After the first appearance of parasites in the films had been found in this manner, more accurate observations were made. The films taken on the day previous to the first appearance of the parasites were each examined during a standard time of thirty minutes. Particular attention was paid to the edges and to the ' tags ' of blood at the end of the film as parasites are often found in greater numbers in these situations than in the remainder of the film. Table IX shows the results obtained. The patients bracketed together were inoculated from the same patient. The total quantity of blood required was withdrawn, divided into the necessary quantities, and injected into the general paralytics to be treated. The relationship between the quantity of blood, and therefore the comparative number of parasites inoculated, and the length of the incubation-period can therefore be studied in each series of cases bracketed together. The table shows the duration of the incubation-period as measured by the date on which the first rise of temperature to 101° F. and to 103° F. occurred.

TABLE IX

Series No.	Patients No.	Sex	Dose in c.cs.	In Days		
				Parasites first found	1st Temp. to 101° F.	1st Temp. to 103° F.
1	1	M	2	17	17	17
	1A	M	2	13	9	9
2	2	M	2	14	11	13
	2A	M	2	16	19	19
3	3	M	1.5	7	7	10
	3A	F	1.5	5	7	8
4	4	M	3	19	21	22
	4A	F	3	19	18	18
5	5	M	5	12	8	9
	5A	F	5	26	26	29
6	6	M	5	6	5	6
	6A	F	4.5	8	6	6
7	7	M	3	18	20	20
	7A	F	5	11	6	8
8	8	M	2.1	9	8	9
	8A	M	4	6	7	8
9	9	M	2	11	6	8
	9A	M	3	11	6	6
	9B	M	4	7	6	11
	9C	M	5	7	8	10
10	10	M	2	21	17	19
	10A	F	4	16	15	15
	10B	M	8	13	12	14

The above table may be divided into two groups: the first consists of the series Nos. 1 to 5, the patients in each series being given the same number of parasites; the second consists of the series Nos. 6 to 10, the patients in each series being given different

numbers of parasites. Series Nos. 1 to 4 show that when there is the same dosage of parasites the length of the incubation-period is somewhat similar in each series. In series Nos. 1, 2, and 4, it is more nearly similar when it is measured by the time that parasites were first found than by the first rises of temperature. In series No. 5 there is a marked difference between the length of the period in the two patients. Patient 5A had been inoculated previously with malaria but had not 'taken.' The resistance of this patient was presumably high. After the second inoculation, however, all the parasites could not have been destroyed but, if a large number were, the same effect would be produced as if a small number had been injected.

The later series of the table, Nos. 6 to 10, show the effect of inoculating different numbers of parasites. It will be observed that, in each series, the cases that received the smaller dose gave the longer incubation-period as measured by the first appearance of parasites. This was also found to hold when the length of the incubation-period is measured by the first rise of temperature except in one series, No. 9. In this series the patients that received the greatest number of parasites showed the longest incubation-periods as regards the first rise of temperature, but the shortest as regards the first appearance of parasites. It will also be observed that the incubation-periods measured by the first time that parasites were found agree more nearly with the dosage, in an inverse relationship, than do the same periods when measured by the first rise of temperature.

SUMMARY

(i) The incubation-period of the naturally-acquired benign tertian malaria is given by most authorities as being from 6 to 21 days with, however, wide variations.

(ii) In 34 cases (chiefly from the literature) inoculated by means of anopheline mosquitos, the incubation-period varied from 7 to 30 days as measured by the first date on which parasites were found, and, in 37 cases, from 7 to 25 days as measured by the first rise of temperature.

(iii) Subcutaneous inoculation of malarial blood gives, according to a number of writers, an incubation-period of from 1 to 32 days. A series of 50 general paralytics inoculated subcutaneously with *Plasmodium vivax* showed that 90 per cent. gave a rise of temperature of from 101° F. to 102·9° F. in less than 21 days after inoculation and 46 per cent. in less than 10 days. The first rise of temperature over 103° F. occurred within 21 days in 84 per cent. and within 10 days in 38 per cent.

(iv) Following subcutaneous inoculation there is a well-marked correlation between the first rises of temperature to 101° F. and to 103° F. and the first finding of parasites in thin-films. The frequency-curves of the first finding of parasites in thick-films and of the first rise of temperature to 101° F. are very similar, although the observations were made upon two different series of cases inoculated with two different strains of parasite. Curves of the first finding of parasites in thin-films and the first rise of temperature to 103° F., in the same series of cases, are also somewhat similar.

(v) Intravenous inoculation of malarial blood gave an incubation-period of from 1 to 19 days in a series of 60 cases collected from the literature.

(vi) In a series of 28 cases inoculated intramuscularly at Bexley and Winwick Mental Hospitals the incubation-period varied from 6 to 23 days.

(vii) In 10 series of cases injected subcutaneously with malarial blood it was found (*a*) that when similar numbers of parasites were injected the incubation-periods were of somewhat similar lengths, and (*b*) that when different numbers of parasites were injected the incubation-periods showed a marked tendency to be shortest when the dosage of parasites was the greatest. In one series of four cases this relationship did not hold as regards the first rises of temperature but only as regards the first dates on which parasites were found. In most series the length of the incubation-period as measured by the first time that parasites were found, under standard conditions, corresponded more nearly to the dosage than did the length of the period as measured by the first rises of temperature.

I have again to thank Lieut.-Col. S. P. James for his kind assistance. My thanks are due to Drs. G. F. Barham and G. Clarke, Medical Superintendents of Claybury and Bexley Mental Hospitals,

for permission to publish records from the two Institutions, and to Dr. M. Greenwood for his kindly help.

I am indebted to Dr. D. Firth, of King's College Hospital, and to Dr. F. Kiddle, of Severalls Mental Hospital, for details of cases.

REFERENCES

Acton, cited by Castellani and Chambers (1919).

Castellani, A., and Chambers (1919). *Manual of Tropical Medicine.*

Davidson, T. W. (1925). Treatment of General Paralysis by Malaria. *Brit. Med. Jour.*, 1925, Vol. I, p. 452.

Donner, S. (1925). Malarial Treatment of Progressive Paralysis. *Finsk. Lakares. Handl.*, Jan., 1925, p. 8.

Gerstmann, J. (1924). The Malarial Treatment of Progressive Paralysis. *Ars Medici*, Vol. II, pp. 345-346.

Gould, G. M. (1915). *A Pocket Medical Dictionary.*

Grant, A. R., and Silverston, J. D. (1924). Malarial Therapy in General Paralysis. *Jour. of Ment. Sc.*, Vol. LXX, pp. 81-89.

James, S. P. (1920). *Malaria at Home and Abroad.*

Korteweg, P. C. (1924). The Initial Fever in Tertian Malaria. *Ned. Tij. v. Geneesk.*, 1924, No. 15.

Macbride, H. J., and Templeton, W. L. (1924). The Treatment of General Paralysis of the Insane by Malaria. *Proc. Roy. Soc. Med.*, Vol. XVII, No. 8.

McAlister, W. M. (1924). The Rôle of Infection in the Treatment of General Paralysis. *Jour. of Ment. Sc.*, Vol. LXX, pp. 76-81.

Nonne, M. (1922). Treatment of General Paralysis and its Relation to Malaria. *Revista Med. de Chile*, Vol. L, p. 481.

Pijper, A., and Russell, B. D. (1924). Incubational Changes in the Red Cell Count in Inoculated Malaria. *Brit. Med. Jour.*, 1924, Vol. II, p. 620.

Plehn, A. (1925). Remarks on Malaria in General Paralysis Patients. *Arch. f. Schiffs. u. Tropenhyg.*, Vol. XXIX, Pt. 2.

Ross, R. (1911). *The Prevention of Malaria.*

Rudolf, G. de M. (1924). The Temperature-Chart in Artificially-Inoculated Malaria. *Jour. of Trop. Med. and Hyg.*, Vol. XXVII, pp. 259-263.

Rudolf, G. de M. (1925). The Malarial Treatment of General Paralysis. *Jour. of Ment. Sc.*, Vol. LXXI, pp. 30-41.

Scripture (1923). *Jour. of Ment. Sc.*, Vol. LXIX, p. 79.

Stitt, E. R. (1922). *Diagnosis and Treatment of Tropical Diseases.*

Templeton, W. L. (1924). The Effect of Malarial Fever upon Dementia Praecox Subjects. *Jour. of Ment. Sc.*, Vol. LXX, pp. 92-95.

Worster-Drought, C., and Beccle, H. (1923). *Brit. Med. Jour.*, 1923, Vol. II, p. 1256.

Yorke, W., and Macfie, J. W. (1924). Certain Observations on Malaria made during Treatment of General Paralysis. *Lancet*, 1924, Vol I, p. 1017.

THE MODE OF ACTION OF BAYER '205' ON TRYPANOSOMES

BY

I. J. KLIGLER AND I. WEITZMAN

(*From the Laboratory of the Malaria Research Unit, Department of Health, Haifa*)

(*Received for publication* 18 *May*, 1925)

Since its introduction the drug Bayer '205' has excited the interest of various workers engaged in the study of trypanosomes or of general chemotherapeutic problems. This interest is due partly to its active trypanocidal properties and partly to the peculiarity of its behaviour *in vivo* and *in vitro*. *In vivo* it excites a profound reaction, modifying the coagulability of the blood (Steppuhn, Zeiss u. Brychonenko, 1923) injuring the red blood cells (Sei, 1923), stimulating a lymphocytic response (Kligler & Weitzman, 1924), and, in larger doses, producing marked toxic effect on the kidneys (Duncan and Manson-Bahr, 1924). Unlike most other drugs it is retained in the body in active form for weeks after the injection (Mayer u. Zeiss, 1920, and Ruppert, 1923). Another striking peculiarity is the apparent difference in its trypanocidal power *in vivo* and *in vitro*; *in vivo* it is active in small doses, while *in vitro* it is apparently ineffective.

All those who have experimented with the drug agree as to its profound effect on the parasites in animals; but there is a considerable amount of controversy as to the mode of action on the trypanosomes. Morphological studies by Steffan (1922) and by Hesselbach (1922) indicated a direct effect on the cell protoplasm, and observations by Mayer and Zeiss (1920) and Shintake (1923) suggested an influence on the process of division. The work by Haendel and Yotten (1920) showed that there is a direct combination between the trypanosomes and the drug and that the latter cannot be released by washing. Ruppert (1923), on the other hand, concluded from his experiments that Bayer in its active form is not fixed *in vitro* although it does exert some effect, and that the action of the drug is indirect.

The nature of the action of the drug is of more than theoretical importance. If its effect is really an indirect one, it follows that the usual *in vitro* estimation of the parasiticidal property of a drug is of little value as an indication of the behaviour of the drug in the animal body. It seemed of interest, therefore, to investigate further the effect of the drug on the trypanosomes *in vitro* and to ascertain whether there is any relation between its effect *in vitro* and *in vivo*.

As our experiments were drawing to a close Nauck (1925) published a paper on the same subject. This article does not, therefore, present any new information, but our experiments serve to supplement as well as confirm Nauck's findings.

Nauck worked with a strain of nagana trypanosome and used mice as his culture medium; infecting rabbits, treating them with Bayer, and then, at varying intervals after treatment, infecting mice with the blood of the treated rabbit. Nauck used large doses of Bayer and carried on most of his experiments *in vivo*.

We used a strain of *Tr. evansi* and our procedure differed from Nauck's in that the exposure of the trypanosomes to the drug were made *in vitro* and only the effect on the organisms tested by inoculation into animals to determine loss of virulence. Our experiments were also designed to obtain an approximate quantitive comparison of the trypanocidal power of the drug *in vitro* and *in vivo*.

The following is a brief presentation of the principal experiments bearing on this question.

The object of the first series of experiments was to ascertain whether exposure to the drug in any way affected the virulence of the trypanosomes. After exposure of the organisms for varying lengths of times in varying dilutions of the drug, the trypanosomes were sedimented and injected into guinea-pigs or rabbits. Adequate controls were always made.

Experiment 1.—The first experiment consisted in exposing suspensions of trypanosomes in serum to which varying dilutions of Bayer were added. The suspension was kept three hours at 25° C. centrifugalized, the supernatant solution containing the drug was decanted, and the sediment inoculated into rabbits and guinea-pigs. The details of this experiment are given in the protocols.

Protocol a, Experiment 1. Date 23.9.24.

4 c.c. blood from a guinea-pig containing 4 trypanosomes per microscopic field, defibrinated; 1 c.c. saline added; centrifuged at 700 revolutions for 5 minutes; opalescent fluid withdrawn; divided into 3 parts; to 1 part added Bayer in concentration 1/200; to 1 part 1/400; 1 part-control; kept 3 hours at 25° C. (incubator); centrifuged; fluid decanted, sediment shaken in 1 c.c. saline, injected half into a rabbit (R.) and half into a guinea-pig (G.p.).

R. 12. Injected tryps. in Bayer 1/200; after 5 days positive.

R. 13. Tryps. in Bayer 1/400; after 9 days positive.

R. Control. After 9 days positive.

G.p. 38. Tryps. in Bayer 1/200, after 5 days positive.

G.p. 39. Tryps. in Bayer 1/400, after 10 days positive.

G.p. Control. After 9 days positive.

Protocol b, Experiment 1. Date, 2.10.24.

Guinea-pig punctured; numerous parasites; 3½ c.c. blood taken; defibrinated by means of beads; 1 c.c. saline added, centrifuged. To the plasma added Bayer to concent. 1/100, 1/200, 1/400; ½ c.c. quantum taken, kept 3 hours, centrifuged, serum decanted; to sediment added ½ c.c. saline and 0·2 c.c. injected into each animal.

R. 15. Bayer 1/100; negative, observed 37 days; 10.11.24 injected 10 c.c. oil; observed 18 days; negative; superinfected; positive after 7 days.

R. 16. 1/200; died after 5 days; intercurrent infection.

R. 16a. 1/400; died after 5 days; intercurrent infection.

R. 17. Control. Died after 5 days; intercurrent infection.

G.p. 41. Bayer 1/100; observed 37 days; results negative. 10.1.24 injected 4 c.c. oil, observed 18 days; negative. 18.11.24 superinfected; positive after 4 days.

G.p. 42. Bayer 1/200; negative; history same as g.p. 41.

G.p. 43. Bayer 1/400; positive after 14 days.

G.p. Control. Positive after 10 days.

5.10.24 preparation of material the same as that of 2.10.24 and injected again into

R. 18. 1/200.

R. 19. 1/400. To replace R. 16 and R. 16a.

R. 18. 1/200, negative; observed 35 days. 10.1.25, 10 c.c. oil; observed 18 days; negative; superinfected; positive after 5 days.

R. 19. 1/400 positive after 14 days.

It appears that contact of trypanosomes for three hours with a 1 : 100 dilution of the drug is sufficient to destroy their virulence; a 1 : 200 dilution gave variable results; in one experiment the organisms were still infective, in the other not; a three-hour exposure to 1 : 400 dilution did not completely destroy the virulence, but the incubation period was prolonged, indicating a certain degree of injury.

Experiment 2.—This experiment was similar to No. 1, except that the exposure was for twenty-four hours. The results as shown in the protocol were negative, even in a dilution of 1 : 400.

Protocol a, Experiment 2. Date, 13.10.24.

Two guinea-pigs punctured; 3½ c.c. and 2½ c.c. blood taken; positive 7 per field; defibrinated; 2 c.c. saline added; centrifuged; supernatant fluid containing tryps. withdrawn; Bayer added to dilution 1/200, 1/400 in quant. of ½ c.c.; fluid left for control. After 24 *hours* suspensions examined; tryps. alive; sluggish motion; tubes centrifuged; clear fluid decanted saline added to sediment and injected with glass capillaries intraperitoneally.

R. 25. 1/200; negative; observed 30 days. 15.11.24 injected 10 c.c. oil; negative. 28.11.24 superinfected; positive; 11 days incubation.

R. 24. 1/400; negative; observed 30 days; 15.11.24 injected 10 c.c. oil; negative. 28.11.24 superinfected; positive; incubation 9 days.

R. 25. Control; positive after 6 days.

Protocol b, Experiment 2. 12.1.24.

Two guinea-pigs bled; 3½ c.c. blood; defibrinated; centrifuged slow speed until fluid opalescent.

0.25 c.c. susp. of tryps. 0.25 c.c. 1/100 Bayer	0.25 c.c. susp. of tryps. 0.25 c.c. 1/200 Bayer	0.25 c.c. susp. of tryps. 0.25 c.c. saline
0.50 c.c. 1/200 After 24 hours at 25° C.—1/200; Alive; persistaltic movements of the undulating membrane. Motion sluggish.	0.50 c.c. 1/400 Control for motility; 1/400; Active movement.	Control Control; Active movement.

Tubes centrifuged 10 minutes at highest speed; clear serum decanted; added ½ c.c. saline to each tube; injected 0·2 c.c. into each animal.

R. 27. 1/200 negative; observed 44 days; superinfected; positive; 5 days incubation.

R. 28. 1/400 negative; after 44 days superinfected; positive after 5 days.

R. 26. Control; positive after 5 days.

Experiment 3.—This was a repetition of Experiment 1, namely, a three-hour exposure, but a smaller number of trypanosomes was injected; the results showed that even an exposure of three hours to 1 : 400 dilution of the drug renders the organisms non-infective.

G.p. 34. 3 c.c. (tryps. 1 per field) taken; defibrinated; added 1 c.c. saline; centrifuged; dilutions to 1/200, 1/400 Bayer made as in experiment date 12.1.25; kept 3·15 hours in incubator at 25° C.; centrifuged, clear fluid decanted; to sediment added ½ c.c. saline; shaken; 0·2 c.c. injected into each animal.

R. 34. 1/200 negative; observed 19 days; superinfected 1.3.25; positive after 7 days.

R. 35. 1/400; negative; observed 19 days; 1.3.25 superinfected; positive; incubation 5 days.

R. Control. Positive; incubation 5 days.

Experiment 4.—This experiment was a repetition of Experiment 2 (twenty-four hours exposure), except that higher dilutions of the drug were used (800 and 1,600). The results indicated that even as low a concentration of the drug as 1 : 1600 is sufficient to destroy the virulence of the organisms. The control trypanosomes in each case were put through the same manipulations as the drug-exposed organisms, so that the possibility of loss of virulence through mechanical injury was eliminated.

Protocol Experiment 4. 7.2.25.

Two guinea-pigs bled 2½ c.c.; tryps. 3 per field; defibrinated; added 1 c.c. saline; centrifuged at 750 revolutions; opalescent fluid still containing few r.b.c used. Dilutions with Bayer made; opalescent fluid taken; 0·9 c.c., ½ c.c., ½, etc., to the first tube added; 0·1 c.c. 10% Bayer; dilution obtained 1/100; ½ c.c. transferred to second tube; etc. Final dilutions 1/100, 1/200, 1/400, 1/800, 1/1600; tubes left for 24 hours in the incubator at 25° C. After 24 hours examined; 1/100—slight undulant movement; 1/200 sluggish movement
1/400 active movement
1/800 active movement
1/1600 active movement
Control active movement.

Tubes 1/800, 1/1600 and control; centrifuged; clear fluid decanted; sediment diluted in ½ c.c. saline; tryps. still active; injected 1/800 into rabbit 48; 1/1600 into rabbit 49; control into rabbit 50.

R. 48. Observed 30 days; negative.
R. 49. Observed 30 days; negative.
R. Control. Positive after 6 days; heavy infection.

This series of experiments showed that Bayer ' 205 ' has a marked effect on trypanosomes *in vitro*. Ordinarily this effect is overlooked because the result is judged by the motility of organisms. The motility is not, however, an index of protoplasmic injury and the principal effect of the drug lies in a lowering or destruction of virulence due presumably to cell injury.

On the basis of these experiments made *in vitro*, it appears that the action of the drug *in vivo* is also direct and that the therapeutic as well as prophylactic action of the drug depends on the concentration of the drug in the body and the rate of elimination by a given host.

Previous therapeutic experiments showed clearly that the drug is active in certain proportional doses, at least in so far as rabbits and guinea-pigs are concerned. A dose of 0·1 gm. per kilo cured all animals; 0·05 gms. per kilo gave about 80 per cent. cures, while 0·005 gms. per kilo was not effective.

This relation of dose to effect is further illustrated by the following experiment. The purpose of this experiment was to see whether an infection can be aborted by a dose of Bayer smaller than the therapeutic dose. As is seen from the protocol below, the abortive dose is the same as the therapeutic dose ; 0·05 gm. per kilo aborted the infection, while 0·005 gm. did not.

Protocol Experiment 5. 13.12.24.

Two rabbits of same weight infected 13.12.24 ; on 16.12.24 rabbit 015 given 0·005 gm. Bayer per kilo and rabbit 016 was given 0·05 gm. per kilo. R. 015, 28.12.24, blood positive. R. 016, blood negative ; observed 32 days and continued negative.

The next series of experiments dealt with the prophylactic property of the drug. The object was to ascertain whether there was any relation between dose and the duration of protection. In other words, we tried to determine the relation between concentration of the drug and prevention of infection.

Experiment 6. Three rabbits injected with different doses of Bayer and at varying intervals ; after treatment the animals were infected.

R. 017. 0·05 gm. Bayer injected 16.12.24 ; infected 35 days later ; negative.

R. 018. 0·05 gm. Bayer injected 16.12.24 ; infected 35 days later ; negative. Infected again after three months ; positive, after incubation of 15 days.

R. 019. Injected 0·1 gm. Bayer per kilo ; one month later infected ; negative ; reinfected after another month ; trypanosomes appeared in the circulation after a delay of three weeks.

This experiment indicates that Bayer apparently confers protection only so long as the drug remains in the body in a concentration sufficient to affect the parasites. This relation between concentration of drug and protection is further emphasised by the subsequent experiment.

Experiment 7.—The object of this experiment was to determine whether the minimal protective dose corresponds to the minimal therapeutic dose. In this experiment the infection was given within a week or two after the injection of the drug. It is evident from the results that a dose of ·005 gm. per kilo failed to give any protection just as this dose is devoid of any therapeutic effect.

R. 022. Given ·005 gms. Bayer ; two weeks later infected ; positive after ten days.

R. 023. Given 0·01 gm. Bayer per kilo and infection followed 8 days later ; results negative ; animal observed two months.

R. 024. Given ·005 gms. Bayer per kilo ; infected 10 days later ; positive after 7 days.

Analysis of Experiments.—The various experiments described above bring out two facts. First that contrary to our previous belief that Bayer exerts little trypanocidal action *in vitro*, it appears that the drug has a marked effect on the cell so that a dilution of 1 : 1600 is sufficient to destroy the virulence of the organisms. The other fact is that, in rabbits at least, the therapeutic, abortive and prophylactic doses are similar.

It is difficult to make comparisons between *in vitro* and *in vivo* effect, because it is not possible to determine the amount of drug which remains in circulation. The work of Mayer and Zeiss indicates that the drug is bound in the blood stream, by the serum, and is thus retained for many weeks. If the weight of the blood is accepted as approximately 1/15 the total body weight, it is possible to make a rough estimate of the effective dilution of the drug in the circulation. Our experiments show that doses of 0·005 gm. per kilo, or a dilution of 1 : 3000, fails either to protect or cure an animal while 0·01 gms. per kilo, or a dilution of 1 : 1500, is effective in a proportion of cases. Even if we assume that only 50 per cent. of the drug is bound in the serum, the effective doses *in vivo* correspond fairly well with those *in vitro*. A still further correspondence is the fact that when small doses of the drug are given the trypanosomes disappear from the circulation only sixteen to eighteen hours after treatment.

The rational conclusion then is that the therapeutic property of Bayer ' 205 ' is due to a direct injury to the trypanosomes which renders them avirulent for the host and thus readily destroyed and eliminated. The difference observed in different hosts are probably due to the rate of elimination of the drug, or in other words, to the residual concentration of the drug in the circulation.

REFERENCES

Duncan, I. T., and Manson-Bahr, P. (1924). *Trans. R.S. Trop. Med.*, Vol. XVII, p. 392.
Haendel u. Yoetten (1920). Berl. Klin. Wochenschr. N. 35.
Hesselbach, K. (1922). Centralbl. f. Bakt. Orig. Bd. LXXXIX, p. 48.
Kligler, I. J., and Weitzman, I. (1924). *Ann. of Trop. Med. and Parasit.*, Vol. XVIII, p. 437.
Mayer, M. u. Zeiss, H. (1920). Arch. f. Schiffs. u. Tropenhyg. Bd. XXIV, p. 25.
Nauck, E. (1925). Arch. f. Schiffs. u. Tropenhyg. Bd. XXIX, p. 1.
Ruppert (1923). Deutsche Tierärztliche Wochenschrift, XXXI, 44, p. 483.
Sei, S. (1923). Arch. f. Schiffs. u. Tropenhyg. Bd. XXVII, p. 130.
Shintake (1923). Arch. f. Schiffs. u. Tropenhyg. Bd. XXVII, p. 433.
Steffan, P. (1922). Zeitschrift f. Hyg. u. Infekt.-Krank. Bd. LXXXXVI, p. 263.
Steppuhn, Zeiss u. Brychonenko (1923). Arch. f. Schiffs. u. Tropenhyg. Bd. XXVII, p. 206.

ON A NEW CESTODE FROM NIGERIA

BY

T. SOUTHWELL.

(*Received for publication* 26 *May*, 1925)

A single specimen of a cestode worm from the small intestine of a ' large grey eagle ' was obtained by Dr. Ll. Lloyd at Sherifun, Northern Nigeria, 24.12.24. The species is new and is described as follows :

LATERIPORUS FUHRMANNI, n.sp. (figs. 1-4)

External Anatomy :—The worm was fragmented but apparently measured about 20 cms. in length ; its maximum breadth is 1 mm. It is composed of a very large number of segments, the posterior margins of which are imbricated ; the most posterior segments are gravid, somewhat bell-shaped and as long as broad. The genital pores are unilateral and are situated just in front of the middle of the lateral margin.

Head. The head is somewhat oval and measures about 450μ by 330μ. It is armed with a single crown of about fourteen hooks, each of which measures about 31μ in length.

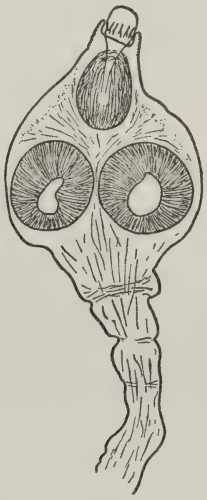


Fig. 1. *Lateriporus fuhrmanni* n.sp. Head. × 75.

Neck. A neck is present but, owing to the fact that the worm was fragmented, its length could not be determined.

INTERNAL ANATOMY :—As only a single worm was available, details relating to the muscular, nervous and excretory systems were not investigated.

FIG. 2. *Lateriporus fuhrmanni* n.sp. Hooks. × 1125.

Testes. There are about twenty-five testes situated posteriorly, behind, and lateral to the ovary. In full development they have a diameter of about 50μ.

Vas deferens. The cirrus pouch is situated anterior to the vagina, and it varies a little in shape ; usually it is a cylindrical organ extending in the median direction to the excretory vessel ; its median extremity appears glandular. The vas deferens is a long coiled tube, situated in front of the ovary and surrounded with a mass of prostatic glands.

Ovary. This is a bilobed organ composed of large acini situated in front of the testes.

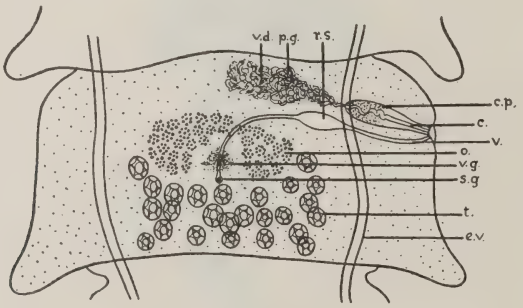


FIG. 3. *Lateriporus fuhrmanni* n.sp. Mature segment. *c.*—cirrus ; *c.p.*—cirrus pouch ; *t.*—testes ; *v.d.*—vas deferens ; *p.g.*—prostatic glands ; *r.s.*—receptaculum seminis ; *v.*—vagina ; *o.*—ovary ; *v.g.*—vitelline glands ; *s.g.*—shell gland ; *e.v.*—excretory vessels. × 75.

Vagina. The vagina runs posterior to the cirrus pouch and, immediately median to the excretory vessels, it dilates into a large pear-shaped receptaculum seminis.

The vitelline and shell glands lie immediately behind the ovary, the shell gland being very small.

Uterus. The uterus consists of a simple sac which completely fills the segment.

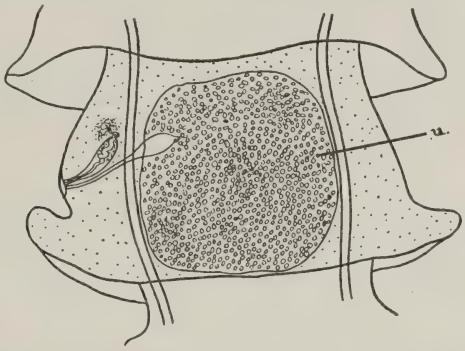


FIG. 4. *Lateriporus fuhrmanni* n.sp. Gravid segment. *u.*—uterus.

Eggs. No fully mature eggs were seen.

DIAGNOSIS. The single crown of hooks on the head, the unilateral pores, the posterior testes and the sac-like uterus place this worm in the genus *Lateriporus* Fuhrmann 1907. Six species of this genus are known.

The following table shows how *L. fuhrmanni* differs from the other species of the same genus, viz., principally in the size of the hook.

	Length of worm	No. of hooks	Size of hooks	No. of testes
cylindrica (Clerc, 1902) ...	25 mm.	16	200-216μ	15
teres (Krabbe, 1869)	42-60 mm.	12-16	150-170	30
biuterinus Fuhrmann, 1908 ...	300 mm.	16	120μ	16-18
spinosus Fuhrmann, 1908 ...	40 mm.	22	50μ	6 (?)
propeteres Fuhrmann, 1907 ...	several centimetres	16	120μ	about 12
geographicus Cooper, 1921 ...	172 mm.	?	?	15-20
fuhrmanni n.sp.	about 200	about 14	31μ	25

The type specimen is in the collection of the Liverpool School of Tropical Medicine.

REFERENCES

Cooper, A. R. (1921). Trematoda and Cestoda. *Rep. Canad. Arctic Exped.* 1913-1918. 9. Part G-H. 27 pp. 2 pls. Ottawa.

Fuhrmann, O. (1907). Bekannte und neue Arten und Genera von Vogeltänien. *Centralbl. f. Bakt., etc. I Abt. Originale.* Bd. XLV. Heft. 6, pp. 516-536.

——— (1908). Nouveaux Ténias d'Oiseaux. *Rev. Suisse de Zool.* Tome, XVI. Fasc. 1. Geneva. pp. 27-73.

Lühe, M. (1920). Parasitische Plattwürmer. II: Cestodes. *Die Süsswasserfauna Deutschlands.* Heft XVIII.

SOME CHARACTERISTICS OF THE FIRST STAGE LARVA OF *DERMATOBIA HOMINIS* Gmelin

BY

R. NEWSTEAD, F.R.S.,

AND

W. H. POTTS, B.A.

(*Received for publication* 8 *June*, 1925)

Plates IV and V.

In Central and South America there is an Oestrid Fly, *Dermatobia hominis*, whose larva is the cause of cutaneous Myiasis in man and animals. These larvae are able to penetrate the unbroken skin, and there, in the course of development to maturity, give rise to tumours similar to those produced by the larvae of the Warble Flies (*Hypoderma* spp.) of Europe and North America. They are a source of considerable loss to cattle-owners, since the hides, riddled by the holes left on the emergence of the fully-grown larvae, are often valueless. According to Da Matta (1920), the proportion of hides thus damaged may be from 5 per cent. to as much as 70 per cent. The larvae are also indirectly responsible for the death of many animals, especially calves, since the tumours caused by them are liable to secondary infection from other myiasis-producing flies, whose larvae are unable themselves to pierce the unbroken skin. There is one fly of this latter type, the ' Screw-worm ' (*Chrysomyia macellaria*), which is very abundant in the Neotropical Regions and which in this way does a great deal of damage. Lastly, the *Dermatobia* larvae are the cause of much pain and inconvenience to man when passing through infected regions, and even give rise to serious illness if present in large numbers.

This Oestrid Fly differs from all other members of its class in that, instead of laying eggs or larvae directly on the hair or skin of the host, it lays batches of its eggs on the bodies of other insects, chiefly mosquitos. The larva of *Dermatobia* does not, apparently, leave the egg until the mosquito alights on a warm-blooded animal

to take a meal. Thus it would seem that the larva must be highly sensitive to a slight rise in temperature, and that the emergence from the egg must of necessity take place during the comparatively brief period in which the mosquito feeds. There is evidence to show that the larva, if unable to emerge completely from the egg during the time the mosquito is feeding, may withdraw itself into the egg and there wait until the mosquito visits another animal. This may occur several times, the larvae being capable of remaining alive for twenty days before reaching a host.

We are indebted to Dr. Nunez Tovar, who himself has done much to elucidate the remarkable life-history of the *Dermatobia*, for his gift of material which has enabled us to study these young larvae which penetrate the skin of their hosts. Before we present a detailed description of its characteristics, it has been thought well to give a short account of the life-history of this fly, as some interesting work has been done since the last comprehensive account in English was written by Sambon in 1915.

THE DISTRIBUTION OF THE FLY

According to Neiva and Gomes (1917), *Dermatobia hominis* occurs in Central and South America from Mexico to the Argentine. It appears to be absent from the United States, for, although cases infested with the larvae have been recorded from that country, it has always been found that the larvae were acquired in Central or South America. The fly seems to need a warm temperature, a certain degree of humidity, and forest country.

HOSTS OF THE FLY

The occurrence of larvae in the skin of man and various animals has long been known. The domestic animals in which they have been found are, in order of importance :—Cattle, dogs (especially hunting dogs), pigs, goats, turkeys, and, rarely, mules. There appears to be some doubt as to whether they occur in sheep, donkeys and horses. It is, in fact, sometimes stated that horses are not infested, but Neiva and Gomes (1917) give one record of the finding of larvae in a horse. The larvae have also been found in the

following wild animals :—Monkeys (whence the name ' Ver Macaque,' frequently given to the larva), jaguar, tapir, coati, agouti, deer (rarely), squirrels, and even birds, e.g., toucans and the ant-thrush (*Formicarius* sp.).

DISPOSAL OF THE EGGS

Although the existence of these larvae has been known for so long, there has been considerable doubt and uncertainty as to the exact way in which they reach the skin of their hosts.

Morales (1911), in Guatemala, was the first to publish a statement to the effect that the eggs were carried, firmly attached to the abdomen of a mosquito, *Psorophora* (then *Janthinosoma*) *lutzi*. From such eggs Morales obtained a larva, which produced in man characteristic tumours, and which presented all the characters of a *Dermatobia* larva. Tovar, in Venezuela, made similar observations two months earlier, but these were not published until 1913, in an article by Gonzales Rincones in the newspaper ' El Universel ' of Caracas.

These observations have since been confirmed by other observers, different flies being found to be *involuntary carriers* of the eggs in different parts of the country. Specimens of *Dermatobia* astride other flies have been caught, and even observed in the act of siezing the flies. According to Neiva and Gomes (1917), the adult *Dermatobia* frequent horses and other animals, and sieze flies which come either to suck blood or to feed upon sweat.

The final piece of evidence, the observation of the act of deposition of the ova by *Dermatobia*, has also been recorded. Neiva and Gomes (*loc. cit.*) enclosed adult females with various flies and found that eggs were laid on *Musca domestica*, *Stomoxys calcitrans*, and also on some Sylvan Muscoids. From these eggs larvae were obtained and were reared to the adult stage in dogs ; the whole process, from the laying of the eggs to the emergence of the adult, occupied 120 to 141 days.

Dr. N. Tovar also (1924) placed captured *Dermatobia* with specimens of the mosquitos—*Psorophora posticata*, *P. lutzi*, *P. tovari*, *Aedes trivittatus*, *Stegomyia calopus*, *Culex scapularis*, and Woodland Muscoids. Bundles of eggs were laid on all the examples of *Psorophora* (fifteen in all), irrespective of sex, whilst on none of

the others (twenty specimens in all) was a single egg to be found. In fact, he stated that although the insects other than *Psorophora* were sometimes seized by the *Dermatobia*, they were treated with violence and discarded damaged, whereas the *Psorophora* were always treated gently and liberated unharmed. He also records that *Dermatobia* eggs were never found in nature save on specimens of *Psorophora* (Plate IV, fig, 1).

In view of the results of modern investigations it is interesting to record some of the names by which the natives of various parts of America referred to the fly. For instance, in Venezuela the worm was commonly known as Gusano de Zancudo, in Colombia as Gusano de Mosquito, and in Trinidad as Ver Marangouin ; all of these terms mean ' Mosquito worm.' In an old book, the ' Historia del Nuevo Mundo,' written in 1653 by a Jesuit, Father Bernabe Cobo, the following statement, doubtless based on information received from the natives, is found :—' In some of the warm lowlands there is a species of mosquito . . . somewhat reddish. In each wound produced by this mosquito, soon grows within the flesh a spine-covered worm the size of a haricot bean or even larger . . . .' (Quoted from Sambon, 1915). Knab (1913) states that in 1905 the natives of the Isthmus of Tehuantepec, Mexico, pointed out to him certain large mosquitos (*Psorophora*) as ' Madre del Gusano.' In the first reference to this fly under the binomial system of nomenclature, that of Linnaeus Junior (1781), we find :—' . . . . the fly deposits on a man's skin, one after another, its eggs, or rather, its living larvae, *of which it carries about* 50 *on its hinder portion.*' (Quoted from Sambon, *loc. cit.*).

Da Matta (1920), in his account of *Dermatobia*, states that the mode of transference of the larvae may also be by direct oviposition on the skin of animals, or by indirect methods, by their deposition on leaves, from which the larvae may be picked up by passing animals, or by deposition on sweaty garments. These have been discussed by Neiva and Gomes (1917), For the first, we have been unable to find any record of a direct observation, either of eggs being found attached to the skin of animals, or of the act of deposition on the skin, although there is a record of *Dermatobia* having been seen hovering over horses with the ovipositor extended.

As to oviposition on leaves, there appear to be no records of

leaves having been found with ' packets ' of eggs adhering to them. Neiva and Gomes (*loc. cit.*) record that eggs were deposited on the sides of the vessel. They offer the explanation that, at a given moment, the female feels the necessity for oviposition irrepressible ; if then the insect which she is attempting to catch escapes, she oviposits on the nearest object. They found that eggs so laid, if kept in a moist place, produced larvae ; if, however, the conditions were dry, the eggs shrivelled and perished. They suggest that this may happen in nature, but the chances of larvae so produced being picked up by appropriate hosts do not seem to be very great. Since, however, it has been shown that the larvae can remain alive for twenty days in the egg without finding a host, this may be an alternative mode of transference.

Oviposition on ' sweaty ' clothes, too, seems to be supported by no direct observation. Neiva (1914) supports the hypothesis, saying that it would account for cases of infection of newly-born children who have never left the house. But he states that such cases are rare.

CARRIERS OF THE EGGS

The following insects have been found bearing batches of *Dermatobia* eggs in nature :—

Brazil :—*Psorophora posticata* (one example only, by Neiva and Gomes, 1917. Also by Peryassu, 1922).
Anthomyia heydenii (Lutz, 1917).
Anthomyia lindigii (Lutz, 1917).
Synthesiomyia brasiliana (Lutz, 1917).
Woodland Muscoids (on numerous occasions, Neiva and Gomes, 1917).

Guatemala :—*Culex* sp. unknown (Morales, 1911).

Panama :—*Goeldia longipes* (a non-bloodsucking mosquito, Shannon, 1925).

Trinidad :—*Psorophora* (then *Janthinosoma*) sp. (collected by Mr. F. Urich. Knab, 1913).

Venezuela :—*Psorophora lutzi*, *Psorophora posticata* (Tovar, 1924).

In captivity, *Dermatobia* has laid packets or batches of eggs on the following insects :—*Musca domestica*, *Stomoxys calcitrans*, Woodland Muscoids. (Neiva and Gomes, 1917). *Psorophora posticata*, *P. lutzi*, and *P. tovari* (Tovar, 1924).

Blanchard (1896) was sent the following flies by Da Silva Araujo in 1893, as being incriminated by the natives as ' parents of the Berne ' (*Dermatobia* larva) :— *Lucilia ruficornis* Macq., *Sarcophaga chrysostoma* Wd., *S. plinthopyga* Wd., and an *Hystricia*.

Neiva (1910) states that in Brazil nearly all species of *Tipulidae*, *Volucella obesa*, and a species of *Mesembrinella*, are accused of producing the warbles, whilst in Matto Grosso, several species of *Echinomyia*, and in Mexico, the beetle *Atractoceros brasiliensis* were also suspected. He, however, considered these popular beliefs to be erroneous.

Finally, Dunn (1918) has suggested the possibility of a tick (probably *Amblyomma cajannense*) being a carrier. The evidence is as follows :—Dr. Clark, in the course of two trips into the interior of Panama, discovered larvae of *Dermatobia* five times in wounds in man caused by tick bites. *Psorophora* were not obtained in collections of mosquitos from the places at that time, and besides, four out of the five sites were protected by clothing so that subsequent infestation of the wound seems improbable.

GENERAL DESCRIPTION OF FIRST INSTAR LARVA (Plate IV)

The general outline is somewhat elliptical, bluntly rounded anteriorly, and gradually attenuated posteriorly, the width of the last two segments being approximately half the width of the mid-thoracic segment, as seen in profile, after maceration in caustic potash (See fig. 1).

The cephalic segment is scantily clothed with very minute spines ; these appear to be more numerous dorsally and bilaterally (fig. 3A). The first thoracic segment bears a continuous band of relatively small and closely set black spines. The second and third thoracic segments are completely clothed with similar spines (figs. 3B and C). First, second and third abdominal segments show a double transverse series of large spines dorsally, and a single series ventrally ; the interspaces are set with smaller spines which are much more numerous in the posterior series than in the anterior one. The fourth to sixth segments inclusive are spineless. The seventh segment is clothed with long and slender, translucent spines (fig. 3D). The terminal segment is almost covered with relatively large strongly hooked and translucent spines (fig. 3E).

The spines on the thoracic and first three abdominal segments are directed backwards, whilst those of the last two segments are directed forwards. This arrangement of the posterior groups of spines enables the larva to retain a firm hold of the inner walls of the egg-shell after partial emergence.

The main tracheal tubes of the respiratory system, which resist the action of caustic potash, show very clearly (fig. 2). On the other hand the posterior stigmata are minute and not very clearly defined; they communicate with the tracheal trunks by two long and slightly narrower felt chambers which extend to the middle of the penultimate segment.

The antennal organs (fig. 2), presumably corresponding to the antenno-maxillary organs of other Dipterous larvae as described by Keilin (1915), are placed well forward in the cephalic segment in a dorso-lateral position; the proximal portion of the organ is strengthened with an incomplete band of dark chitin, the terminal portion being translucent.

THE MOUTH PARTS (Plate V)

The mouth parts consist of the following paired appendages:—

(1) Mouth hooks (*mh* in all figures).
(2) ' Prestomal sclerites ' (*ps* in all figures).
(3) Stomal plates (*sp* in all figures).
(4) Membranous bands (*mb* in all figures).
(5) Rudimentary Hypo-pharyngeal sclerite (fig. 1C, *hs*).
(6) Cephalo-pharyngeal sclerites (*cs* in all figures).

(1) *The Mouth Hooks.*

These are highly chitinised, blackish and strongly falciform structures, the inner edge being finely though somewhat irregularly serrated. Proximally the anterior portion is strongly produced (figs. 1A, *mh*, and 1D). There are two centrally placed foramina.

(2) *The ' Prestomal sclerites.'*

These appear to consist of very thinly chitinised, translucent plates, which may act as a sheath to the tips of the mouth hooks. They are not apparent in fig. 1A, being hidden by the stomal plates, but they are indicated in fig. 1C, *ps*.

(3) *The Stomal Plates.*

These are relatively large cone-like processes, converging distally, and with longitudinal but somewhat indefinite ridges ; proximally these structures are partly surrounded by a strongly chitinised plate, which is toothed on its distal or anterior margin (figs. 1A and B, *sp*.1, and fig. 1E). Below the cones is a mass of tissue with an irregular outline, portions of which seem to bear chitinous bodies, possibly muscle attachments.

(4) *Membranous Bands.*

These very thin and very slightly chitinised structures appear to arise towards the base of the mouth hooks ; they are curved, and directed outwards and slightly backwards, the tips in some cases being slightly curved inwards, and somewhat strongly chitinsed.

(5) *Hypo-pharyngeal Sclerite.*

This consists of a median and very thinly chitinised plate with a pair of sub-median foramina, and lies between the anterior processes of the cephalo-pharyngeal sclerite, at the articulation with the mouth-hooks.

(6) *Cephalo-pharyngeal Sclerites.*

These consist of two plates, which are free dorsally, each consisting of three processes : a long, fairly heavily chitinised, anterior, inferior one, a short, fairly heavily chitinised, dorsal one, and a ventral one so lightly chitinised that it is difficult to see how far it extends into the thoracic region.

SOME AFFINITIES AND RELATIONSHIPS WITH OTHER FORMS

The most marked characteristics of the buccal organs of the first instar of *Dermatobia hominis*, are the presence of—

(1) the paired and well-developed mouth hooks (*mh*) ;
(2) the cone-shaped stomal plates (*sp* and *sp*1)

Another noteworthy feature is the absence of an unpaired median tooth, such as is found in most other first stage larvae, and is shown in *Hypoderma bovis* (Plate V, fig. 3, *mt*).

In his extensive paper on the larvae of Cyclorhaphous Diptera, Keilin (1915) states that in certain Acalyptrates, especially those with carnivorous larvae, one finds a precocious development of the paired mouth hooks of later stages. This condition also obtains in the first stage larva of *Calliphora*, where, however, as generally, a median tooth is present as well; *Hypoderma bovis* (Pl. V, fig. 3, *mt*, *mh*) shows both paired hooks and median tooth. No trace of the latter structure is to be seen either in *Dermatobia hominis* or in *Cordylobia anthropophaga* (Plate V, 2A-C), and this is paralleled in a figure given by Keilin of *Onesia sepulchralis* (*loc. cit.*, Pl. X, fig. 49C).

The paired mouth hooks of *Cordylobia anthropophaga* ('median buccal spine' of Blacklock, 1923, fig. 1, 3C and D) show a very remarkable modification. These structures, of which four aspects are shown in our illustration (Plate V, figs. 2A-D) are broadly dilated unilaterally at the tips and strongly toothed on the distal margin (figs. 2A-C, *mh*, and fig. 2D). Seen in profile (fig. 2B) they are strongly directed upwards, and according to Blacklock lie, when at rest, at right angles to the cephalo-pharyngeal sclerite. These processes are also very strongly developed dorsally (figs. 2B and C, *mh* 3) and bilaterally are broadly expanded (fig. 2A, *mh*1). Further, ventrally there is a thin and broadly dilated flange (figs. 2A and C, *mh* 2) which appears to be connected with the finely spinose lower lip of the buccal cavity (fig. 2C, *bs*).

In the larva of *Hypoderma bovis*, which shows both median unpaired spines (fig. 3, *mt*) and paired mouth hooks (fig. 3, *mh*), the latter, as Carpenter and Hewitt (1914) have pointed out, are remarkable for being widely separated, directed laterally, and pointing outwards instead of upwards as in *Cordylobia anthropophaga*, or downwards and normally, as in *Dermatobia hominis*.

The peculiar form and high development of the structures we have called the stomal plates seem to be without exact parallel in other first stage larvae. They may be represented in a rudimentary form by some of the accessory pieces which Keilin has described (*loc. cit.*). For instance, there is an appendage which Keilin terms 'pièce en brosse' (*loc. cit.*, Plate VIII, fig. 37, *f*, and *f* in other figures) which may be homologous, but as we have been unable to study these forms we cannot give a definite opinion as to their homologies.

Again there are the paired structures to which we have given

the term ' membranous lobes.' These are very indefinite structures. They appear in the case of *Hypoderma bovis* (fig. 3, *mb*) to correspond in position with the parastomal sclerites described by Lowne (1890) in the third stage larva of *Calliphora erythrocephala*, whilst in *Dermatobia hominis* they appear to be in a more anterior position, lying well in front of the bases of the mouth hooks.

It is interesting to note that in these three closely allied forms, all adapted to the same mode of life, quite different dispositions of the mouth parts exist. Thus the larva of *Dermatobia* penetrates the unbroken skin of man and animals, that of *Cordylobia* the skin of rats and sometimes man, and that of *Hypoderma* the tough hide of cattle. Of the three, *Dermatobia* perhaps approximates most closely to the condition shown by *Calliphora*, differing from it markedly by the absence of the median tooth, and by the strong development of the stomal plates; *Hypoderma* agrees with *Calliphora* in the possession of lateral hooks and median tooth, but differs in the disposition of these organs, the lateral hooks being directed outwards instead of downwards. *Cordylobia* is the most aberrant of the three, the mouth hooks being modified in a most extraordinary way, and directed dorsally instead of ventrally; the latter character is no doubt correlated with the mode of penetration of the larva in a horizonal direction under the skin, as has been well described by Blacklock (*loc. cit.*).

REFERENCES

I. LIFE-HISTORY

Da Matta, A. (1920). Considerações sobre a Dermatobiose (Ura ou Berne no Brasil). *Amazonas Medico*, Manaos, III, p. 2.

Dunn, L. H. (1918). The Tick as a possible agent in the collocation of the eggs of *Dermatobia hominis*. *Jl. of Parasitology*, IV, p. 154.

Knab, F., (1913). The Life-History of *Dermatobia hominis*. *Amer. Jl. Trop Dis. & Preventive Med.*, I, p. 464.

——— (1916). Egg disposal in *Dermatobia hominis*. *Proc. Ent. Soc. Washington, D.C.*, XVIII, p. 179.

Lutz, A. (1917). Contribuições ao Conhecimento dos Oestrideos brazileiros. *Mem. Inst. Oswaldo Cruz*. Rio de Janeiro, IX, p. 94.

Morales, R. (1911). *El Nacional* (a newspaper of Guatemala. This has not been consulted; our information is derived from Sambon, 1915).

Neiva, A. (1914). Informações sobre o berne. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, VI, p. 206.

Neiva, A., and Gomes, J. F. (1917). Biologia da Mosca do Berne (*Dermatobia hominis*) observada em todas suas phases. *Annaes Paulistas de Medicina e Cirurgia*, VIII, p. 197.

Peryassu, A. (1922). Os mosquitos portadores de ovas da *Mosca* do 'Berne.' (Nota Previa). *A Folha Medica*, Rio de Janeiro, III, p. 105.

*Sambon, L. W. (1915). Observations on the Life History of *Dermatobia hominis* (Linnaeus, jun., 1781). *Rep. Advisory Committee for Trop. Dis. Res. Fund* for 1914, App. VII, p. 119.

Shannon, R. C. (1925). Brief History of Egg-laying Habits of *Dermatobia*. *Jl. Washington Acad. Sci.*, XV, p. 137.

Surcouf, J. (1913). La transmission du ver macaque par un moustique. *C. R. Hebd. Acad. Sci.*, Paris, CLVI, p. 1406.

Tovar, N. (1924). Notas de Historia Natural Medico. Experienca para determinar que zancudo transmite el Gusano de Monte. *Boletin de la Camara de Comercio de Caracas*, XIII, p. 2540.

*Ward, H. B. (1903). On the Development of *Dermatobia hominis*. *Mark Anniversary Volume*, Article XXV, p. 483.

* These two papers contain excellent accounts, with full bibliographies, of the early literature.

II. ANATOMY OF THE MOUTH PARTS

Blacklock, B., and Thompson, M. G. (1923). A study of the Tumbu-Fly, *Cordylobia anthropophaga*, Grünberg, in Sierra Leone. *Ann. Trop. Med. and Parasit.*, XVII, p. 443.

Carpenter, G. H., and Hewitt, T. R. (1914). The reproductive organs and the newly hatched larva of the Warble Fly (*Hypoderma*). *Sci. Proc. of the Roy. Dublin Soc.*, XIV, p. 268.

Hewitt, C. G. (1914). The House Fly. Cambridge University Press, p. 102.

Keilin, D. (1915). Recherches sur les larves de diptères cyclorhaphes. *Bull. Sci. de la France et de la Belgique*, XLIX, p. 16.

Lowne, B. T. (1890-1892). The Blow Fly. London. Vol. I, p. 41 et seq.

Wahl, B. (1915). Über die Kopf-bildung cyclorhapher Dipteren—larven und die post-embryonale Entwicklung des Fliegen kopfes. *Arb. Zool. Instit.*, Wien XX, p. 159.

EXPLANATION OF PLATE IV.

Dermatobia hominis.

FIG. 1. Adult mosquito (*Psorophora posticata*) carrying a batch of eggs of *Dermatobia hominis* on the ventral surface of the abdomen. Note that in one instance the embryo larva is shown partly protruding from the egg. × 10.

FIG. 2. Larva, First Instar. Lateral view. From a specimen macerated in caustic potash. Showing the arrangement of the dermal spines and the main sub-lying tracheal tubes. × 140.

FIG. 3A. Dermal spines from the cephalic segment.

FIG. 3B. Dermal spines from a thoracic segment.

FIG. 3C. Dermal spines from a thoracic segment.

FIG. 3D. Dermal spines from the penultimate abdominal segment.

FIG. 3E. Dermal spines from the terminal abdominal segment.

Figs. 3A-3E (inclusive) × 500.


1
= 2 mm


= 0·2 mm
2

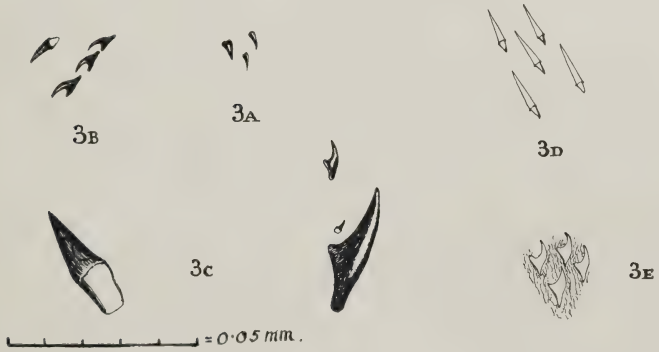

3B
3A
3D
3C
3E
= 0·05 mm.

PLATE V

EXPLANATION OF PLATE V.

Mouth Parts of First Instar Larva of Dermatobia hominis.

FIG. 1A. Profile.

FIG. 1B. Ventral.

FIG. 1C. Dorsal.

FIG. 1D. Mouth hook.

FIG. 1E. Two views of the proximal portions of the stomal plates, detached from the cone-shaped process.

Mouth Parts of First Instar Larva of Cordylobia anthropophaga.

FIG. 2A. Ventral.

FIG. 2B. Dorsal.

FIG. 2C. Profile.

FIG. 2D. Terminal portion of mouth hooks, ventral view, showing the arrangement of the teeth × 1000.

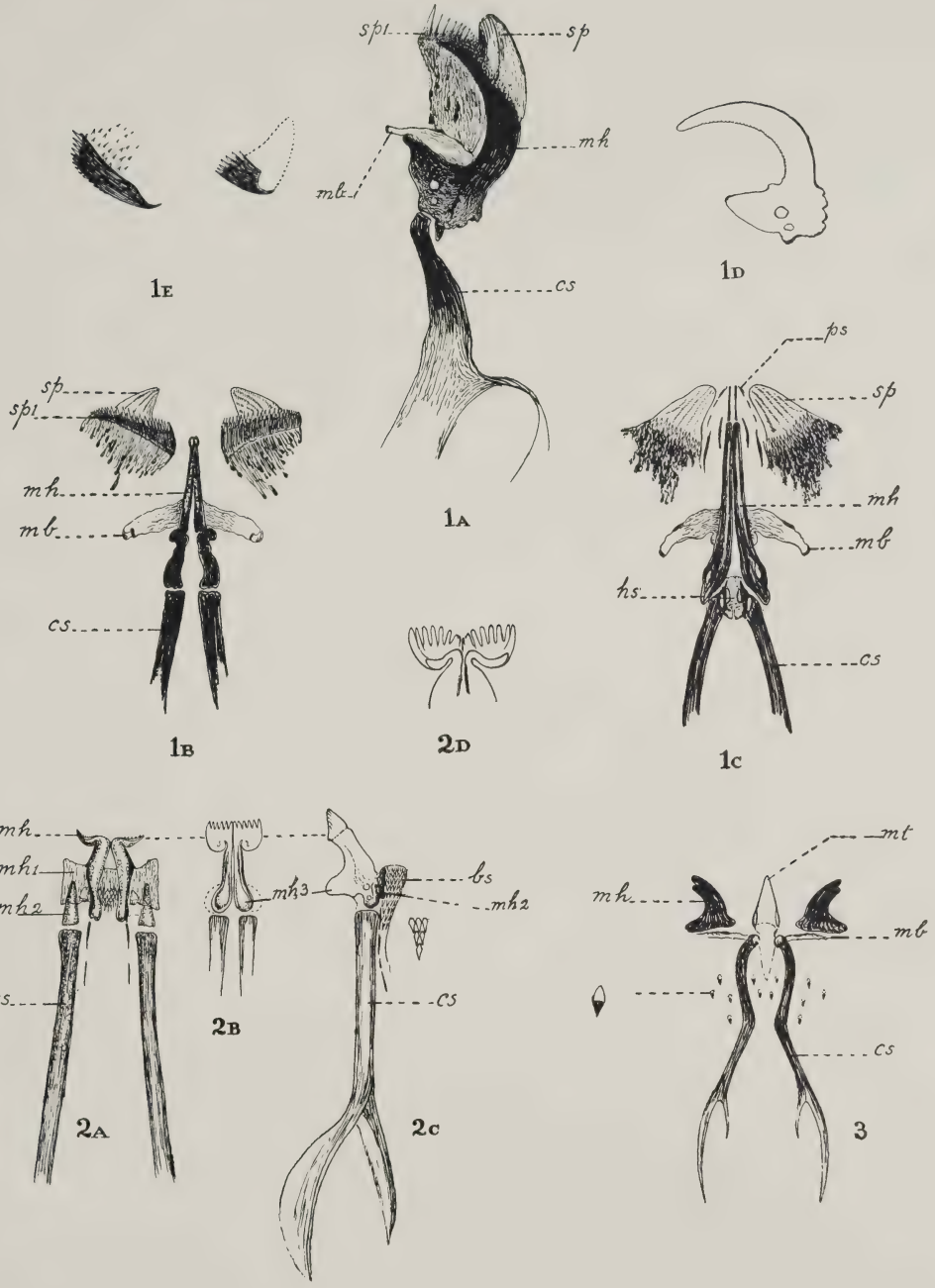
Mouth Parts of Third Instar of Hypoderma bovis.

FIG. 3. Dorsal.

All the figures with the exception of Fig. 2D. × 500.

EXPLANATION OF LETTERING.

cs = cephalo-pharyngeal sclerites.
bs = buccal spines.
hs = rudimentary hypo-pharyngeal sclerite.
mb = paired membranous bands.
mh = mouth hooks.
*mb*1 = lateral proximal extension of the mouth hooks.
*mb*2 = ventral proximal flange of the mouth hooks.
*mb*3 = dorsal proximal process of the mouth hooks.
mt = median tooth.
ps = prestomal sclerites.
sp = stomal plates.
spl = proximal pieces of stomal plates.


spl
sp
mh
mb
cs
1E
1A
1D
sp
spl
mh
mb
cs
1B
2D
ps
sp
mh
mb
hs
cs
1C
mh
mh1
mh2
cs
2B
mh3
bs
mh2
cs
2A
2C
mt
mh
mb
cs
3

MISCELLANEA

PLACOBDELLA PARASITICA

Dr. Aitken Clark has presented to the Liverpool School of Tropical Medicine a large leech measuring, when fully expanded, about seven inches, which was found sucking human blood, in Para, Brazil.

The specimen proved to be *Placobdella parasitica* (Say) 1824. It is most commonly found attached to species of *Chelydra*, etc.

T. SOUTHWELL.

A CASE OF EMPYEMA SIMULATING ABSCESS OF THE LIVER

The patient, a male aged thirty, Brazilian, who had resided in Amazonas for the past ten years, was admitted to hospital 29.12.21.

History.—Patient dates his present illness from an attack of dysentery six months ago. Symptoms began with pain in the right hypochondriac region, debility, fever every afternoon, profuse sweating, loss of weight and swelling of the abdomen.

On admission.—Patient very much emaciated; abdomen generally distended, but markedly so in the right hypochondriac and epigastric regions. The right hypochondrium is occupied by a swelling, resistant, somewhat resilient, dull on percussion, reaching almost to the level of the right anterior spine of the ilium and across the mid-line; the swelling bulges in the right flank. Dullness on percussion is complete as high as the right nipple in front, and almost to the inferior angle of the scapula posteriorly. No fluctuation is perceptible over the swelling, which moves slightly with respiration.

No cough; no sputum; dullness and faint breath sounds over the base of the right lung as high as the inferior angle of the scapula.

Treatment and Progress.—On 31.12.21 an exploratory puncture was made one inch below the right costal margin in the anterior axillary lines, in the most prominent and most tender part of the tumour. One-and-a-half litres of reddish brown pus were aspirated with great relief of symptoms. Although no amoebae were present, on the assumption that the condition was probably liver abscess, a

grain of Emetine was administered hypodermically, and repeated daily till 21.1.22. The evening temperature was normal for a week, but then rose again. Another litre of pus was aspirated, but again two days later the temperature rose. It was then considered advisable to operate. On 21.1.22 a needle was inserted one inch below the costal margin in the anterior axillary line and a small amount of pus aspirated with a syringe. Under chloroform anaesthesia an incision was made, four inches long, parallel to the costal margin, its centre being just below the insertion of the needle. When the incision was deepened it was seen that the needle penetrated the diaphragm, which bulged down below the level of the incision. The diaphragm was incised and two litres of pus were evacuated. A finger passed through the incision and upwards encountered a large empty space, with the lung collapsed towards the apex. Inferiorly the diaphragm, partly adherent to the upper surface of the liver, was found to bulge downwards, below the level of the skin incision, to a distance of about two inches. A drainage tube was inserted and the remainder of the incision closed. For a week a fair amount of pus was discharged, the patient being encouraged to do breathing exercises to expand the lung.

2.2.22. Discharge much diminished ; drain and stitches removed.

5.3.22. Patient left hospital ; practically no discharge ; feeling very well and weighing 20 kilos more than on admission.

15.3.22. Patient returned to report. In the interval he had been riding on horseback every day. Wound closed and patient feeling very fit.

R. M. Burnie.

THE GOLUBACSER FLY

'. . . There is, in Servia and the Banat, a minute fly,* from whose destructive assaults on the cattle the inhabitants have suffered immense losses. A traveller, arriving at Golubacs, on the Danube, thus speaks of it :—

" Near this place we found a range of caverns, famous for producing the poisonous fly, too well known in Servia and Hungary

* *Simulium columbaschense* Köll.

under the name of the Golubacser fly. These singular and venomous insects, somewhat resembling mosquitos, generally make their appearance during the first great heat of the summer, in such numbers as to appear like vast volumes of smoke. Their attacks are always directed against every description of quadruped, and so potent is the poison they communicate, that even an ox is unable to withstand its influence, for he always expires in less than two hours. This results, not so much from the virulence of the poison, as that every vulnerable part is simultaneously covered with these most destructive insects; when the wretched animals, frenzied with pain, rush wild through the fields till death puts a period to their sufferings, or they accelerate dissolution by plunging headlong into the rivers '' '*

'The Romance of Natural History,' by P. H. Gosse, F.R.S., 2nd ed., 1861, p. 111. Compare these ANNALS, XVIII, No. 3, 1924, p. 323.

J. W. W. STEPHENS.

* Spence's *Travels in Circassia*, i, p. 59.

ON *PROTEOCEPHALUS MARENZELLERI*, *P. NAIAE* AND *P. VIPERIS*

BY

W. N. F. WOODLAND

Wellcome Bureau of Scientific Research, Endsleigh Gardens, London, N.W. 1

(*Received for publication May* 25, 1925)

PROTEOCEPHALUS MARENZELLERI (Barrois, 1898)

This species, one of the largest known Proteocephalids, was first proposed (as *Ichthyotaenia marenzelleri*) by Barrois (1898) who supplied a very brief account of its structure from material collected by Calmette in 1897 from *Ancistrodon piscivorous* Holbr., the 'Water Viper,' a snake found in the southern United States. Ten years later, Schwarz (1908) published a more complete description, with three figures, solely based, however, upon the identical material studied by Barrois. Five years later still, Beddard (1913b) supplied some further details of structure from the examination of a number of immature specimens (the longest measuring about 250 mm.) found in a water viper which had died in the London Zoological Gardens. Since Beddard's specimens were immature and La Rue (1914) expressly recommends a further study of new material, the following description of the anatomy of one large fully-mature example, which I have found recently in the Wellcome Bureau collection of Helminths and which was collected from a water viper which had also died in the London Zoological Gardens, is worth publishing.

My single specimen measured between 300 mm. and 400 mm. in length and was well preserved in spirit. It shows well a striking feature of this species, viz., the very small proportion of the strobila which consists of mature and ripe proglottides. As Beddard remarks concerning his specimens, 'in proglottides situated 8 inches or so

from the scolex [the largest worm being 10 inches in length in spirit] there were merely traces of the reproductive organs,' and in my own single specimen, though it must have measured about 350 mm. (i.e., over 14 inches, in spirit) in total length, yet I did not obtain from the strobila more than about a dozen ripe proglottides and as many which could be described as mature, the rest of the strobila consisting of immature proglottides.

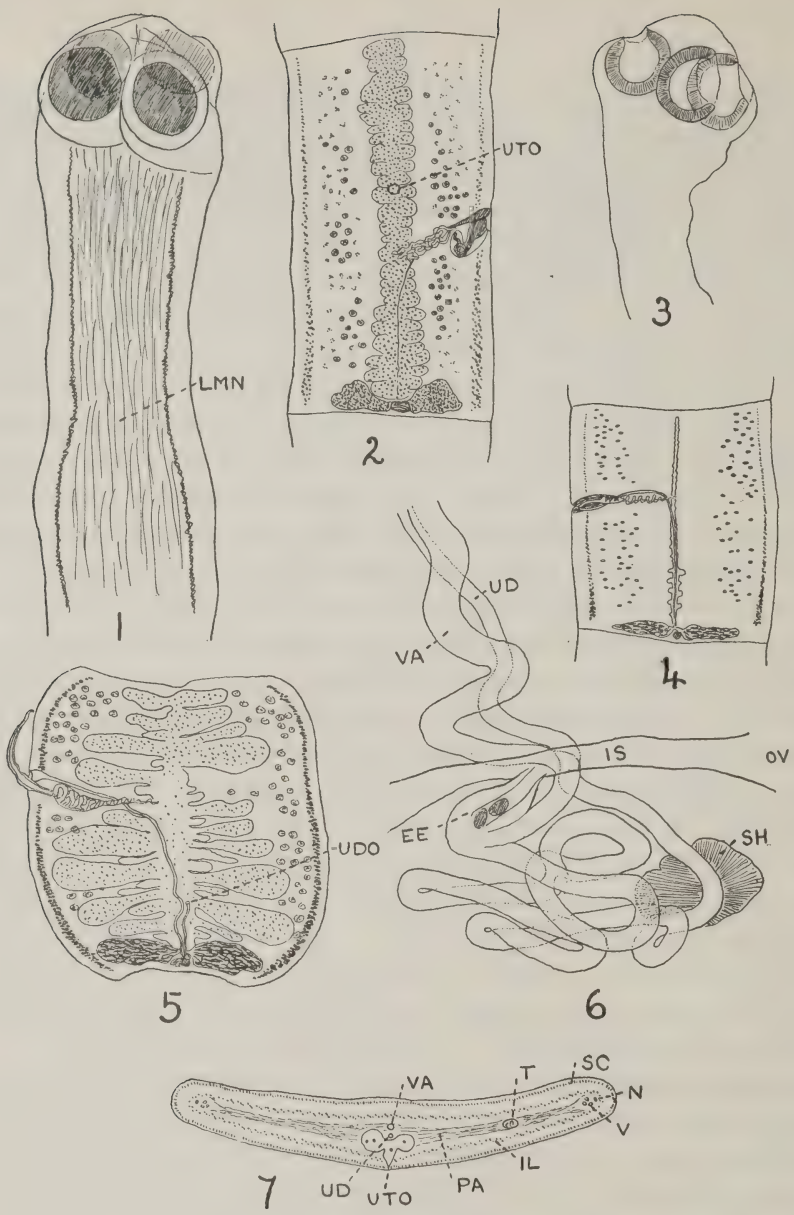
The scolex (fig. 1) was present and measured about 1·9 mm. in breadth. The four large suckers are borne on protrusible lobes on the anterior end of the scolex and face upwards and outwards, the apex of the scolex being quite insignificant, i.e., no ' rostellum ' is present. I did not sectionize the scolex to ascertain if a minute apical organ were present. The suckers measure about 0·913 mm. in breadth. Spinelets are entirely absent. The unsegmented neck is about 7 mm. long, with an average breadth of about 1·4 mm.

The strobila is, in transverse section, extremely flat and has a maximum breadth of a little over 3 mm. (3·068 mm.). The immature proglottides which, as already stated, compose by far the greater part of the strobila, vary in size and shape from $\frac{1\cdot9 \text{ mm. broad}}{0\cdot767 \text{ mm. long}}$ in front to $\frac{3\cdot068 \text{ mm. broad}}{1\cdot180 \text{ mm. long}}$ behind, and thus are all broader than long. Mature proglottides are more square in shape ($\frac{2\cdot95 \text{ mm. broad}}{1\cdot71 \text{ mm. long}}$ and $\frac{2\cdot8 \text{ mm. broad}}{2\cdot0 \text{ mm. long}}$), while ripe proglottides are, more anteriorly, distinctly broader than long ($\frac{3\cdot068 \text{ mm. broad}}{1\cdot8 \text{ mm. long}}$) and, more posteriorly, longer than broad ($\frac{2\cdot124 \text{ mm. broad}}{4\cdot0 \text{ mm. long}}$). The genital apertures are, as usual, irregularly alternate and open midway in the lengths of the proglottides, and the cirrus and vaginal apertures irregularly alternate as to which is anterior. The cirrus sac in mature and ripe segments is extremely broad antero-posteriorly, measuring 0·448 to 0·680 mm. in length and 0·149 to 0·298 mm. in maximum breadth. In some proglottides the sac stretches over a quarter of the breadth of the proglottis but usually a somewhat less distance. The contained cirrus is, next the opening, bulbous in form, and continuous with a straight portion which is connected with several coils of the ductus at the base of the sac. The cirrus was not everted in any of my preparations. The vas deferens

outside the sac is not very voluminous and stretches to the middle line of the proglottis. The vagina is dilated only next its opening. Occasionally a small genital atrium or depression seems to be present, but usually the two apertures appear to lie next the surface at the same horizontal level. The young ovary is rather flattened antero-posteriorly but becomes less so in ripe proglottides, and is of the lateral extent shown in fig. 2. The isthmus joining the two lobes of the ovary is thin and canalicular when seen in toto-preparations and the entire ovary is seen to be very flat in transverse sections. Both the uterine canal and the vagina lie dorsal to the ovarian isthmus (the vagina being ventral to the canal in front of the ovary) and a shell-gland is present. The testes are numerous and lie in two lateral fields and measure on an average about 44 by 22 microns. The vitelline strands are thicker posteriorly than anteriorly.

The uterus in mature segments is of the usual type, viz., a narrow hollow stem lying in the median longitudinal line of the proglottis, continuous with the uterine canal, and with no openings to the exterior. In ripe proglottides, on the other hand (fig. 2), the hollow median stem has become dilated into a broad trunk of considerable size (occupying a fifth or sixth of the breadth of the proglottis and most of the space between the dorsal and ventral surfaces), the wall of which bears irregular lateral very short lobose protuberances, the whole cavity being filled with eggs. Serial transverse sections also reveal the fact that at this stage the uterus opens to the exterior by a single very large and conspicuous ventral pore, situated anterior to the cirrus sac level and nearly midway between this and the anterior limit of the proglottis. I am not certain as to whether other additional pores are subsequently formed (and the ventral uterine wall approaches very close to the ventral subcuticula in two or three places, though no openings were present in my sections) but I doubt it, both because the proglottides appear to be fully ripe and because of the large size of the very well-defined single existing pore. The uterine eggs measure about 22 microns in external diameter, the embryos about 11 microns.

In transverse section the mature and ripe proglottides are seen to be extremely flat. Beddard remarks that in his immature specimens he could find ' no marked layer of longitudinal fibres in the body generally ' and quotes Schwarz as saying that ' die innere



Figs. 1, 2. *Proteocephalus marenzelleri.*

„ 3 to 7. *Proteocephalus naiae.*

Fig. 1 (× 12). Scolex. Compare the magnifications of this and fig. 2, and figs. 3, 4 and 5.

Fig. 2 (× 12). Ripe proglottis. Dorsal view. The testes are degenerating. Note the small size of the uterine diverticula.

Fig. 3 (× 87·5). Scolex in outline.

Fig. 4 (× 12). Mature proglottis, unflattened. Dorsal view.

Fig. 5 (× 12). Ripe proglottis, much flattened. Dorsal view.

Fig. 6 (× 180). Ducts in the region of the ovarian isthmus. Ventral view.

Fig. 7 (× 27·5). Transverse section through a young ripe proglottis immediately in front of the ovary.

EE.—egg-ejector ('schluckapparat'); *IL.*—internal layer (sheath) of longitudinal muscles; *IS.*—isthmus of ovary; *LMN.*—longitudinal muscles of neck; *N.*—nerve; *OV.*—ovary; *PA.*—modified parenchymal core in medulla; *SC.*—nuclear layer of subcuticula; *SH.*—shell gland; *T.*—testes; *UD.*—uterine canal; *UDO.*—opening of uterine canal into median chamber of uterus sac; *UTO.*—opening of uterus to exterior; *V.*—vitellaria; *Va.*—vagina.

Längsmuskulatur ist schwach.' In all my sections through mature and ripe proglottides there is a very distinct layer of internal longitudinal muscles—much more distinct than that in Beddard's '*Solenotaenia*' *viperis* (*vide infra*), because definite bundles of two, three or more fibres are present and are relatively numerous. The medulla contains a core of specialized parenchyma in which the meshes are transversely elongated. I only observed the specialized longitudinal musculature of the neck region in a toto-preparation.

PROTEOCEPHALUS NAIAE (Beddard, 1913)

Syn., *Ophidotaenia naiae* Beddard, 1913.

Of this species I possess more than two dozen specimens, all taken from the anterior and middle intestines of ten (fourteen examined) full-sized cobras (*Naia tripudians*), supplied by snake-charmers of the United Provinces, India. This species has already been described by Beddard (1913a) from three specimens obtained from a cobra which died in the Zoological Gardens, London, but a re-description is necessary owing to the original account being deficient in some respects. Most of my specimens were found in the intestine just behind the stomach and isolated detached proglottides were also found in the faeces on several occasions. My largest (unflattened) specimen measured 180 mm. in total length, with a maximum breadth of 2·5 mm., but other specimens (mostly without ripe proglottides) measured considerably less and one immature specimen only measured 43 mm. Beddard's largest specimen measured 110 mm., with a maximum breadth of 1·5 mm.

In the following account of the species I shall for the most part only deal with those features which require a more complete description than Beddard has given, or which, in my opinion, have been misunderstood by him. As Beddard remarks, the 'rostellum' (by which term I mean simply the terminal part of the scolex, anterior to the suckers) is never very conspicuous (fig. 3) and when retracted consists solely of a very restricted non-projecting area lying between the suckers. In my specimens (toto- and in sections), as in Beddard's, an apical body is absent, though a number of gland-like cells appear to be clustered in the position normally occupied by

the apical organ. Cuticular spinelets were absent. In seven of my balsam preparations, the scolex measured 0·248 to 0·303 mm. in breadth and 0·153 to 0·201 mm. in length (from apex to lower edge of suckers), and the maximum diameter of the suckers varied between 0·106 mm. and 0·153 mm. The suckers are borne on lobes of the scolex base (each sucker, however, occupying the bulk of the lobe) and are undoubtedly protrusible. The unsegmented neck in my specimens is of considerable length, varying between 3·5 mm. (in one case) and 6 mm. (in most cases) according to the state of contraction, and 0·116 to 0·614 mm. in breadth. According to Beddard the neck is ' short.'

The mature and ripe proglottides are of considerable size and are only found in the extreme hind regions of most worms, the greater part of the strobila being composed of large and yet immature proglottides. Only in one of my two dozen specimens were the proglottides in a ripe condition. The immature proglottides (unflattened) in most worms varied between $\frac{2{\cdot}242 \text{ mm. broad}}{0{\cdot}295 \text{ mm. long}}$ and $\frac{1{\cdot}770 \text{ mm. broad}}{1{\cdot}121 \text{ mm. long}}$, and more or less mature proglottides between $\frac{2{\cdot}655 \text{ mm. broad}}{0{\cdot}413 \text{ mm. long}}$ (considerable contraction) and $\frac{0{\cdot}531 \text{ mm. broad}}{3{\cdot}009 \text{ mm. long}}$ (considerable extension), but the average shape of the mature and ripe proglottides is square or a little longer than broad (figs. 4, 5). The genital openings are irregularly alternate and open, often on a distinct projection, either midway in the length of the proglottis or a little anterior to this point. The cirrus sac and vagina irregularly alternate as to which is anterior. The cirrus sac, in unflattened and not unduly contracted or extended proglottides, extends across about a quarter of the breadth of the proglottis and, when fully developed, measures about 0·498 to 0·531 mm. long and 0·083 to 0·107 mm. broad. In flattened (between glass slides) and very contracted or extended proglottides the sac varies enormously in size, from being almost globular in form and therefore very short, to very elongated in form and extending across at least one-third the width of the proglottis. In the cirrus sac both the cirrus (unarmed) and the ductus are usually coiled. In many of my flattened proglottides the cirrus is everted to its full extent and in some cases is longer than one-third the width of the proglottis,

and then the sac is practically invisible, from which I conclude (though the eversion may have been due to the artificial flattening) that, in these cases at least, the cirrus sac itself has been everted, though Beddard says that he has seen no evidence of this. The wall of the cirrus sac is thin but muscular. A small cloaca genitalis is present. The coils of the vas deferens in unflattened preparations are not very voluminous and are just visible as far as the middle line of the proglottis. In elongated proglottides the vas deferens coils form a bunch in the middle line. The vagina opens at the same horizontal level as the cirrus, but away from the opening it lies ventral to the cirrus sac and to the vas deferens coils. The vagina is very slightly dilated near its opening but in no other region and, except in very elongated proglottides, is sinuous or slightly convoluted just anterior to the ovary. The testes are about 120 in number and in unflattened preparations measure, on the average, about 62 by 36 microns. They are situated in two quite separate lateral fields. The vitellaria (cir. 14 by 11 microns) are, as usual, arranged in two thin lateral strands, which, however, are distinctly broader posteriorly than anteriorly. The ovary consists, as usual, of two lobes connected medianly by an isthmus. In surface view the lobes are narrow antero-posteriorly and extend laterally, in mature proglottides, only a little more than half-way to the proglottis edge. In sections they are seen to be very thin dorso-ventrally and to lie nearer the dorsal than the ventral surface of the strobila, though the isthmus connecting the lobes bends ventrally to allow of the dorsal passage of the uterine canal and vagina. The lobes are distinctly follicular. Beddard says that he ' could not find any signs of a shell-gland ' and he endeavours to correlate its supposed absence with the presence of a ' glandular investment ' on the walls of the uterine diverticula, which he assumes to take the place of a shell-gland. For my part, I have had no difficulty in finding a very distinct shell-gland (diagrammatically represented in fig. 6) in most of my preparations, and, on the other hand, I have been unable to find any investment of the uterine walls with cells which can be described as glandular. A distinct egg-ejector (' schluckapparat ') is also present. The uterus in my ripe proglottides (fig. 5) consists of (*a*) a wide median uterine sac extending the whole length of the proglottis from the ovary anteriorly, which carries on either side from 16 to 25 lobose diverticula

of very different sizes (I could not detect any diverticula ventral to the ovary), and (*b*) a uterine canal, which, with the vagina, passes *dorsal* to the ovarian isthmus, and opens into the median uterine sac some distance in front of the ovary. Nearly all the eggs are collected in the diverticula, in which they are freely scattered (not in clusters), and they possess two distinct shells, the outermost thick shell measuring about 25·6 microns in diameter, and the contained embryo 9 to 11 microns. The median sac of the uterus has a number of pointed downgrowths (fig. 7) which open on what is usually considered to be the ventral surface of the strobila. The position of the uterine pores is indeed the sole certain criterion of determining the orientation of the strobila.

In transverse sections (fig. 7) through mature proglottides the usual two layers of longitudinal muscles are to be seen—a very thin layer just external to the nucleated region of the subcuticula and internal to an equally thin circular muscle layer, and a thicker, though somewhat attenuated, internal layer of longitudinal muscles, demarcating the cortex from the medulla. The parenchyma is in most regions of a uniform wide-meshed character, but in the centre of the medulla there is a kind of core of closer-meshed parenchyma, with the meshes transversely elongated. This core of differentiated parenchyma is apparently identical with that figured by Beddard (1913b, p. 167, text-fig. 38) for '*Ichthyotaenia* sp.' (i.e., *P. marenzelleri*) only Beddard assumes (in the absence in his immature specimens of an internal longitudinal muscle sheath) that it represents the whole of the medulla, whereas in *P. naiae* and in my mature specimens of *P. marenzelleri* (*vide supra*) it is obviously only the internal region of it. I am ignorant of the significance of this altered parenchymal core.

PROTEOCEPHALUS VIPERIS (Beddard, 1913)

Syn., *Solenotaenia viperis* Beddard, 1913.

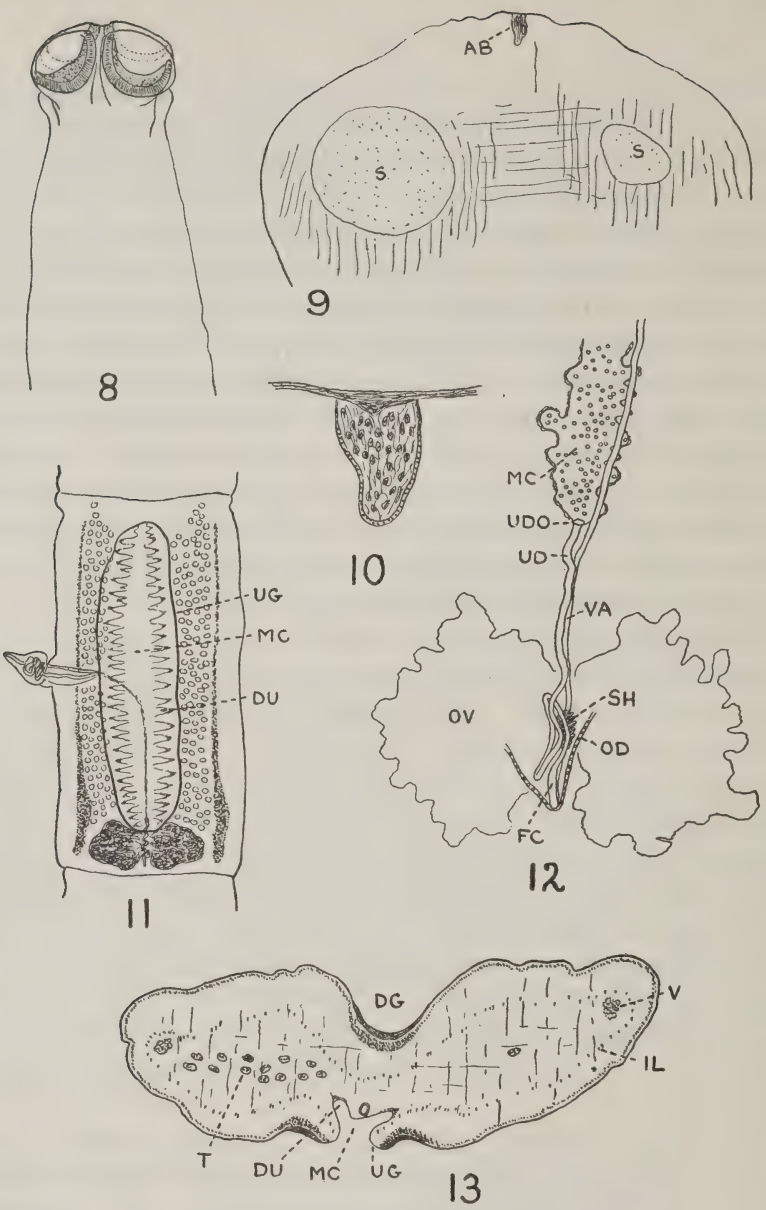
Beddard (1913c) has provided a full description of this remarkable species, and the following account only professes to supply details which Beddard has omitted and to confirm and, if possible, emphasize, his statement of the peculiar character upon which he founded his new genus *Solenotaenia*. I possess a large number of specimens

of this species contained in the helminthological collection made by Dr. L. W. Sambon almost entirely from animals which had died in the London Zoological Gardens, and now in the Wellcome Bureau of Scientific Research.

Proteocephalus viperis (as I propose to call this species) is a parasite of the Crossed Viper, *Lachesis alternatus*, from Central or South America. One nearly-entire worm in my collection measured 170 mm. in total length (in spirit) and others must have reached 200 mm. and possibly more, so that my specimens were somewhat longer than those studied by Beddard. The maximum breadth of my specimens was 2·41 mm., and was always found in the region of immature proglottides. Mature and ripe proglottides only occur in about the last quarter of the worm's length. There are no external signs of segmentation, save the small lateral notches and the uterine grooves which demarcate ripe proglottides.

The scolex (fig. 8) consists almost entirely of the four large hemispherical suckers, which occupy the greater part of the four lobes which bear them. The terminal area between the suckers is extremely small and does not protrude. The scolex measures 0·860 to 1·527 mm. in breadth and 0·531 to 0·713 mm. in length (from tops to bases of suckers). The suckers measure 0·415 to 0·664 mm. in breadth and look upwards and outwards. Spinelets are entirely absent. As Beddard remarks, a minute funnel-shaped apical organ is present, which is so small that it is almost invisible in toto-preparations. Its appearance, in longitudinal section, is shown in figs. 9 and 10. An unsegmented neck is present, varying in different specimens from 1·7 mm. to 4·7 mm. in length and 0·767 to 1·534 mm. in breadth, but the average length is about 3 mm.

As already remarked, the broadest part of the strobila is in the region of immature proglottides. The proglottides are here indicated by the presence of faint transverse segmentation lines and more posteriorly by genital rudiments and are all much broader than long, measuring from $\frac{2{\cdot}419 \text{ mm. broad}}{0{\cdot}236 \text{ mm. long}}$ and $\frac{2{\cdot}124 \text{ mm. broad}}{0{\cdot}088 \text{ mm. long}}$ to $\frac{1{\cdot}534 \text{ mm. broad}}{0{\cdot}354 \text{ mm. long}}$ (all measurements of unflattened material). Mature and ripe proglottides are not nearly so broad, the former being either



FIGS. 8 to 13. *Proteocephalus viperis.*

Fig. 8 (× 17·5). Scolex in outline.

Fig. 9 (× 56). Longitudinal section through the scolex showing the minute apical organ.

Fig. 10 (× 260). The apical organ in longitudinal section.

Fig. 11 (× 27·5). Ripe proglottis with the uterus entirely split along its whole length and empty of eggs. Note the small diverticula.

Fig. 12 (× 56). Ducts in the region of the ovary, from the dorsal view. In this proglottis the uterus has not yet split to the exterior.

Fig. 13 (× 39). Transverse section through a ripe proglottis behind the cirrus sac. Note the open uterus, the small uterine diverticula and the weak internal longitudinal muscle sheath.

AB.—apical organ ; *DG.*—dorsal groove (artefact ?) ; *DU.*—diverticula of uterus ; *FC.*—fertilization chamber ; *IL.*—internal layer (sheath) of longitudinal muscles ; *MC.*—median chamber of uterus ; *OD.*—oviduct (?) ; *OV.*—ovary ; *S.*—sucker ; *SH.*—shell-gland ; *T.*—testes ; *UD.*—uterine canal ; *UDO.*—opening of uterine canal into median chamber of uterus sac ; *UG.*—uterine groove, edge of ; *V.*—vitellaria ; *VA.*—vagina.

approximately square in shape or longer than broad, and measuring from $\frac{1{\cdot}121 \text{ mm. broad}}{0{\cdot}885 \text{ mm. long}}$ to $\frac{1{\cdot}534 \text{ mm. broad}}{1{\cdot}888 \text{ mm. long}}$ and $\frac{1{\cdot}230 \text{ mm. broad}}{2{\cdot}006 \text{ mm. long}}$, and the latter (fig. 11) always being longer than broad and measuring from $\frac{0{\cdot}885 \text{ mm. broad}}{1{\cdot}652 \text{ mm. long}}$ to $\frac{1{\cdot}357 \text{ mm. broad}}{4{\cdot}838 \text{ mm. long}}$. In my material the genital apertures are situated almost on the middle transverse line of the proglottis but not quite, being a little in front, and the cirrus aperture is usually in front of the vaginal, though the reverse condition does occur. The cirrus sac is very broad antero-posteriorly and measures in my preparations 0·298 to 0·365 mm. in length and 0·149 to 0·182 mm. in breadth, and it extends across from one quarter to one-third of the breadth of the proglottis according to the state of contraction of the latter. The cirrus sac wall is very thin, though quite well-defined, and apparently contains no muscle-fibres. The sac contains three parts of the cirrus apparatus: (*a*) an external thick-walled convoluted part which forms the outer walls of the extruded cirrus, (*b*) a long thick-walled (less thick than the first part) straight tube (the cirrus canal), and (*c*) coils of the ductus. The first two parts have attached to them ejector muscle-fibres (Beddard's ' layer of glandular cells ' ?). The cirrus when everted (which may equal in length half the breadth of the proglottis) is slender distally but dilated at the base which contains the ductus coils (fig. 11), and the sac, contained inside the proglottis in this condition, is relatively narrow (only 0·041 mm. broad and 0·298 mm. long in one specimen which reached a quarter of the distance across the proglottis). The sac itself then is not everted. The cirrus is not armed. The coils of the vas deferens are not very voluminous and extend to about the middle of the proglottis. The vagina opens on the same horizontal level as the cirrus and shows no marked dilatation anywhere in its course, though in contracted proglottides (not in extended) it becomes convoluted anterior to the ovary. It occasionally opens on a papilla and there is no genital atrium. The ovary in mature non-elongated proglottides is only a little more than half the breadth of the proglottis and is of the shape shown in fig. 11. There is no narrow canalicular isthmus, the follicles of the ovary extending across the middle line over a broad area. The vitellarian strands (thickened posteriorly)

have been sufficiently described by Beddard, and are of course medullary in position. The testes, as Beddard states, are very numerous, lie in two distinct fields, and measure in toto-preparations about 44 by 25 microns. I must also mention that both the vagina and the uterine canal (*vide infra*) lie on the dorsal side of the ovary (the former lying for the most part ventral to the latter), that in two of my preparations the oviducts* apparently join the vagina at the level of the hind end of the ovary (an unusual position) and that there is a recognizable shell-gland. In fig. 12 I have depicted these ducts as well as I am able to make them out, but I cannot guarantee the exact positions of the coils, nor could I detect the vitelline ducts.

Beddard has fully described the uterus and its extraordinary later development in this species, and I intend only to make one or two corrections in his account and to emphasize the features in which this uterus differs from that of other known Proteocephalidae. As Beddard says, the early development of the uterus as a median hollow stem is like that of other Proteocephalids, but he omits to lay stress upon the fact that whereas the uterus of most other Proteocephalids remains devoid of eggs until the diverticula are well developed, the uterus of '*Solenotaenia*' (like that of *P. marenzelleri* and some other snake Proteocephalids) becomes crammed with eggs while the diverticula are either entirely absent or only represented by minute irregularities of the wall (fig. 12). This fact in itself indicates that the '*Solenotaenia*' uterus is distinct from that of the majority of Proteocephalids. The next stage of development of the '*Solenotaenia*' uterus is, not the development of large diverticula, but the splitting and opening to the exterior of its entire ventral wall (the process commencing anteriorly and proceeding posteriorly, until the entire length is exposed), so that the whole cavity of the stem uterus becomes continuous with the outer world and in consequence devoid of the eggs which are at once liberated (figs. 11, 13). There is thus formed, as the final stage of development of the uterus, a deep and broad uterine groove, with smooth thickened edges, situated on the ventral side of the proglottis along nearly its entire length, i.e., from the posterior opening of the uterine duct to near

* These ducts (which are very distinct in one preparation) may possibly be the vitelline ducts, though they appear to come from the ovary and I cannot trace any connection with the vitellaria. I also admit that I cannot see these ducts in most of my preparations, nor in serial transverse sections.

the anterior end of the proglottis. This conspicuous uterine groove, as Beddard points out, is quite distinct morphologically from the apparently similar longitudinal grooves in certain Bothriocephalids and in many Proteocephalidae, since in these latter it is only a continuous depression of the body-wall which harbours the uterine pores, whereas in the former it is equivalent to the fused uterine pores themselves and represents the actual cavity of the uterus. Correlated with this formation of the uterine groove*

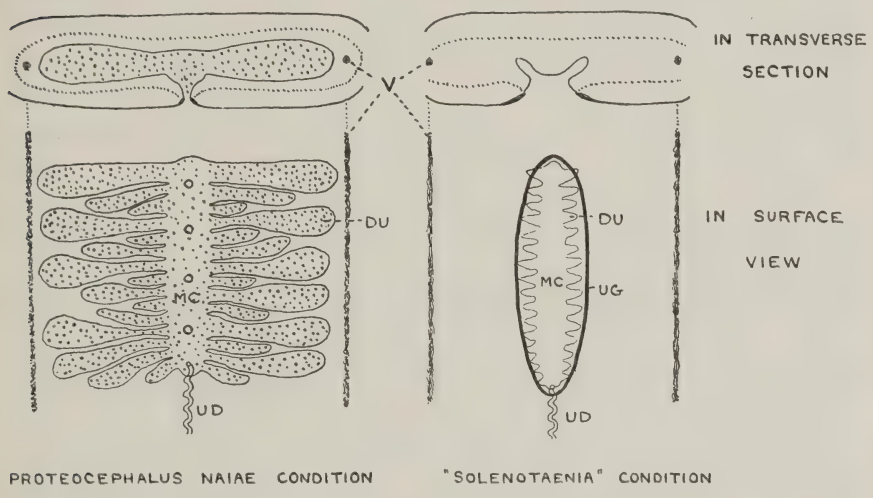


Diagram to contrast the conditions of the ripe uteri of a normal Proteocephalid and of "*Solenotaenia*."

DU.—diverticula of uterus; *MC.*—median chamber of uterus; *UD.*—Uterine canal; *UG.*—uterine groove, edge of; *V.*—vitellaria.

in '*Solenotaenia*' and the early formation of the large uterine pores in allied species is the stunted development of the diverticula which are so conspicuous a feature in the fully-formed uteri of most other Proteocephalids. This difference of development of the uterus in this species, compared with the developments of the uteri of most other Proteocephalids, would afford a very much better basis for the founding of a distinct genus (cf. Lühe's characterization of the genera of the Ptychobothriinae e.g.) than the trivial scolex characters,

* In my figure 13 of a transverse section it will be observed that there is, in my material, a very distinct dorsal groove bordered by a thickened area of the subcuticula. This dorsal groove is possibly the result of local contraction, since Beddard's figures do not indicate its presence in his material.

and even the testes distribution, which have been utilized up to the present, and I would readily adopt Beddard's new genus *Solenotaenia* were it not for the facts that: (1) the stunted uterine diverticula found in '*Solenotaenia*' are also to be found in several other Ophidian Proteocephalids which do not possess the uterine groove (e.g., in *P. marenzelleri*, *P. calmettei* and '*Crepidobothrium*' *gerrardii*), and that (2) there appears to be every transition from these stunted diverticula (cf. e.g., *P. racemosa*, *P. nattereri* and the '*O*' *monnigi* recently described by Fuhrmann, 1924) up to fully-developed diverticula (as in the '*O*' *punica* recently re-described by Southwell and Adler, 1923, and many other species of '*Ophiotaenia*'), and that (3) the uterine groove represents physiologically, if not morphologically, after all only a fusion of uterine pores and is therefore only an individual, i.e., specific, peculiarity of an external character.

Beddard says nothing about the development of the uterus into two definitive portions—the dorsal *uterine canal** (representing the posterior portion of the primitive stem) and the ventral *uterine sac*—a development common to many other Proteocephalids, though not to all. He, however, states that in this species and in '*Ophidotaenia*' *naiae* (= *Proteocephalus naiae*, *vide supra*) the minute and large uterine diverticula respectively are invested with cells which seem to be 'exactly like those of the shell-gland in other tape-worms,' and he suggests that they may have a similar function, i.e., that the eggs may, in these two species, acquire their shells in the uterus instead of in the usual place. I have observed these cells investing the uterine wall in *P. viperis*, but I am not convinced of their glandular nature, and, as Beddard remarks, a shell-gland is apparently present in '*Solenotaenia*,' and is certainly present in *Proteocephalus naiae*, though Beddard failed to observe it in this latter case.

The fully-formed eggs of *Proteocephalis viperis* are fairly thick-shelled and measure about 21·7 microns in diameter, and the contained hooked embryos about 12·8 microns. In transverse section (fig. 13) the general parenchyma of the proglottis is seen to be wide-meshed and to be divided into the usual two regions of cortex and medulla by the presence of a very weakly-developed internal

* Beddard apparently figures this canal in transverse section in his text-figure 50 (p. 252) but is under the impression that it represents a convolution of the vagina. In my serial transverse sections I have followed both ducts through their entire course and have seen the uterine canal opening into the uterus sac.

layer of longitudinal muscles, a layer consisting of widely-separated small bundles, each containing only two or three fibres, and occasionally of single fibres.

The foregoing three species are provisionally placed in the genus *Proteocephalus* (syn. *Ichthyotaenia*) and not in '*Ophiotaenia*' (La Rue, 1911) or '*Crepidobothrium*' (*vide* Nybelin, 1917) for the reasons stated by me in a paper published elsewhere (Woodland. 1925).

REFERENCES

Barrois, T. (1898). 'Sur quelques Ichthyoténias parasites des Serpents.' *Bull. Soc. Sci. Agric. et Arts.* Lille. T. 2, p. 4. (The present writer has not been able to see this paper.)

Beddard, F. E. (1913a). 'Contributions to the Anatomy and Systematic Arrangement of the Cestoidea. VII. On Six Species of Tapeworms from Reptiles belonging to the Genus *Ichthyotaenia* (s.l.).' *Proc. Zool. Soc. London*, Part I, p. 4.

——— (1913b). 'Contributions, etc. VIII. On some Species of *Ichthyotaenia* and *Ophidotaenia* from Ophidia.' *Ibid.*, p. 153.

——— (1913c). 'Contributions, etc. IX. On a new Genus of Ichthyotaeniids.' *Ibid.*, p. 243.

Fuhrmann, O. (1924). 'Two new Species of Reptilian Cestodes.' *Ann. Trop. Med. and Parasitol.*, Vol. XVIII, No. 4, p. 505.

La Rue, G. R. (1911). 'A Revision of the Cestode Family Proteocephalidae.' *Zool. Anzeig.*, Bd. 38, p. 473.

——— (1914). 'A Revision of the Cestode Family Proteocephalidae.' *Illinois Biological Monographs*, Univ. of Illinois, Vol I, Nos. 1 and 2.

Nybelin, O. (1917). 'Results of Dr. E. Mjoberg's Swedish Scientific Expedition to Australia, 1910-1913. XIV. Australische Cestoden.' *Kungl. Svenska Veten. Handl.* Bd. 52, No. 14.

Schwarz, R. (1908). 'Die Ichthyotaenien der Reptilien und Beiträge zur Kenntnis der Bothriocephalen.' *Inaug.-Dissert. Univ. Basle.* 52 pp. and 7 plates.

Southwell, T., and Adler, S. (1923). 'A Note on *Ophiotaenia punica* (Cholodovski, 1908) La Rue 1911.' *Ann. Trop. Med. and Parasitol.*, Vol. XVII, p. 333.

Woodland, W. N. F. (1925). 'On three new Proteocephalids and a Revision of the Genera of the Family.' *Parasitology*, Vol. XVII, p. 295.

HUMAN TRYPANOSOMIASIS IN THE LUANGWA VALLEY, NORTHERN RHODESIA

BY

ALLAN KINGHORN

(*Received for publication June* 25, 1925)

I. PHYSICAL ASPECTS OF THE VALLEY

The Luangwa river rises in the hilly country near the junction of the Rhodesia-Nyasaland-Tanganyika Territory boundary at an altitude of from 5,500 to 5,900 feet and flows in a general south-westerly direction to its confluence with the Zambesi at Feira, 1,500 feet above sea level. On either side its valley is bounded by ranges of hills which run roughly parallel to the course of the river and which lie at distances varying from a few to as many as 40 or 50 miles from it. The country with which I am more immediately concerned in this report extends from Fundu at the southern end to the confluence of the Wira with the main river at the northern end, a distance of approximately 400 miles. Taking the average width of the valley to be 50 miles, the area thus included is at least 20,000 square miles. The Luangwa is fed by a large number of tributaries which enter it more or less at right-angles ; but whereas many of those on the right side are large and permanent streams, those on the eastern bank, with one exception, flow only in the rains, and then only assume perceptible volume when carrying flood water. In general the floor of the valley is level and is covered to a very large extent by ' mopani ' bush which becomes water-logged, if not actually flooded, during the rains, and in which the grass is short and of scanty growth. This mopani bush usually ends rather abruptly at some distance from the streams and is replaced by more or less open country covered by dense and luxuriant growths of grass. As it is only in these situations that the soil repays cultivation, the villages, which are small collections of from 20 to 200 inhabitants, are found strung along the courses of the larger streams. This is more particularly the case on the eastern

side of the Luangwa, as it is only in the beds of the larger streams that water can be obtained by digging in the dry season. It may be noted that at this time of the year the Luangwa itself dries up in stretches in the upper reaches.

The mean altitude of the area under discussion may be taken as about 2,300 to 2,500 feet, so that the meteorological conditions are much more tropical than in the other portions of Northern Rhodesia. At Nawalia, which is about centrally placed at an altitude of about 2,100 feet, the mean temperature varied from 67° F. in July to 87° F. in November, and the relative humidity from 31 per cent. in September and October to 78 per cent. in January. The rain-fall averages about 40 inches and is spread over the six months, November to April, though the bulk is precipitated during the first three months of the year.

II. DISTRIBUTION OF GAME AND FLY

Game is both extremely abundant and varied throughout the whole of the valley. In the rains it ranges through the whole of the area, but in the dry season, more particularly after the grass has been burnt (July to September), it tends to collect along the courses of the streams where the grazing is better than in the mopani and where alone a supply of water is assured. At this time of the year it is not uncommon to find a relatively large animal population in fairly close proximity to the villages, as many of the species, e.g., waterbuck, roan, eland and bushbuck, are fond of feeding in the old gardens. I think this point should be emphasised, as it was demonstrated at Nawalia that waterbuck and bushbuck, amongst others, were infected by *Trypanosoma rhodesiense*, the first-mentioned to the extent of between 17·8 per cent. and 25 per cent. of the total number examined. It may here be noted that in the immediate neighbourhood of some villages in Kambombo's country which have suffered severely from sleeping sickness, waterbuck are extremely plentiful. There are grounds, therefore, for regarding this species of buck with particular suspicion.

Fly. *Glossina morsitans* is to be found throughout the whole of the valley and has been observed to show much the same seasonal variation as the game, i.e., in the dry season it is abundant near

the streams and hence near the villages, but after the rains have commenced tends to become more uniformly scattered over the country as a whole. This peculiarity has been observed in other parts of Africa. Owing to the fact that the villages are, as a rule, built in clearings and are surrounded by gardens, it is unusual for the fly to invade them, though an occasional specimen may follow the natives in. If so, it soon disappears again.

III. HISTORY OF SLEEPING SICKNESS

Attention was first drawn to the occurrence of human trypanosomiasis in the valley by the diagnosis of the disease in several Europeans about the year 1909. Investigations commenced then and carried out in the succeeding years showed that it was scattered over the whole of the area though the number of cases found was comparatively small. They demonstrated further that the infection displayed no tendency to assume epidemic proportions. Writing at the end of 1912, the Principal Medical Officer stated : ' In the light of recent knowledge as to the presence of the necessary factors and apparently very suitable conditions for the production of an epidemic, it is difficult to understand why after four years there should be no evidence that such is likely to occur. It can be most reasonably concluded that there is some unknown but necessary factor wanting or some inhibitory influence present, i.e., that the disease is an old one and that there may be a certain immunity present which is limiting its spread.' This is in strict accordance with the native evidence, for those I have questioned have always consistently maintained that they have always known of the existence of the disease as far back as their memories carry, in some cases a matter of seventy years or so. They state that it cropped up only as isolated cases which were drastically disposed of in some localities. Amongst the Bawisa and Bansenga the terms ' Chilotera ' and ' Nyamakazi ' are used to denote the symptom-complex of fever, oedemata of the extremities, protuberant abdomen, diarrhoea and emaciation, all commonly observed in sleeping sickness, though, of course, they do not associate the disease with tsetse flies. My own belief coincides with that of the natives, that the disease is an old one and not of recent introduction.

IV. INCIDENCE OF THE DISEASE

The observations with which I am now about to deal are all, with few exceptions, derived from data collected by me during 1913 and the early part of 1914, and after the conclusion of the war, from 1920 onwards. In this work I have relied on gland palpation and puncture as the means of diagnosis and only departed from it in exceptional cases and for special reasons, e.g., where a native's illness appeared to be due clinically to sleeping sickness but no palpable glands were found. Under these conditions, I claim that the various sets of figures may be properly compared and that they give a real indication of the relative incidence of the disease in the different years and in the same localities. I do not claim, however, that they indicate the absolute incidence of the disease. While I am of the opinion that gland palpation and puncture is the quickest and only feasible method of examining large bodies of natives, and that the great majority of cases will be found by its employment, I admit that some cases, particularly those in the very early stages of the infection, before the lymphatic glands have hypertrophied and in which no other symptoms are present, will be missed.

As mentioned earlier, the disease is very widely distributed and has been found in the Petauke, Serenje, Fort Jameson, Lundazi, Mpika and Chinsali portions of the valley. Further it is, as a general rule, comparatively rare, as the following figures will demonstrate :—

Year examined	District	Natives examined	Cases	% infected
1913	Mpika	2,613	2	0·08
	Lundazi	13,100	9	0·07
	Chinsali	1,465	4	0·26
1914	Serenje	1,981	2	0·10
	Petauke	3,654	2	0·05
		23,113	19	0 08
1921	Lundazi, Part	3,723	1	0·03
	Mpika „	1,311	0	0·00
	Chinsali „	913	1	0·11
		5,947	2	0·03

As illustrating the general tendency for the disease to remain stationary over a period of years the following figures may be quoted. In every instance they are for the same villages in the respective years.

District	Year	Natives examined	Cases	Year	Natives examined	Cases
Lundazi	1913	2,812	0	1921	3,530	1
Mpika		1,233	0		1,261	0
Chinsali		776	3		913	1

A further example may be quoted from Dr. May's investigations in one particular area in the Petauke Sub-District where, in the three successive years 1910, 1911, and 1912 respectively, 7, 7, and 3 cases were found.

It is difficult, in the absence of a complete census and the registration of births and deaths, to estimate not alone the general death rate amongst these natives but also that more specifically due to sleeping sickness. The following calculations, however, may be given. At the end of 1912, Dr. May computed the adult death rate in a portion of the Petauke Sub-District to be 28 per 1,000 and the incidence of sleeping sickness to be 8 per 1,000, also amongst the adults only. In 1913 I made similar enquiries as carefully as was possible in the valley portion of the Mpika Sub-District and estimated the general death rate for the year 1912-1913, exclusive of accidents, to be 23·7 per 1,000, the adult rate to be 47·7 per 1,000 and the incidence of sleeping sickness to be 3 per 1,000 of the whole population seen. Speaking of the valley generally, I should be inclined to say that under ordinary circumstances the incidence of the disease is not in excess of 3 to 4 per 1,000 of the total population per annum, though of course it may be exceeded temporarily in those localities in which exacerbations of the infection occur (for example, the estimated death rate for 1920-21 of 25 per 1,000 in the countries of chiefs Tembwe and Kambombo).

A very striking feature of the infection is the extraordinarily sporadic manner in which it is found. Not only are the cases found in villages widely separated but they are also usually found occurring singly, and cases in the same village may be separated by an interval of years. Examples of this are given in the following tables, and it should be noted that approximately the same number of natives were examined on the various occasions.

Village	Wallace, 1912	Kinghorn, 1913
Mumamba	1	0
Daroba	1	0
Chuni	0	1
Mkasanga	0	1
Chombero	0	1
Kundawamawe	0	1
Temba	0	1
Chinyondo	0	1
Luchenga	0	1
Kampuzunga	0	1
Mulumgu	0	1

Village	1920	1921	1922	1924	1925
Tembwe Virizi	0	2	1	0	0
Katangalika	0	1	0	0	0
Ng'anjo	1	0	0	0	0
Mwimba	0	1	0	0	0
Kajumba...	1	1	0	1	0
Kambombo	0	1	0	0	0
Chizonde...	0	1	0	0	0
Kambwiri	1	0	0	0	0
Kazembe...	0	0	1	0	0
Dungulungu	0	1	0	0	0
Chama	1	1	0	0	0
Kawanda...	1	1	1	0	0
Kapalakonje	1	0	0	0	0
Chitimbe...	1	1	1	0	0
Nyika	2	1	0	0	0
Luambo	2	0	1	0	0
Chileta	0	0	1	0	0
Hunga	1	2	0	0	0
Buli	1	0	0	0	0
Chitukula	1	0	0	0	0
Chiruarua	2	0	0	0	0
Zowole	1	0	0	0	0
Mtonya	1	0	0	0	0
Mkunguwe	1	0	0	0	0
Luchenga	0	0	2	0	1
Kapotwe	0	1	0	0	0
Makondola	1	0	2	0	0
Kakuni	0	0	2	0	0
Marunga	1	0	0	0	0

How is this peculiar distribution and incidence to be explained ? As is well known, some observers, chiefly the Germans, maintain that there are two distinct trypanosomes, *brucei* and *rhodesiense*, existing side by side in tropical Africa, which are indistinguishable except for the fact that *T. rhodesiense* is capable of infecting man while *T. brucei* is not, but is restricted to game and stock. The other school, chiefly British, maintain that the two trypanosomes in question are identical ; that it is essentially a parasite of game ; and that man is ordinarily resistant to infection, though this may occur. Exactly what conditions are necessary before man does become infected are uncertain. The chief arguments of those who favour the non-identity hypothesis are : (1) Dr. Taute's experiments, and (2) the geographical argument, that in many localities where *T. brucei* is found cases of sleeping sickness have never been diagnosed. With reference to the first of these, I think the experiments may be held quite permissibly to prove the truth of the contention that man is naturally resistant to infection by *T. brucei vel rhodesiense* and that, in any event, they are not sufficiently extensive to prove indisputably the truth of the negative statement that the human and game trypanosomes are not identical. As regards the geographical argument, I am not aware that the localities usually cited have been thoroughly and repeatedly examined over a period of years, and that, at the same time, such important factors as the abundance of fly and game, the percentages of both harbouring *T. brucei, sensu strictu,* and the closeness of contact existing between them and the native population have been taken into consideration. Without departing from the confines of Northern Rhodesia, however, it appears to me to be a difficult matter to explain, on the assumption that human cases of the disease are invariably due to a specific human as distinct from the ordinary game trypanosome, the occurrence of one European and two or three native cases in the western part of the Serenje sub-district with an interval of years between them, more particularly as the examinations of the suspected area carried out by Dr. Masters prior to 1912, by Dr. Ellacombe in 1912, and again by Dr. Powell in 1920, gave negative results as far as the indigenous population was concerned. And further, it is peculiar that in the other area in this country in which the disease has been found

to occur much as it does in the Luangwa valley the same conditions of an abundance of game and fly co-exist in close contact with the natives. I refer to the focus in the south-western corner of the Ndola sub-district. Prof. Kleine, one of the protagonists of the non-identity theory, admitted in conversation that the local game was susceptible to infection by *T. rhodesiense, sensu strictu,* and this being the case it follows that it would only be a matter of time until this parasite was widely distributed amongst the fauna of the valley and one would then expect to find human cases to be both more numerous and more uniformly spread over the country as a whole. As pointed out above, there is a concentration of both the game and the fly in the vicinity of the villages in the dry season, and while a certain amount of evidence exists to show that the risks of infection are then greater it is not extensive enough to permit of any dogmatic statement on the point. I believe, personally, that the sporadic appearance and erratic distribution of the infection as it is generally seen and has been seen since 1909, with no tendency to assume epidemic proportions, is more satisfactorily explained by the theory that the human and game trypanosomes are one and the same parasite and that man is ordinarily resistant to infection by it than by the theory that the human and game trypanosomes are distinct entities.

While the normal incidence of the infection is as shown above, exacerbations have been observed from time to time in localized areas of the valley, but these, after the lapse of a few years, have always ended spontaneously and the disease has then reverted to what may be termed its equilibrium. The most pronounced of these has been the one which started early in 1918, in the contiguous territories of the three chiefs Chikwa, Tembwe and Kambombo towards the Northern end of the Lundazi sub-district. Of the three, Kambombo suffered most. Thus writing in May, 1920, the Native Commissioner, Lundazi, reported that since the commencement of this outbreak some 131 deaths had occurred to date from what appeared to be sleeping sickness, amongst an adult population estimated at 1,455 on March 31st, 1920, in the villages under that chief. It may be noted that at the end of 1917, or beginning of 1918, plague appeared in this same area and was not stamped out until 1919. This coincidence was probably quite fortuitous except that many of the

villages were burnt and the natives forced to build temporary quarters in the bush. This, of course, brought them into closer and more continuous contact with fly than is ordinarily the rule, and to some extent an increase in the number of cases of sleeping sickness may be ascribed to this cause, but it cannot have been of general application, as those villages which suffered most severely from sleeping sickness were not destroyed. In 1920, and the succeeding years, I examined and re-examined the natives in this particular area with the following results :—

Year	Natives examined	Cases	% infection
1920	5,756	25	0·43
1921	5,634	18	0·32
1922	5,317	9	0·17
1924	4,347	1	0·02
1925	4,301	2	0·04
Compare 1913	5,122	3	0·06

Or if we consider the three sets of natives separately we have the following percentages of infection in the various years :—

Year	Percentage Infection		
	Chikwa	Tembwe	Kambombo
1920	0·25	0·73	0·36
1921	0·15	0·27	0·57
1922	0·22	0·12	0·15
1924	0·00	0·00	0·06
1925	0·07	0·00	0·06

It should be noted that these percentages only represent the incidence of the disease at the actual time of examination, and, while under ' normal ' conditions they might afford an approximate idea of its frequency, they are much too low for ' Epidemic ' conditions. Thus in 1921, by tracing the causes of deaths in the interval between that and the former visit, I estimated that the then rate of infection was about 2·5 per cent. of the whole population per annum. I am not fully satisfied that the 1924 figures give anything like a true indication of the percentage of infection in that year, as I saw about a thousand fewer natives than in former trips, and amongst those who evaded examination cases may have existed ; but it may be noted that the deaths between 1922 and 1924, which could be attributed to sleeping sickness were comparatively few ; hence it seems reasonable to conclude that this localized ' semi-epidemic ' is behaving like the others and that the infection is becoming stabilised again. That this conclusion is essentially true is borne out by the results of the trip I have just finished through this area. As will be seen above, 4,301 natives were seen and 2 cases of the disease diagnosed, a percentage of 0·04. In the six months which elapsed between my 1924 and 1925 trips some nine deaths, which might possibly have been due to sleeping sickness, occurred, so that it would appear that the annual incidence of the disease in this particular area is now not in excess of 5 per 1,000 of the total population.

I am of the opinion that these epidemics are due largely to the superimposition of a man-fly-man cycle of transmission of the parasite on the more ordinary game-fly-man cycle and the occurrence of this is often hastened by some of the habits of these natives. For instance, cases may be carried from one village to another owing to the custom of returning to the original home when a native falls sick. Again, during the rains the villages split up, each family living in the middle of its gardens in order to protect them from the depredations of monkeys and elephants, and cases of infection in man and wife and parent and child have been observed under these conditions. Further, the habit of growing crops between the huts in the villages themselves increases the liability of their invasion by fly with the consequent danger of their becoming infected should a case of sleeping sickness exist.

V. THE DISEASE IN HUMAN BEINGS

The incubation period is short, probably between one and two weeks. At first the only symptom is fever with its concomitants, followed by enlargement of the lymphatic glands, particularly in the basal portion of the posterior neck triangles, progressive emaciation, oedemata of the extremities and face, protuberant abdomen, diarrhoea, anaemia, muscular tremors, inco-ordination, mental hebetude and somnolence deepening into coma. In untreated cases, death is the inevitable result and the whole duration of the infection is short, on the average from about three to six months. An occasional case may live for ten or twelve months, though this is very exceptional, and only one, in my experience, has ever exceeded this period.

This native, Chimwila, was found to be infected on the 18th November, 1920, and then gave a history of having been ill only one month, complaining of headache. This was probably an underestimate as his neck glands were markedly enlarged. He was seen again on the 9th May, 1921, and appeared to be in perfect health saying himself that he was not sick. His neck glands were now smaller though the juice still contained parasites. His condition remained the same on the 11th March, 1922, and trypanosomes were still found in the gland juice. He was then sent to Prof. Kleine for treatment with Bayer 205, i.e., sixteen months after he had been found to be infected and probably eighteen at least after he contracted the disease. Prof. Kleine states in one of his reports that Chimwila appeared to be physically in good health on his arrival but that some mental dullness was noticed. After receiving treatment he returned to his village later in 1922 and was apparently quite well when last seen in March, 1925. No glands were palpable and no parasites were found in the blood.

The disease is very decidedly one of adolescence and adult life. I have never seen a case in a young child, and only comparatively rarely under the age of about fifteen. This is well brought out in the following tables from two independent sources, and in view of the great difficulty of estimating at all accurately the ages of natives and the part the personal equation must play in such estimations, the general agreement of the two sets of figures is very striking.

Age	Kinghorn		Fischer	
	Cases	%	Cases	%
6-15	5	6·66	1	3·57
16-25	20	26·66	9	32·14
26-35	27	36·00	11	39·28
36-45	17	26·66	5	17·85
46-55	5	6·66	1	3·57
Over 55	1	1·33	1	3·57
	75	99·97	28	99·98

It will be noticed from these tables that there is a steadily increasing susceptibility to infection from the earlier ages to about the 35th year and that thereafter the decline in susceptibility is as equally and steadily marked; further, that from 85 to 89 per cent. of all the cases occur in the age group 16 to 45. These remarks apply with equal force to the two sexes considered separately, as shown below.

Age	Males		Females	
	Cases	%	Cases	%
6-15	4	8·88	1	3·33
16-25	11	24·44	9	30·00
26-35	15	33·33	12	40·00
36-45	12	26·66	5	16·66
46-55	3	6·66	2	6·66
Over 55	0	0·00	1	3·33
	45	99·97	30	99·98

This table also brings out the fact that the disease is commoner in men than in women, the proportion being as 3 : 2, and this is corroborated by an analysis of Dr. Fischer's cases, which gives a proportion of $3\frac{1}{2}$ males to 2 females. Probably the greater number of male cases may be accounted for on the basis that the men, on the whole, spend more time in the bush, hunting, collecting honey, poles for building, reeds and other material for mat and basket-making, and so on, than the women do in their daily trips to collect firewood. It is more difficult to explain why, approximately after the age of puberty is passed, the susceptibility to infection should increase so perceptibly and increase in an ascending curve to the age of thirty-five and then decrease steadily. Babies and very young children are always carried by their mothers in a calico or sling and are thus fairly effectively protected, but the older children who also usually accompany their mothers wherever they go are naked and unprotected. The older children, aged from ten to twelve onwards, are, at best, very scantily dressed and have to assist their parents in the various duties outlined above. They are thus frequently exposed to the risk of infection and yet it is only very rarely that this occurs. It may be argued that the discrepancies in the age-incidence of the disease are more apparent than real and that if the actual age distribution of the whole population could be plotted, these would become obvious, but in the absence of an accurate census this is impossible. I am, however, inclined to doubt this. Certainly as between children and adults it does not apply, as it may be said generally that the number of children in a village will about equal the number of adults. Thus an analysis of the two groups in the villages under Chikwa, Tembwe and Kambombo, gives 2,741 children to 3,015 adults. I am convinced that the varying rates of infection amongst the different age-groups cannot be explained wholly and satisfactorily on the assumption that they are in direct ratio to the risks of infection run by the respective groups, though it is difficult to suggest other factors which may influence the occurrence of the disease in the light of our present knowledge. It may here be pointed out that hunger with its consequent lowering of vitality does not increase susceptibility to infection. In this area the 1923-24 crops were bad, with the result that, towards the end of 1924, acute hunger,

amounting in some villages to actual starvation, became apparent, and several deaths from this cause were reported. The results were particularly noticeable in Chikwa's country, where all of the natives showed emaciation varying from slight to extreme, yet only one case of sleeping sickness was found, and only five deaths from what may have been sleeping sickness were reported amongst 1,400 natives.

There is no evidence that acquired immunity to the infection is found in man though, as stated above, I am of the opinion that he is naturally very resistant to it.

VI. TREATMENT WITH 'BAYER 205'

Early in 1922, a commission composed of Prof. Kleine and Dr. Fischer came out to this country to try the effects of the drug known as 'Bayer 205' on human trypanosomiasis and a camp was established in the Luangwa valley at Ndombo, about thirty-five miles east of Mpika. In all, 38 cases were treated, and of these one man went later to Southern Rhodesia and has not been traced, five are still alive, and the remaining 32 are dead. Five of the deaths occurred at the Commission's camp from intercurrent affections and the other 27 in the villages at varying periods after the parents had returned there on leaving Ndombo in October, 1922. The following is the complete list :—

Name	Sex	Age	Date of death	Duration of life after treatment	Remarks
1. Pitala	M	25	February, 1924	16 months	
2. Yatula	F	30	—	—	Alive
3. Chimwila	M	35	—	—	Alive
4. Chizilicho	M	35	March, 1924	17 months	
5. Isake	M	15	July, 1923	9 „	
6. Chilikupata	M	25	April, 1924	17 „	
7. Mofati...	M	35	?	?	Untraced
8. Lamek	M	16	—	—	Alive
9. Samuel	M	16	February, 1924	16 months	
10. Tadeyu	M	27	February, 1924	16 months	

Name	Sex	Age	Date of death	Duration of life after treatment	Remarks
11. Kajawa	M	38	—	—	Alive
12. Vioka	F	22	October, 1923	12 months	
13. Mateyo	M	35	February, 1923	4 months	
14. Chifundulwa	M	60	—	—	Alive
15. Lasalu...	M	25	February, 1924	16 months	
16. Vilauli	M	38	July, 1924	19 months	
17. Malita	F	22	November, 1923	13 ,,	
18. Mwipi	M	12	December, 1923	14 ,,	
19. Murere	F	45	October, 1923	12 ,,	
20. Nderema	F	40	October, 1923	12 ,,	
21. Ntanda	F	27	October, 1923	12 ,,	
22. Yumba	F	45	November, 1923	13 ,,	
23. Ndabeya	F	54	April, 1923	6 ,,	
24. Mage	F	26	February, 1924	16 ,,	
25. Marata	M	28	July, 1923	9 ,,	
26. Mapulanga	M	35	February, 1923	5 ,,	
27. Kabrieni	M	18	November, 1923	13 ,,	
28. Chikoti	M	35	October, 1923	12 ,,	
29. Kamchepa	F	32	October, 1923	12 ,,	
30. Zakeyo...	M	35	October, 1923	12 ,,	
31. Wayilipa	F	35	October, 1923	12 ,,	
32. Sabeta	F	20	October, 1923		Died on road home
33. Thomas	M	17	February, 1924	16 months	
34. Thomas	M				
35. Ngoza...	F				
36. Chiweza	M	died at Ndomba from intercurrent disease			
37. Tepatepa	M				
38. Puntayila	M				

As will be noticed, the cases were of both sexes and of all ages and it may be added that they were in various stages of the disease from early to late. With regard to the cases which died in the villages, the native evidence is that at varying periods after they had returned they started to become ill again and presented the ordinary symptoms of sleeping sickness, e.g., progressive emaciation, oedemata, and so on. This applies equally to the eight cases which died in October, 1923, but there is a possibility that in these death was hastened by influenza of which an epidemic swept through the valley in August and September of that year. This reappearance of symptoms must be regarded as due to relapses of the original, and not to fresh infection. In the treatment of sleeping sickness the greatest drawback to success is the difficulty of attacking effectively the parasites in the cerebro-spinal fluid, and evidence of this with 'Bayer 205' is found in the reports of the German Commission. Thus it is stated that of twenty-one patients examined by lumbar puncture before their discharge from the Ndombo Camp in October, 1922, eight were found to harbour trypanosomes. Under such circumstances it would only be a matter of time until the trypanosomes re-invaded the blood stream and set up symptoms of the disease. The only definite result claimed by the Commission for 'Bayer 205' is that by its use it is possible to sterilise the blood for a long period even in those cases which are not clinically cured ; and while this claim seems to be established I question, in view of the demonstrated fact that the bulk of the cases did ultimately relapse, whether there are any substantial grounds for stating that ' if in districts infected with sleeping sickness all suspected natives receive treatment . . . the source of infection for the tsetses will gradually disappear and in time the disease must die.' It is assumed that man is the only reservoir and that the game is negligible. This I cannot admit.

The routine method of treatment adopted by the Commission was three subcutaneous or intravenous injections of 1·2 gm. in normal saline at intervals of ten and eighteen days, though those cases in which the parasites persisted in the blood and cerebro-spinal fluid received a fourth and fifth injection. It is apparent, therefore, that a dosage of from 3·6 to 6 grammes is insufficient to cure the majority of cases of *T. rhodesiense* infections in natives. Better

results might, of course, follow the adoption of an increased dosage up to 10 or 12 grammes as has been advised by some experienced workers, but at the price of 6/6 a gramme it is questionable whether the expenditure of £3/5/– to £4 per head on drugs alone would be legitimate, in the light of our present knowledge, on any very wide scale, more particularly as reports from the Congo would indicate that in tryparsamide we possess an equally, if not more, efficacious drug which possesses the advantage of being cheaper. It is of interest to note that in these reports it is said that the results of treatment there with 'Bayer 205' had been 'frankly disappointing.'

I saw and examined the five cases which are still alive in August, 1924, and again last month. All of them appeared to be in perfect health, presented no signs or symptoms of sleeping sickness, and showed no parasites in the blood stream. It was not possible to perform lumbar puncture. I believe, therefore, that these natives may now be regarded as being definite cures. Disregarding the five cases which died from intercurrent disease and the one which has not been traced, this represents a percentage of 15·6 cured. In view of the fact that all previous methods of treatment for the Rhodesian type of the disease have been failures, this must be admitted to be a considerable advance, but these results do not justify the very optimistic claims which are still being made for this drug.

VII. PROPHYLAXIS

It is a definite fact, which must be recognised, that no active assistance can be expected from these natives for any measures designed either to combat a specific infection, or generally to improve the sanitation of the villages. For some years movement into and out of the valley was prohibited, and even though the sleeping sickness regulations have fallen into comparative desuetude very few Europeans travel there now. Thus to a marked degree the natives have remained under the influence of their ancient tribal beliefs and treat European ideas of the etiology of disease and the methods to be adopted in treating them with undoubted, if unexpressed, disbelief. The general attitude, therefore, becomes one of passive resistance which can only be overcome by a certain

amount of compulsion, and when force has to be employed the results are usually unsatisfactory. Not only does the native revert to his own ideas and habits as soon as he thinks he can safely do so, but there is also a tendency to evade actively the application of disagreeable rules and regulations by running away and hiding as soon as an official appears in his district. Of this I have had personal experience. This becomes more pronounced when the results of European regulations and treatment are unsatisfactory, as it must be admitted they have so far been with particular reference to sleeping sickness. My experience is that the few cures are overlooked and attention is concentrated on the failures, and that indeed the natives really believe that the deaths have been caused by the use of hypodermic needles. In time, and with the increase of education, this general attitude may be modified but it will necessarily be a very slow process, and in the interval it is not apparent that much can be done. Something might be done to hasten this, by arrangement with the various missions having schools in the valley, if the teachers were given instruction in the essential facts of the etiology of sleeping sickness and other infections, told to explain these facts to their classes at frequent intervals, and to urge patients to go to hospital for treatment. No immediate results could be expected in view of the ingrained instinct of the native to return to his home as soon as he falls sick, and in view of the widespread aversion to going into hospital. I think, however, it would be a step in the right direction.

In view of the facts, which I think have been brought out earlier, that sleeping sickness ordinarily is one of the less important causes of death in the valley and that in general it occurs only in widely-scattered, sporadic cases, I am inclined to doubt whether it is incumbent on the Government to provide special facilities for treatment beyond those which already exist in the various hospitals. When the enormous area of the valley is recalled it is obvious that to do so would entail, if success is to be obtained, the appointment of at least three special medical officers with hospital and staffs, and the expense of this would, of course, be very large. Success could only be anticipated if the theory is true that *T. rhodesiense* is a parasite of human beings alone, that man is the sole reservoir and that the game plays no part in the perpetuation of the infection.

If so, then it appears to me that the obligation to deal with the disease by special measures is greatly strengthened. If, however, it is correct that the game and human parasites are identical, or alternatively that the game may act as a reservoir for a specific human trypanosome, then success in combating sleeping sickness can only be assured by simultaneously segregating and treating all the human cases of the disease and killing off the whole animal fauna. This is not a practicable proposition in the valley.

In the event of the occurrence of one of the localized epidemics it would, I think, be advisable to institute local treatment, as I believe that in these man does play a part as a direct reservoir of infection and it would be a matter of some importance to break the man-fly-man cycle.

I also think that it would be advisable to keep on the statute book the Sleeping Sickness Regulations, not with any idea of interfering with the natives, but chiefly for the power they confer of regulating the movements of Europeans. If all the regulations were rescinded, there would probably be an influx of professional hunters in pursuit of elephant, and the possible occurrence of cases amongst them might entail an unnecessary expense on the Government.

Lundazi, N. Rhodesia,
April 10, 1925.

THE DESTRUCTION OF ASCARIS EGGS

BY

SEIJI OGATA

PATHOLOGICAL DEPARTMENT, OSAKA MEDICAL COLLEGE, JAPAN

(*Received for publication* 13 *July*, 1925)

Most investigators are agreed that Ascaris eggs have strong powers of resistance to chemical fluids of various kinds. I also have tested the resistance of Ascaris eggs containing embryos by soaking them in various disinfectants, acid or alkaline, and have found that in every experiment they retained for many days the ability to infect.

I found, however, that Ascaris eggs were easily destroyed by heat. In September, 1921, I poured hot water at a temperature of 100° C. over Ascaris eggs collected from human dejecta and found that the eggs lost their power of development. In November of the same year I noticed that Ascaris eggs containing embryos, after hot water had been poured on them, lost the ability to infect.

Having ascertained that Ascaris eggs were instantly destroyed by water at 100° C., I attempted to discover how long they would remain alive in water at varying degrees of temperature. These tests, however, failed on account of the difficulty of finding any effective means of immersing in hot water Ascaris eggs dispersed among human dejecta. Again, if eggs mingled with human dejecta are dispersed in boiling water, it is not always possible to get them out at once.

At length, a method was discovered which seemed to me entirely effective. The following experiments were than carried out.

Method : In testing the resistance of Ascaris eggs to heat, I employed matches in order to prevent the dispersal of the eggs. The human dejecta were washed in water and the sediment obtained by centrifuging was smeared on the matches to half their length, and left to dry. Then each match, held with the forceps, was soaked for the desired period in hot water at varying temperatures. They were then put into cold water to cool. The matches were then subjected to observation.

The observations were carried out in three ways, (1) staining ; (2) development ; (3) infection.

(1) I use Sudan III staining for the purpose of distinguishing between live and dead Ascaris eggs. Presuming that fatty

degeneration might possibly occur in the liver of a patient infected with Ascaris, we applied Sudan III staining to the liver of an animal which was experimentally infected with Ascaris. We found that Ascaris larvae in the liver were distinctly stained by Sudan III staining, and that living larvae separated from the liver or lungs could be similarly stained. The red-stained granules of 'fat-corpuscles,' as I provisionally call them, gradually decrease in size as the size of the larva increases at each stage of its development.

With the object of making clear the true nature of the ' fat-corpuscles ' of the Ascaris larva, I tried to ascertain if there were any substance in Ascaris eggs which could be stained with Sudan III. It was necessary to make sections for the staining of the eggs. The Ascaris eggs, collected from human dejecta, were embedded in gelatine and cut into thin sections with the freezing microtome. They were then stained with Sudan III. By this means the staining of eggs was obtained, but not of the albuminous membrane. I further noticed that fat-corpuscles were abundant in the eggs.

When I applied Sudan staining to Ascaris eggs collected from human dejecta, I found that unfertilised eggs were stained with red granular spots, while healthy fertilised eggs were not stained at all. Therefore, presuming that Ascaris eggs might be made stainable by killing them, I applied Sudan III staining to eggs killed by boiling water. As I expected, all the eggs were seen under the microscope with fine red colouring.

From the results of my repeated experiments, I conclude that the fat-corpuscles are produced in the blastomeres as the egg-cells grow and segment, and that gradually these fat-corpuscles gather much more in the hypoblastic than in the epiblastic cells ; thus very young U-shaped embryos are full of fat-corpuscles from head to tail ; but these fat-corpuscles are ranged along the intestine of the worms as they grow. We consider, therefore, that the above-mentioned fat-corpuscles may be the yolk.

Having found, therefore, that (*a*) healthy fertilised eggs are unstainable with Sudan III, that (*b*) unfertilised eggs are stainable, and that (*c*) fertilised eggs become stainable with that dye if hot water is poured over them, we used Sudan III for the study of Ascaris eggs after immersion in hot water at temperatures varying from 40° C. to boiling point, and for periods varying from one second

to an hour. We found that Ascaris eggs become stainable with Sudan III after being immersed for one second in water at over 75° C., almost all become stainable after being immersed for ten seconds in water at 70° C., while in water at 65° C. they had to be immersed for over ten minutes before becoming stainable. In water at temperatures lower than 60° C. over one hour's immersion is not sufficient to render them stainable.

The above experiment was repeated twenty times with material from the same patient and from twenty-one others, but the results showed no great difference.

(2) In order to prove that Ascaris eggs which have become stainable are really dead, we cultivated, in 4 per cent. formalin solution, Ascaris eggs which had been immersed in water at varying temperatures for varying periods of time as in Experiment 1, and examined their developmental condition at the end of stated periods. This was repeated some thirty times with material from the same patient and from many others, but all the results were practically the same, namely, that all the eggs lost their power of development after being immersed in water at over 70° C. for one second; a few retained it after being dipped in water at 65° C. for one second; embryos developed from all eggs immersed for three seconds in water at 60° C., for 40 seconds in water at 55° C., and for thirty minutes in water at 50° C.; all eggs would develop into embryos after being soaked for one hour in water at temperatures lower than 45° C.

The difference between the results of Experiments 1 and 2 is noteworthy.

Ascaris eggs which have lost their power of development can be divided into two groups, one stainable with Sudan III, the other unstainable with that dye. The former show a morphological change (i.e., fatty degeneration and distortion of the eggs) after immersion in hot water; the latter do not differ from healthy eggs, and yet are in a state of suspended development. This condition of suspended development may continue for as long as twenty days or even longer, but if left alone for long they are likely to perish gradually.

Ascaris eggs are instantly killed by very hot water; by water at lower temperatures they are merely deprived of their ability to develop; and after being immersed in water at temperatures below 45° C. the eggs develop in the usual manner.

(3) As a result of Experiments 1 and 2 I could definitely fix the temperature at which hot water will destroy Ascaris eggs containing embryos capable of infection.

I cultivated for a month, in 4 per cent. formalin solution, Ascaris eggs collected from human dejecta. When matured, I placed them on matches and left them to dry until the following day. I then soaked these matches in hot water at various temperatures and fed mice with the eggs. After three days I examined the mice with a view to ascertaining whether liver, heart, or lungs were infected. I repeated this experiment many times with 515 mice and arrived finally at the following results.

The mature Ascaris eggs, cultivated in 4 per cent. formalin solution, lose their infecting power after remaining for one second in water at 70° C. or more ; almost all lose it after remaining for one second in water at 65° C., for five seconds in water at 60° C., for forty seconds in water at 55° C., or for fifteen minutes in water at 50° C. Even after remaining for one hour, however, in water at temperatures below 45° C. the eggs did not lose their power to infect.

SUMMARY

A minute examination of the power of resistance to heat of Ascaris eggs by means of the above experiments has led to the conclusion that the method of destruction of Ascaris eggs by boiling water can be effected also with hot water at lower temperatures. Generally speaking, the ideal to be aimed at in disinfection is simplicity of method and rapidity of action.

To prevent Ascaris infection it is safest to kill the eggs by immersion in hot water at the temperature at which the egg content changes morphologically and becomes stainable with Sudan III ; though the power of development and the power to infect may be destroyed by immersion in water at over 70° C. for one second, at 65° C. for two seconds, at 60° C. for five seconds, at 55° C. for fifty seconds and at 50° C. for forty-five minutes.

In conclusion, I wish to acknowledge my great indebtedness to Dr. S. Yoshida who kindly superintended my work and gave me all facilities for completing the present paper.

AN EXPERIMENTAL STUDY ON THE DEVELOPMENT OF THE DWARF TAPEWORM (*HYMENOLEPIS NANA*)

BY

YOSHIHISA SAHEKI

RED CROSS HOSPITAL, NAGANO, JAPAN

(*Pediatrics Laboratory of Kanazawa Medical College, Kanazawa, Japan*)

(*Received for publication* 10 *May*, 1925)

The course of infection and the development of the dwarf tapeworm have been much studied and discussed, especially as to whether this species has any intermediate host or not, but as yet no definite conclusion has been reached. I have studied this subject extensively, both in animals and in man.

From my experiments it appears that the eggs of this tapeworm, unlike those of others, can hatch and develop without any intermediate host.

ON THE DEVELOPMENT OF THE DWARF TAPEWORM IN ITS HOSTS

1. *From five hours to twenty-four hours after feeding.*

On examining mice which had swallowed dwarf tapeworm eggs five or six hours previously, I observed in their small intestines six-hooked larvae, all of which had emerged from the egg-shells. After ten hours the mice had larvae which had already entered into the villi in the upper part of the small intestine and from hour to hour, up to twenty to twenty-four hours, larvae penetrated the villi. These larvae were oval or round in shape, their surface was granulated and they had six hooks near one end.

2. *Three days after feeding.*

I found many larvae in the villi; their shape was the same as noticed above but they had increased in size. Most of them were somewhat pear-shaped. In the centre of their bodies the granules

became somewhat coarse and I observed some small calcareous corpuscles which reflected the light strongly, but as yet the larvae were not encysted.

3. *Four days after feeding.*

The six-hooked larvae, which had entered the villi, had increased much in size and became bean-shaped encysted larvae (Cysticercus), the space between the body of the larva and the cyst being filled with granular material. Some, still with six hooks at one end of the cyst, were moving in the villi.

In the centre of the body of the larvae was a ring of regularly arranged wedge-shaped hooks, which reflected the light strongly ; but the roots of the hooks were not yet separated and the differentiation of the suckers was not clearly defined ; however, by carefully adjusting the light, I could see some encysted larvae with two suckers in front and some with only one ; and among the radiating fibres running from the caudal extremity of the encysted larva to the cyst, could be found irregularly-shaped calcareous corpuscles, some large, some small.

4. *Five days after feeding.*

Well developed encysted larvae had emerged from their cysts, left the villi, descended to the lower part of the small intestine and were actively moving about, extending and contracting themselves ; some were in process of emergence from their cysts and some had already emerged, still having parts of the cysts attached at the rear. In these larvae I could see four suckers ; a single ring of hooks, an excretory tube, running from the rear of the rostellum to the caudal extremity, and some irregularly-shaped calcareous corpuscles which reflected the light strongly.

5. *Eight days after feeding.*

The size has increased and segments are clearly seen, but there is no differentiation of the reproductive organs.

6. *Ten days after feeding.*

A gradual increase in size ; the uteri being filled with what seemed to be primitive eggs, the structure of which was not very clear.

7. *Fourteen days after feeding.*

Growth is complete and the posterior gravid segments are so full of eggs that the testes are compressed and the excretory tubes obscure.

8. *Fifteen to nineteen days after feeding.*

Although there was some difference in the development of the dwarf tapeworms according to the host employed (mice, rats) they grew little by little, and about the seventeenth or eighteenth day, the experimental animals began to evacuate eggs in the faeces.

After eighteen days the last segments of the adult tapeworms become more slender, as the result of having discharged many eggs.

EXPERIMENTAL INFECTION IN MONKEYS

15 June, 1919. Two young male monkeys (one year and two months old), showing no tapeworms' eggs in their faeces, were obtained. I made one of the monkeys swallow many eggs of the dwarf tapeworm, but found no eggs on examining its faeces fifteen to sixteen days after the feeding ; so I again made it swallow many eggs. It was killed seven days later ; the contents and villi of its small intestine were closely examined, but no dwarf tapeworms were to be found.

26 July, 1919. The other monkey was fed with many dwarf tapeworms' eggs ; it became gradually feeble, and died in about six days. Examination of its small intestine showed thirteen young dwarf tapeworms in the lower part, the shape and size being no different from those of the young dwarf tapeworms in the small intestine of the mice and rats.

SWALLOWING OF EGGS BY MAN

29 January, 1919. Taking great care not to destroy the eggs of the dwarf tapeworm, I washed them with water, put many of them into capsules and swallowed them myself.

As a control, a part of the same material was given to a rat and a white rat which seven days later proved to be infected, but in spite of having examined my own faecal matter several times, for fourteen or fifteen days after ingestion, I could not find any dwarf

tapeworm eggs. Then I tried to expel tapeworms from my intestine, but unsuccessfully. Afterwards I swallowed eggs three times but neither eggs nor worms were found in my faeces.

Fortunately, a girl four years old was available for experiment; she was strongly-built, well nourished and healthy. I carefully examined her faeces several times, and each time found a few eggs of Ascaris but none of the dwarf tapeworm. On 12 April, 1919, she swallowed many eggs of the dwarf tapeworm in capsules and afterwards her faeces were examined on many occasions. On the first of May, nineteen days later, I found a few eggs of the dwarf tapeworm, and upon expelling the worms from her small intestine, I secured ninety-seven adult dwarf tapeworms.

SUMMARY

1. The six-hooked larvae of the dwarf tapeworm, about ten hours after ingestion, penetrate the villi in the upper part of the small intestine and four days later become encysted larvae (Cysticercus). Five days after ingestion, well developed larvae emerge from their cysts and leave the villi.

2. After seven days, well developed young dwarf tapeworms have some segments at the end of the body.

3. After nine days, their reproductive organs become visible.

4. About fourteen days after ingestion, the segments are full of eggs, in each of which can be seen a six-hooked larva (Onchosphere).

5. After about seventeen days, the ripe eggs of the dwarf tapeworms are found in the faeces of the experimental animals.

6. These experiments show that dwarf tapeworm eggs which have been evacuated with the faeces of the host, are swallowed by animals or man with their food, and that the eggs hatch and the six-hooked larvae are liberated in the small intestines, and later enter the villi of the small intestines where they become encysted larvae; then emerging from the cysts they grow into young dwarf tapeworms and upon maturity evacuate the ripe eggs.

7. Therefore, without any intermediate host, the dwarf tapeworm can directly develop in the body of mice, rats, young monkeys, or man, especially children.

A SPOROZOON OF *PHLEBOTOMUS PAPATASII*

BY

S. ADLER

AND

O. THEODOR

(*Microbiological Institute, Jerusalem*)

(*Received for publication* 9 *July*, 1925)

During a routine examination of 1,037 *Phlebotomus papatasii*, of which 939 were females, one female was found to contain oocysts. All the insects were collected in Jericho between April and June, 1925. The infected specimen was one of a number used for experimental purposes and dissected immediately after a feed on a laboratory assistant on 25th May, 1925.

On dissecting the head from the thorax a large number of small glistening bodies were seen to emerge from the thorax and these on examination proved to be sporocysts from a ruptured oocyst.

Further dissection revealed the fact that thorax and abdomen contained four ripe oocysts, one in the thorax and three in the abdomen. The oocysts were 130μ by 95μ in size and contained about a hundred sporocysts. Between the sporocysts were a number of round refractile bodies up to 8μ in diameter. The sporocysts varied in size from 21·4μ to 36·4μ in length by 15·7μ to 20μ in breadth and contained four to sixteen sporozoites and a residual body, from 3·6μ to 6·4μ in diameter, enclosed in a definite membrane. Apart from the residual body each sporocyst contained a number of small refractile granules lying apparently in the sporozoites.

In the sporocysts the sporozoites were seen to be actively motile, in some cases sufficiently so to cause the whole sporocyst to spin.

From the ruptured sporocysts sporozoites were seen emerging; each sporozoite was then observed to be lying in a membrane which when released from the sporocyst assumed the form of an elongated

spindle about 35μ in length and 6·4μ in breadth (figs. 6 to 8, and 12). The small refractile granules noted in the sporocyst were found to lie in the membrane outside the sporozoite, each membrane containing two to four granules.

The membranes, including the sporozoites, were all seen to be divided longitudinally by two fine lines (fig. 12). The sporozoites were actively motile within their membranes, constantly changing their shape and size by a series of contractile movements, so that it is difficult to give definite measurements. When fully stretched out the sporozoites were sickle-shaped with one end pointed and the other blunt, and then measured 34μ in length by 5μ in their thickest part (fig. 9). Each sporozoite contained a round nucleus.

By contracting and thus increasing their transverse diameter the sporozoites stretched the enclosing membrane and created a gap between the two longitudinal lines of the membrane (fig. 8). The sporozoites then slowly worked their way through the gap, leaving an empty husk containing several refractile granules (fig. 12).

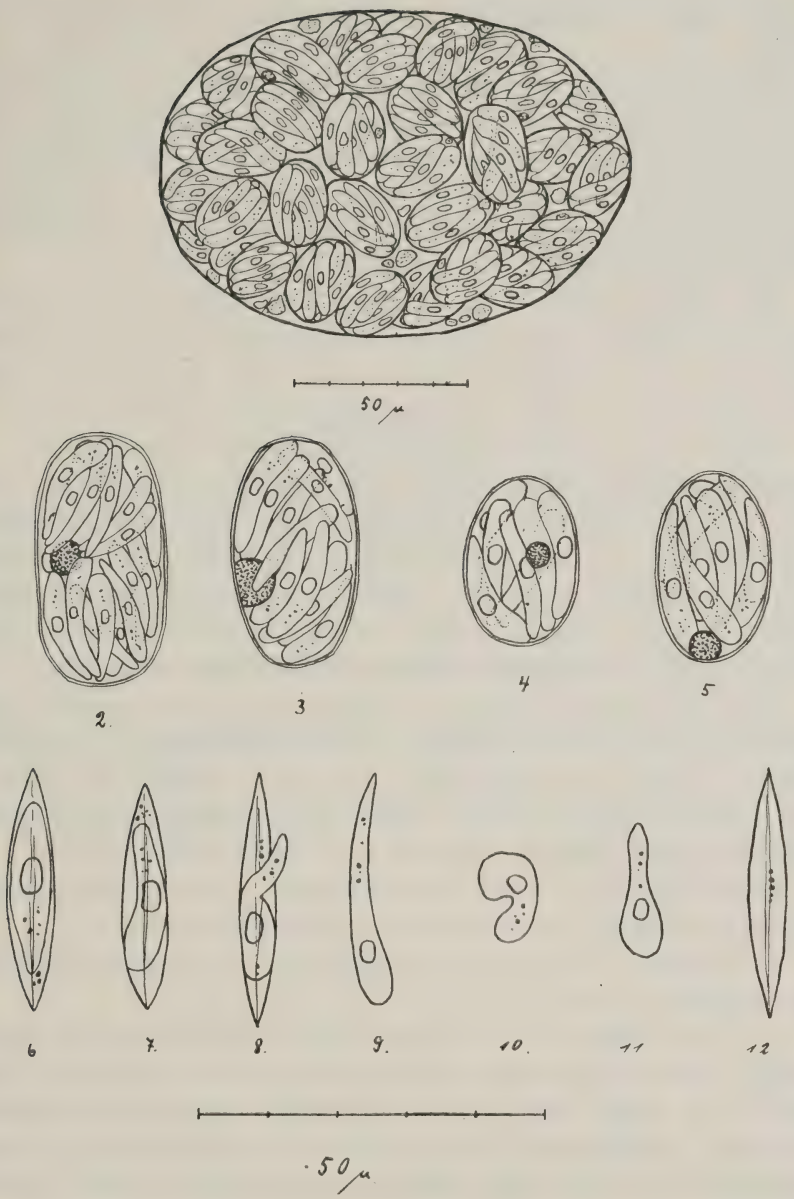
Having escaped, the sporozoites continue their contractile movements, constantly changing their shape and, at the same time, performing a slow translatory movement.

All the material was transferred to two slides and examined in the fresh.

As it seemed obvious that the oocyst above described formed a part of the life-cycle of a haemogregarine of a vertebrate, the following experiments were immediately performed.

(i) The contents of one fresh preparation containing intact sporocysts and numerous free sporozoites from ruptured sporocysts were carefully washed off the slide in 0·5 c.c. normal saline ; 0·25 c.c. of the resulting mixture was injected intraperitoneally into a specimen of *Gongylus ocellatus*, and the remainder intraperitoneally into a specimen of *Mabuia quinquefasciata*. The above two lizards were both free from haemogregarines at the time of the experiment. The gecko *Hemidactylus turcicus*, which is common in houses in Jericho and feeds on sandflies, would have been a more suitable animal for the experiment, but unfortunately no specimen of this animal was at the moment available in the laboratory.

(ii) The material from the second fresh preparation containing numerous sporocysts and free sporozoites was rubbed into puncture



FIGS. 1-12.

1. A complete oocyst.
2-5. Sporocysts.
6-7. Sporozoites in their enclosing membrane.
8. Sporozoite escaping from membrane.
9-11. Change in shape of a sporozoite.
12. Empty membrane with refractile granules.

wounds made by a needle into the forearms of two healthy human beings.

The lizards were examined at intervals during an observation period of five weeks and the peripheral blood was found to contain no haemogregarines. At the end of this period the two animals were killed and the liver, lungs and bone marrow were examined for schizonts with a negative result. The blood of the two human beings was also found to be negative during this period.

The oocyst described above apparently belongs to the genus *Hepatozoon* (Miller 1908) since it contains numerous sporocysts and each sporocyst contains a number of sporozoites, the formula for the genus *Hepatozoon* being, according to Reichenow (1921) :

' Oocysts with *n* sporocysts, sporocysts with *n* sporozoites.'

Infection of a new vertebrate host with *Hepatozoon* sp. takes place by the accidental ingestion of the transmitting arthropod containing ripe oocysts, e.g., *Mus rattus* becomes infected with *Hepatozoon perniciosum* (Miller 1908) by swallowing the mite *Lelaps echidnius* containing ripe oocysts, and dogs are infected with *Hepatozoon canis* (James 1905) by swallowing infected *Rhipicephalus sanguineus*.

Up to the present the genus *Hepatozoon* has been recorded only from mammals, but the finding of oocysts belonging to the genus *Hepatozoon* free in the abdominal cavity of *Gl. palpalis* by Chatton and Roubaud (1913) and by Macfie (1916) points to the possible presence of this genus in lizards or birds which feed on the fly.

In the case of the oocyst of *Phlebotomus papatasii*, two possible modes of infecting a vertebrate host suggest themselves :

1. Ingestion by a lizard and liberation of the sporozoites into the alimentary canal.

2. The crushing of an infected Phlebotomus during the act of feeding, and the liberation of sporozoites into the wound, i.e., the method by which Phlebotomus is generally assumed to transmit cutaneous leishmaniasis. The experiment on two human beings described above thus approximates to an infection under natural conditions.

A number of observers (Krempf 1917, Dimond 1917, Sergent, Et. and Ed. and Parrot 1922, Noc 1922, Nattan-Larrier 1922), have described haemogregarines from man. The findings of all these authors have been subjected to a destructive criticism by

Wenyon (1923), who concluded that ' the haemogregarines of man have still to be found.'

The bionomics of *P. papatasii* render it an eminently suitable transmitting agent of a haemogregarine to man if such occur and the result of the experiment on two human beings is therefore of interest.

The above is the first record of an oocyst in *Phlebotomus papatasii*.

REFERENCES

Chatton, E., and Roubaud, E. (1913). Sporogénie d'une Hémogregarine chez une Tsétsé. (Glossina palpalis R. Desv.)—*Bull. Soc. Path. Exot.*, Vol. VI, No. 3, pp. 226-233.

Macfie, J. W. S. (1916). The results of dissection of Tsetse flies at Accra. Report of the Accra Laboratory for the year 1915. London (J. and A. Churchill).

Reichenow, E. (1920). Die Haemogregarinen. Handbuch der pathogenen Protozoen. Prowazek. Vol. II, pp. 602-632. Leipzig.

——— (1921). Die Coccidien. Handbuch der pathogenen Protozoen. Prowazek. Vol. III, pp. 1136-1207.

Wenyon, C. M. (1911). Oriental sore in Bagdad, together with observations on a gregarine in *Stegomyia fasciata*, the haemogregarine of dogs and the flagellates of house flies. *Parasitology*, Vol. IV, pp. 273-344.

——— (1923). ' Haemogregarines ' in man, with notes on some other supposed parasites. *Trop. Dis. Bull.*, Vol. 20, No. 7, pp. 527-550.

THE GENUS *TETRACAMPOS* WEDL, 1861

BY
T. SOUTHWELL

(*Received for publication* 23 *July*, 1925)

Woodland, in the '*Annals of Tropical Medicine and Parasitology*,' Vol. XIX, No. 2, p. 185, refers the above genus to the *Bothriocephalidae*.

In a former issue of the 'Annals,' I gave certain reasons for referring the genus to the Order *Cyclophyllidea*.

This difference of opinion cannot, unfortunately, be settled by an examination of the worm in question, viz., *T. ciliotheca* Wedl, 1861, because the material is not available.

As the species is stated by Wedl to possess four lappets or bothridia which are figured, it should, on that account, be referred to that Order of Cestodes which is characterised by the possession of four bothridia, viz., the *Tetraphyllidea*. Owing to the fact that Wedl's figure of the head leaves one in considerable doubt as to whether the so-called bothridia are really bothridia, or whether, on the other hand, they are badly figured acetabula; and also having in mind the fact that other cestode parasites with armed heads bearing true acetabula, and with ventral pores, have been repeatedly obtained from fish closely related to that in which *T. ciliotheca* was found, the writer concluded that Wedl's genus *Tetracampos* belonged to the *Proteocephalidae;* and, as in this family the head is armed with four suckers, it was referred to the Order *Cyclophyllidea*.

Up to the present helminthologists have agreed, and rightly, that the primary divisions of the polyzootic cestodes should be made on the character of the head. Thus, in the *Cyclophyllidea* the head bears four suckers, in the *Tetraphyllidea* four bothridia or lappets, in the *Trypanorhyncha* four proboscides, and in the *Pseudophyllidea* sometimes one or more, but usually two, bothria (or grooves).

The head thus provides a ready and eminently satisfactory means of effecting a natural classification of this group of worms into

Orders, and the utility and simplicity of this means of classification justifies us in retaining it, until a better system is provided.

In the absence of a head, it is frequently impossible to refer a cestode worm to the Order to which it belongs. If the genital pores (excluding the uterine pore or pores, whether primary or secondary) are situated on the ventral surface, the worm is placed in the Order *Pseudophyllidea ;* there are, however, exceptions to this rule.

If the genital pores are lateral, then it is necessary to locate the position of the vitelline glands. If this organ consists of numerous follicles situated laterally, it is still impossible to say whether the worm belongs to the Order *Tetraphyllidea* or to the Order *Trypanorhyncha.*

If the gland is single, the worm is referred to the Order *Cyclophyllidea.* Unfortunately, however, there are a number of species which, although they possess a head typical of the *Cyclophyllidea,* have the vitelline glands arranged along the lateral margins, and there are also a few species which, while characterised by having a Tetraphyllidean head, have the vitelline glands condensed into a single mass situated behind the ovary.

The male and female genital organs are of the same type, especially in species of all the three Orders, *Cyclophyllidea, Tetraphyllidea* and *Trypanorhyncha,* the trivial differences which exist being limited to the disposition of the musculature, the number of testes, the size of the cirrus pouch, the position of the pore on the lateral margin, etc. —points obviously only of importance in the differentiation of species, or at most of genera. The form of the uterus in the *Pseudophyllidea* is, however, usually characteristic in that Order.

In spite of the fact that in *T. ciliotheca* the head bears four bothridia, or four suckers, Woodland refers the genus to the Order *Pseudophyllidea,* and states that ' scolex characters count for very little.'

Woodland realises that the head of a Bothriocephalid usually possesses two bothria, for he states that the four bothridia in *T. ciliotheca* ' are evidently the four walls bordering the bothriae or sucking grooves.' For a similar reason one could consider the Order *Tetraphyllidea* identical with the *Pseudophyllidea.*

It is true that Wedl states that in *T. ciliotheca* the embryophore is ciliated exactly as it is in *D. latus.* Practically nothing is known

regarding the eggs of the *Tetraphyllidea*, and for this reason one cannot say whether the fact that the embryophore in *T. ciliotheca* is ciliated, has any particular significance or not.

Woodland states that other typically Bothriocephalid features of *T. ciliotheca* are : (1) the shape of the anterior proglottides ; it is not stated what this character is, and the writer's experience is that the anterior segments are almost always featureless ; and (2) the ventral position of the genital apertures. It has already been pointed out that the uterus in many species of *Proteocephalidae* bursts to the exterior by a slit or a number of slits on the ventral surface, and it is not impossible that what Wedl called a genital pore was a uterine opening.

Referring to the *Proteocephalidae*, Woodland further writes ' for me the possession of lateral vitelline strands and of ventral uterine pores affords two very good reasons for relegating the family to the *Tetraphyllidea*.' It is common knowledge amongst all who have worked with worms of this order, that although in gravid segments the uterus sometimes bursts to the exterior by a slit or slits situated on the ventral surface, the presence of true uterine pores has only been established in about six species. Further, the vitelline glands are not in every case situated laterally.

Woodland's paper is useful in that his figures help one to realise pointedly the wide difference between the head of *T. ciliotheca* and those of the two other species which he considers so closely allied to it.

ERRATA

VOL. XIX. No. 3

Page 319. For 'recommended by a Committee of the Royal Institute of Public Health in 1903' read 'recommended by a Committee of the Royal Institute of Public Health in 1914.'

A NEW MEDIUM FOR THE DIFFERENTIATION OF *B. COLI* IN WATER ANALYSIS

BY

J. L. PAWAN

GOVERNMENT BACTERIOLOGIST, TRINIDAD, B.W.I.

(*Received for publication* 28 *July*, 1925)

During the course of an investigation into the bacteriology of the water supplies of the colony the repeated isolation of *B. coli* (lactose +, indol +) and of Houston's atypical *B. coli* from small quantities (1 c.c., ·1 c.c., and ·01 c.c.) of raw natural waters which were declared on sanitary survey to be free from all possibility of human faecal pollution and very remotely, if at all, liable to any animal contamination, centred attention on the sufficiency of the methods for the bacterioscopic examination of waters recommended by a Committee of the Royal Institute of Public Health in 1903, and adopted by bacteriologists in England and elsewhere as the standard method, and the interpretation of the results obtained by this method.

The literature of the bacteriology of tropical waters reveals frequent references to the presence of *B. coli* as detected by the standard method in waters which would be certified as pure and free from objectionable pollution by the comparative freedom from water-borne diseases among those drinking such waters, and by the results of sanitary surveys.

Daniels (1908), working on the waters from jungle streams of the Federated Malay States, observed that it was exceptional to find a jungle stream from which *B. coli* could not be isolated in 2 c.c., even though such waters would appear to be free from human and animal faecal pollution. Archibald (1910), commenting on the waters of the Soudan, remarked :

> ' If samples of water taken from shallow wells or rivers in the Tropics are subjected to a few simple tests for the presence of faecal contamination, the results will often show such a state of things that no analyst in England would ever consider the passing of such waters as fit for human consumption, and yet the water from those sources is used daily by both Europeans and natives alike without any ill effects to health as far as can be told. The question naturally arises whether in the face of these existing conditions one would be justified in using European standards of water purity as a guide or whether some modification of the European standard could be generally employed in tropical climes ? '

Wise and Minett (1912) isolated *B. coli* in from 1 to ·001 c.c. of raw waters from various sources used for drinking purposes by the inhabitants of British Guiana. Clemesha in India (1908-1912), struck by the high degree of 'faecal' (?) pollution in the drinking water supplies of the Madras Presidency as evidenced by the presence of *B. coli* isolated by the standard method, conducted a series of interesting experiments demonstrating the value of sunlight in the process of purification of the waters of India.

B. coli as defined by the standard method, and by Houston, embraces more than ten varieties of organisms, and though one or more varieties may be present in a water supply, the natural purification which that water undergoes from exposure to sunlight destroys those organisms which are objectionable or are evidence of objectionable pollution.

Hence the mere statement by a water analyst of the isolation of *B. coli* in a certain quantity of water is, as regards the Tropics, at all events, of comparatively little value from the sanitary point of view unless the effects of self-purification are taken into account. If, he says, the standard method be applied in its entirety to India, nearly every drop of drinking water in that country would be condemned.

In England the raw waters of the Thames investigated by Houston are waters to which sewage and other pollution gain constant access and *B. coli* in such waters would almost invariably be of faecal origin. Unfortunately the literature available locally does not record the result of quantitative examinations for the presence of *B. coli* in the raw natural waters of England, waters obtained from uninhabited regions and not exposed to human or animal faecal pollution.

Thresh, however, refers to a public water supply from the Welsh moorlands *in which no sewage contamination* was possible, but in which *B. coli* (lactose +, indol +) was present in 1 c.c. Such a water, he recommends, should be filtered before being delivered to the consumer. Does this example, though solitary, indicate that *B. coli* may perhaps be present in raw natural waters not exposed to human or animal pollution in temperate regions as is the case in the Tropics?

The standard method postulates that all *B. coli* are of faecal

origin without regard to the fact that such organisms are frequently found not only in small quantities of raw natural waters free from all faecal pollution, but also in *unpolluted soil*. From the soil free from faecal contamination these organisms may gain entrance to water supplies, and such supplies may be condemned by the standard method as polluted and unfit for human consumption. Chen and Rettger (1919) found 156 out of 467 (33·4 per cent.) coli-like organisms isolated from unpolluted soil to be lactose +, indol + organisms and Max Levine 37·3 per cent. out of 177 lactose fermenters of the soil to be also indol producers.

In Trinidad, of 120 cultures isolated from unpolluted soil by the standard method 42 per cent. were typical *B. coli* (lactose +, indol +) and if Houston's atypical *B. coli* be included, the percentage of *B. coli* regarded as an index of faecal pollution would be considerably higher.

If lactose +, indol + *B. coli* may be isolated by the standard method from soils to which no faecal matter has gained entrance, is a method which does not attempt to differentiate between faecal and non-faecal *B. coli* sufficient to justify an analyst in expressing an opinion upon the sanitary quality of a water, particularly if the sanitary control of that water is liable to variation ? At all events, should the water analyst not attempt to indicate the faecal or non-faecal origin of *B. coli* isolated from water ?

Keyes, Rogers, Clarke and others (1909-1914) showed by accurate determination of the gas volumes and gas ratios produced in the anaerobic fermentations of glucose that the non-spore-bearing lactose fermenters of faeces can be divided into two groups, one a low ratio or *B. coli* group in which the proportion of CO_2 to H_2 is almost constantly equal to 1·06 and the other a high ratio or B. aerogenes-cloacae group which produces considerably more CO_2 than H_2, with a wide range of ratio between these gases.

Clark and Lubs (1915) showed that in a carefully adjusted sugar medium the low ratio organisms produce a relatively high hydrogen ion concentration which can be recognised by an indicator, such as methyl red becoming red, whilst the high ratio organisms produce a low hydrogen ion concentration and methyl red becomes yellow.

In human faeces, according to Rogers, Clarke and Lubs, the low ratio group (methyl red +, Voges-Proskauer −) constitutes

74 per cent., and the high ratio group 26 per cent., of the lactose fermenters, whilst in bovine faeces the low ratio group constitutes 99·4 per cent., and the high ratio group ·6 per cent. (Rogers).

Chen and Rettger, in 1919, found all of 173 organisms from faeces to be methyl red positive and Voges-Proskauer negative.

In Trinidad, of 740 cultures isolated from human, bovine, and equine faeces, 94 per cent. were methyl red positive and all Voges-Proskauer negative.

On the other hand, Chen and Rettger found that of 467 coli-like organisms isolated from unpolluted soils, 430 belonged to the high ratio (methyl red —, V.-P. +) or B. aerogenes-cloacae group, and 20 to the low ratio *B. coli* group and, as stated above, 33·4 per cent. of these 467 were lactose +, indol +.

In Trinidad, of 120 cultures isolated from unpolluted soil, 85 per cent. were methyl red negative and 15 per cent. methyl red positive, and as previously pointed out 42 per cent. were lactose +, indol +.

But the gas ratio determination is not possible in the ordinary laboratory analysis of water, and whilst the methyl red and Voges-Proskauer tests have been found in the case of faeces and soil to indicate fairly accurately the habitat of the organism under investigation, their application in the bacteriological analysis of waters has been shown to afford no clue as to the source (faeces or soil) of the organism isolated from a water. Thus Winslow and Cohen found the percentage of methyl red positive, Voges-Proskauer negative organisms to be practically the same in polluted, unpolluted and stored raw waters. Out of 255 coli-like organisms, 76 per cent. from unpolluted, 77 per cent. from polluted and 85 per cent. from stored rain water were methyl red positive and V.-P. negative. Stewart Koser found 80·4 per cent. of the colon group cultures obtained from polluted waters and 73·3 per cent. from unpolluted waters to be methyl red positive and Voges-Proskauer negative.

In Trinidad, of 220 organisms isolated from polluted waters, 87·3 per cent. were methyl red positive, 6·3 per cent. methyl red negative, and 6·4 per cent. doubtful ; and of 240 cultures obtained from sanitarily pure waters 42·5 per cent. were methyl red positive and 57·5 per cent. negative. Though the American Public Health Association (1923 ed.) recommends the methyl red and Voges-Proskauer tests in the bacteriological examination of water, local

experience supports the conclusion of Winslow, Cohen, Stewart Koser and others that the lack of correlation between these tests and the sanitary qualities of waters justifies little reliance being placed upon them as indices of sanitary purity.

Stewart Koser (1923), in a study of the utilisation of salts of various organic acids, found that the two sections of the colon group of organisms could be clearly distinguished by the use of a chemically definite medium containing sodium, potassium or ammonium citrate as the only source of carbon. Such a synthetic medium can be made by dissolving 1·5 grammes microcosmic salt Na (NH_4) PO_4 + H_2O, 1 gramme KH_2 PO_4, 0·2 gramme $MgSO_4$ and 2 grammes sodium citrate in 1000 c.c. distilled water, tubing, and autoclaving at 120° C. for fifteen minutes. A clear colourless liquid is obtained. In this medium Stewart Koser found that 90·7 per cent. of *B. coli* isolated from faeces failed to develop, whilst the B. aerogenes-cloacae group produced a visible turbidity within forty-eight hours at 30° C. This differentiation correlated with the methyl red and Voges-Proskauer tests as far as the typical *B. coli* type and the aerogenes section in faeces are concerned.

With regard, however, to organisms isolated from the soil, he found that a number were consistently methyl red positive and Voges-Proskauer negative, although they had been obtained from soils regarded as free from pollution. When tested in the citrate medium these soil coli were found to utilise it. Of 72 cultures obtained from unpolluted soils, 97·2 per cent. utilised the citrate with the production of a visible turbidity and were distinct from faecal *B. coli*, whilst the methyl red test showed 51·4 per cent. alkaline to methyl red and the Voges-Proskauer 52·8 per cent. positive.

In Trinidad, of 432 cultures isolated from human, bovine, and equine faeces, 96·3 per cent. failed to develop in the citrate medium, while 3·7 per cent. did so; and in the case of unpolluted soils, of 214 cultures of the coli group, 90 per cent. utilised the citrate medium in forty-eight hours and 10 per cent. failed to do so. The citrate medium as a biological test is thus an accurate indicator of the habitat of the coli organism isolated from faeces and soil. In the application of the citrate medium for the differentiation of faecal and non-faecal *B. coli* obtained from waters, very striking results have been obtained. Samples of waters were secured from localities

in Trinidad where the chances of human intestinal pollution were impossible and contamination by birds and an occasional wild animal practically negligible ; by the standard method, *B. coli* were isolated and put through the citrate, methyl red and Voges-Proskauer tests. Of 240 cultures thus obtained, 81·3 per cent. grew in the citrate medium and 18·7 per cent. failed to do so; whilst of 210 *B. coli* isolated at various periods from polluted streams below villages 90·9 per cent. failed to utilise the citrate and 9·1 per cent. produced a distinct turbidity. The citrate utilisation by *B. coli* is thus seen to afford some degree of correlation with the sanitary survey of a water supply. Further, the *B. coli* colonies (lactose +, indol +) isolated from a certain quantity of water, say 1 c.c., by the standard method, may, by the citrate test, be shown to be of non-faecal origin and it is only in a larger quantity of water (5·10 or 25 c.c.) that faecal (citrate —) *B. coli* (lactose +, indol +) is found. Whilst, therefore, by the routine standard method a water may be condemned, by the use of the citrate test the bacteriological analysis of a water supply may be found to harmonise with epidemiological and sanitary conditions. To those, therefore, engaged in water analysis in the Tropics, particularly of those waters where that perfect sanitary control obtained by Sir Alexander Houston for the waters of the Metropolitan Water Board can only be an impossible vision, Stewart Koser's remarks should be of special interest. He says :

' the primary results shewn by the citrate medium indicate that this method of differentiation is deserving of further study with regard to its usefulness and application in the sanitary examination of water supplies, though the final acceptance of any such test must of course await general confirmation at the hands of different workers.'

Such a test is necessary. For is the relatively low incidence, in certain parts of the Tropics, of water-borne diseases, in contrast with the high degree of faecal pollution as evidenced by the presence of *B. coli*, detected by the standard method, due to the constant accidental absence of the specific pathogenic organisms or to the natural purification which waters in the Tropics undergo from exposure to sunlight in addition to the fact that by the standard method no attempt is made to differentiate between faecal and non-faecal *B. coli* ?

SUMMARY

1. *B. coli* (lactose +, indol +) may be isolated by the standard method not only from faeces and polluted waters, but also from unpolluted soils and unpolluted waters.

2. As a standard indicator of faecal contamination its value is not therefore unquestionable.

3. Local experience indicates that the utilisation of citrate by *B. coli* may be of value in differentiating faecal from non-faecal *B. coli* in water analysis.

UNPOLLUTED SOIL

	In Trinidad		In America (Chen and Rettger)		In America (Levine)	
		No. of Colonies studied		No. of Colonies studied		No. of Colonies studied
Lactose + Indol + *B. coli*	42 per cent.	214	33·4 per cent.	467	37·3 per cent.	177

CITRATE TEST IN AMERICA (STEWART KOSER)

	Growth in Citrate	No growth in Citrate	No. of Colonies studied
Faeces	9·3 per cent.	90·7 per cent.	118
Unpolluted Soil ...	97·2 per cent.	2·8 per cent.	72

CITRATE TEST IN TRINIDAD

	Growth in Citrate	No growth in Citrate	No. of Colonies studied
Faeces	3·7 per cent	96·3 per cent.	432
Polluted Water ...	9·1 per cent.	90·9 per cent.	210
Unpolluted Soil ...	90 per cent.	10 per cent.	214
Unpolluted Water ...	81·3 per cent.	18·7 per cent.	240

REFERENCES

Archibald, R. G. (1911). The Presence, Type, and Possible Significance of Lactose-fermenting Bacilli in Surface Waters. *4th Report Wellcome Tropical Research Laboratories*, pp. 319-334.

Clark, W. M., and Lubs, H. A. (1915). The Differentiation of Bacteria of the Colon Aerogenes Family by the Use of Indicators. *Journ. of Infectious Diseases*, Vol. XVII, pp. 160-173.

Clemesha, W. W. (1912). The Bacteriology of Surface Water Supplies in the Tropics. Calcutta.

Daniels, C. W. (1908). A Preliminary Report on Natural Waters and the Effects of the Method of Purification Adopted. *Studies from the Institute for Medical Research, Federated Malay States*, Vol. III, Part 2.

Keyes (1913). A Contribution to our Knowledge of the Gas Metabolism of Bacteria. *Journ. of Biol.*, Vol. XIII, p. 291.

Koser, S. A. (1924). The Correlation of the Citrate Utilisation by Members of the Colon Aerogenes Group with other Differential Characteristics and with Habitat. *Journ. of Bact.*, Vol. IX, p. 59.

Rogers, L. A., Clark, W. M., and Evans, A. C. (1914). The Characteristics of Bacteria of the Colon Type found in Bovine Faeces. *Journ. of Infectious Diseases*, Vol. XV, p. 100.

Thresh, J. C. (1913). The Examination of Waters and Water Supplies, p. 239.

Wise, K. S., and Minett, E. P. (1912). Rain as a Drinking-Water Supply in British Guiana. *Journ. London Sch. Trop. Med.*, Vol. II, Part i, pp. 74-88.

MALARIA INFECTION AS IT OCCURS IN LATE PREGNANCY; ITS RELATIONSHIP TO LABOUR AND EARLY INFANCY

BY
D. B. BLACKLOCK
AND
R. M. GORDON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication* 13 *July*, 1925)

PLATE VI

I. INTRODUCTION

In a previous paper (1925) we published an account of the malaria incidence in a series of twenty-six placentas of native women in Freetown. The investigation of placental malaria has been continued on all material available since then, so as to eliminate seasonable variations, and the records now cover a period of a complete year, i.e., from July, 1924, to July, 1925. Examinations have been made not only of films of the placental blood and of the peripheral blood of the mother at and about the time of labour, but also of cord and peripheral blood films of the children born of mothers with infected placentas. A small amount of material has been obtained from post mortem examination of children born dead or who died within a period of seven days. For purposes of comparison certain figures of the infection rate of the adult male population have been introduced.

The distribution of parasites in infected placentas has been studied with a view to discovering whether the whole placenta is equally infected or whether there is special concentration of the parasites in any particular areas.

Evidence of the transmission of parasites from the placenta to the child has again been sought. Although evidence of such transmission of parasites has never once been obtained throughout the whole series, yet there are certain facts which strongly suggest that the presence of malaria in the placenta is frequently associated with abnormal labour, that the death-rate among children born of mothers with infected placentas is unusually high, and that the

blood of the child is deleteriously affected by the parasitic invasion of the placenta. Little material from cases of abortion was available and what was obtained was not usually suitable for examination. It is not possible, therefore, to adduce any facts to show whether malaria is an important factor in the causation of abortion in Freetown or not. This is an aspect of the case which clearly requires attention, but until greater facilities for obtaining material are made it is not possible to advance much in this direction.

In our previous paper, we discussed the view held by some observers that new-born children of infected mothers possess a temporary and partial tolerance as regards malaria, so that a new-born child, although congenitally infected, does not present parasites in the peripheral blood at birth nor until after the lapse of a certain time. We argued that there was no direct evidence of the existence of such a partial tolerance on the part of the child, and that there was evidence against its existence at least in some cases, i.e., where authentic congenital malaria has been demonstrated. We noted, however, that of 41 children of one month or under, only one, a child between three and four weeks old, had parasites in the peripheral blood. In this present series it will again be seen that children under one month rarely show parasites in the peripheral circulation. This freedom from parasites in the peripheral blood may be due to freedom of the child from infection. If this is so, it may merely result from the fact that, for some unexplained reason, children up to a week or two old are little exposed to the bites of infected anophelines. On the other hand, it would be compatible with either a temporary general immunity, i.e., a condition during the existence of which the child is totally incapable of developing infection anywhere, or a condition of local immunity with partial tolerance, i.e., a condition in which the child is in fact infected, but in which the infection does not appear equally distributed throughout the body, but only in certain parts, of which the peripheral circulation is not one. That such local infections do occur in adult women, and, moreover, that they can very frequently exist without the production of obvious constitutional symptoms, we are able to prove conclusively from the present series.

We shall show that of 150 parturient native women, aged 15 to 42, examined during the period of twelve months, 55 proved to be

infected with *P. falciparum*. Of those infected, however, only 10 showed infection of both peripheral and placenta blood. In the remaining 45 only the placenta was infected.

It must be concluded, therefore, either that in these latter cases the parasites remain localised in the placenta, and never leave it, or else that if they do leave the placenta on their way to the peripheral blood via the vena cava, they are rapidly destroyed ; for it seems impossible to explain merely on the ground of dilution, the non-appearance of parasites in films from the peripheral circulation, when we consider that the placenta is a highly vascular organ, that it represents some $\frac{1}{120}$th of the total body weight and that of the maternal erythrocytes which it contains as many as 65 per cent. may be infected, as was shown by us previously (1925). See Plate VI, fig. 1.

It seems not only legitimate but necessary to believe that in pregnant native women infected with malaria, there are certain portions of the circulatory system which are immune from infection ; while at the same time, in the same individuals, other portions, far from being immune, exhibit massive infection, accompanied by active sporulation. If we admit a local immunity in the case of the mother, it must be admitted that a similar condition may exist in the child. Although in no case in a child born of a mother with placenta infected were parasites found either in the cord, peripheral blood, or in such organ smears as were available, we are not in a position to deny the possibility of malaria parasites establishing themselves in the internal organs of the child although not appearing in its peripheral blood. We can say, however, that such a condition, while it would be in accordance with the idea of the existence of local immunity in one portion of the child's circulation, namely, the peripheral blood, would equally imply the absence of such an immunity in another portion, namely, the umbilical cord. This question will be referred to again in discussing the age incidence of malaria infection in the children, and the fact that in a few cases of children born dead or who died immediately after birth, there was found in smears made from the internal organs, pigment which could not be distinguished from malaria pigment.

Before proceeding to a detailed account of the facts obtained, it may be noted as somewhat extraordinary that since the observa-

tions made on placental infection by the Greek observers Pezopoulos and Cardamatis (1907), little attention has been given to the discrepancy which appears to exist between the infection rate of males and females as judged by the peripheral blood rate of the former and the placental blood rate of the latter; nor, in our opinion, has sufficient attention been attached to this method of diagnosing malaria in the case of parturient women whose peripheral blood has yielded no evidence of it.

II. EXAMINATION OF THE PERIPHERAL BLOOD

A. Of mothers.

Thin film preparations of blood were made from the peripheral blood of 173 mothers at the time of labour; in addition to thin films, thick films were also examined in 71 of these cases. The number of cases in which malarial infection was diagnosed by examination of the peripheral blood was 12, of which 9 had parasites of *P. falciparum*, and 3 pigmented leucocytes only; it is noteworthy that in no case were gametes found, although in 5 of the 9 positive parasitic cases, one or more thick films were also examined.

Seasonal incidence of infection in the peripheral blood of mothers.

The number of mothers examined by films of the peripheral blood varied from 5 to 24 in a month; the total number examined and the approximate percentage found positive in each month are shown in Table I.

TABLE I.

Showing monthly total of mothers examined and percentage positive.

	Total	Percentage positive		Total	Percentage positive
July	8	25	January	17	6
August	5	20	February ...	5	0
September ...	10	10	March	7	0
October	16	0	April	27	7
November... ...	24	8	May	25	4
December ...	13	8	June	16	13

B. Of non-parturient women.

Of 43 women of the age of 16 and upwards, all, however, examined by means of thick film preparations from the peripheral blood, three had parasites. One of these was a *Plasmodium vivax* infection, the other two were *P. falciparum* infections, and in one of the latter crescents were present.

C. Of Adult males.

In a series of 150 males of the age of 16 and upwards, all examined by the thick film method, three had trophozoites of *P. falciparum* in the peripheral blood ; in no case were crescents found.

D. Of children.

Each one of a series of 809 children of all ages up to two years and a half was examined, on its first appearance, by thin film preparations of the peripheral blood ; an additional examination was made at the same time in the case of 100 of these children by the thick film method. Of the 809 children, 169, i.e., 20.9 per cent. had parasites in the peripheral blood ; *P. falciparum* occurred alone in 149 cases, *P. malariae* alone in 12 cases, and *P. vivax* alone in 2 cases ; mixed infection of *P. falciparum* and *P. vivax* occurred in 2 cases, of *P. falciparum* and *P. malariae* in 2 cases, and of *P. falciparum*, *P. malariae*, and *P. vivax* in 1 case. One case diagnosed by pigment alone cannot be classified. *P. falciparum* infection was found therefore in 19.0 per cent. of the 809 cases examined. Crescents were present in 23 cases, i.e., 14.9 per cent. of the 154 *P. falciparum* cases; this percentage is low, as in cases in which trophozoites were found at once, the examination was not continued to the time limit prearranged.

Seasonal incidence of malaria in the peripheral blood of 809 children up to 2½ years.

The monthly total number of new children examined by films of the peripheral blood varied from 36 to 104 ; the total examined and the percentage found positive in each month are shown in Table II.

TABLE II.

Showing monthly total of new cases examined and percentage positive.

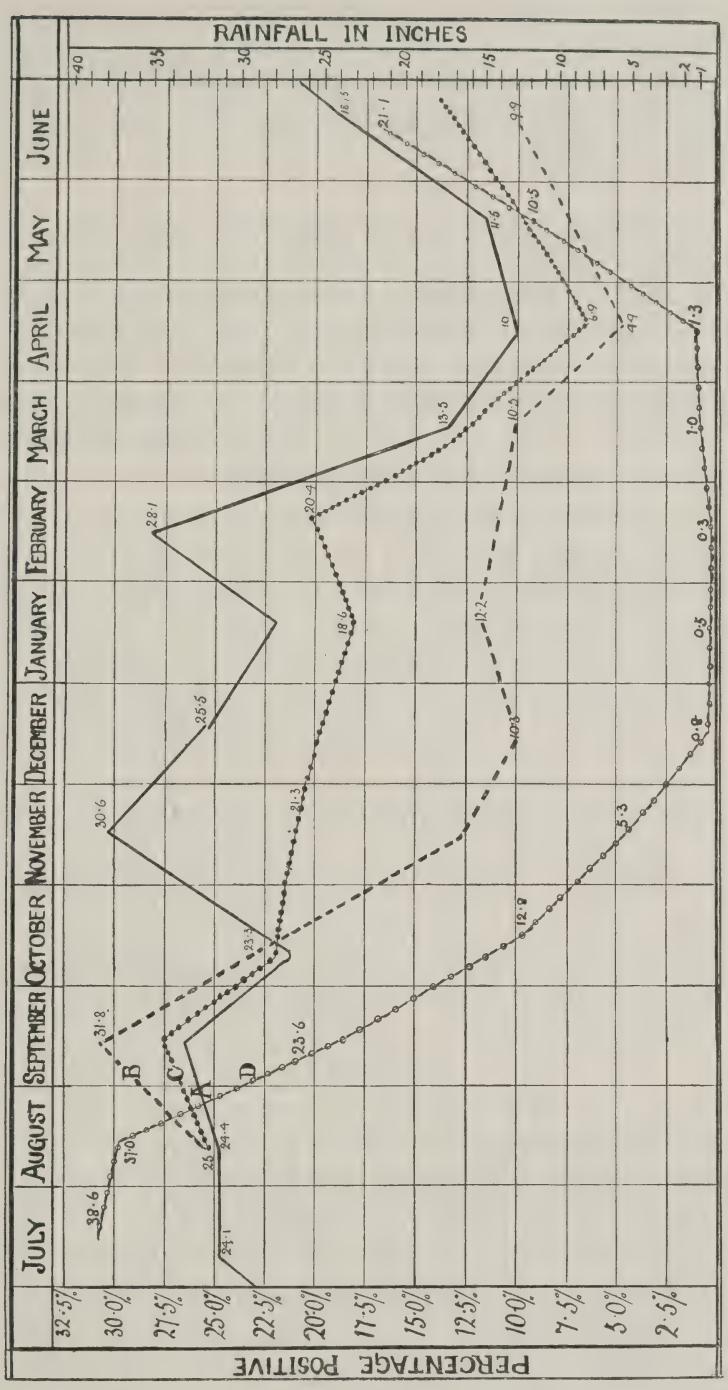
Month ...	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June
Total ...	62	78	93	60	36	51	77	57	104	40	87	64
Per Cent. positive...	24·1	24·4	26·8	21·7	30·6	25·5	22·1	28·1	13·5	10·0	17·2	18·7

Each case on its first appearance is classified here as new; on any subsequent appearance, therefore, it is classified as old, and the parasitic findings require separate record, as is shewn in the graph given below.

The number found positive among new cases in each month is expressed in Graph I A as a percentage of the total new cases appearing for examination for that month. The number of old cases found positive for the first time in each month is expressed in Graph I B as a percentage of the corrected total of old cases seen in that month. The corrected total of old cases is arrived at by excluding all cases which had previously been found positive. The numbers found positive in the above two categories are added together and expressed in Graph I C as a percentage of the total cases, i.e., new cases plus corrected old cases, appearing for examination in each month. The rainfall in inches in an average year is shown in Graph I D.

Table II and Graph I A, which give the same information, represent the results of a single examination; Graph I B represents the results of at least two, and it may be numerous examinations and includes all cases which, negative on first examination, proved positive at a later time; the resultant curve of the summation of the positives in A and B shown in Graph I C gives a more accurate impression of the seasonal incidence of malaria in the children than does A alone or B alone.

It is seen that the form of curve C which we regard as yielding the most reliable information on the question of seasonal incidence in children, presents a fairly definite relationship to the rainfall curve. The relationship is such as to show that soon after the commencement of the rains the malaria incidence rises. If we compare the peripheral



GRAPH I.

Showing seasonal incidence of Malaria and average rainfall in inches.

blood of children with the placentas of mothers (Section IV, Table V) we see that in the latter a rise occurs antecedent to the rains and before the rise in the children's infection. This suggests that the placental rise represents a seasonal relapse, while that of the children represents a seasonal infection.

III. EXAMINATION OF BLOOD FROM THE UMBILICAL CORD

Thin films of blood from a vessel in the umbilical cord were examined in 162 umbilical cords. In no cord was malaria infection found, in spite of the fact mentioned below that the examination of 155 of the placentas belonging to the cords revealed 59, i.e., 38 per cent. positive ; further, in spite of careful search, no pigmented leucocyte was ever found in the cord blood.

Maternal leucocytes in the foetal circulation.

It is stated that leucocytes can penetrate from the maternal into the foetal circulation ; this statement is based upon the relative numbers of leucocytes found in the umbilical arteries and vein. It has been found that the umbilical vein blood contains per volume a greater number of leucocytes than the umbilical arterial blood. This passage of leucocytes presumably occurs through the walls of the villi and is a matter which requires further investigation where placental infection with malaria occurs. It may not be justifiable to argue that where leucocytes can pass through, erythrocytes can also pass through ; still less is it justifiable to assume that infected erythrocytes can pass ; for it was observed by Marchiafava and Bignami (1894) that in capillary apoplexies almost all the extravasated red blood-corpuscles were without parasites, while the cerebral vessels contained immense numbers of red blood-corpuscles having parasites in them. It is, however, important to note that in these heavily infected placentas it is by no means rare to find leucocytes which contain within them parasites. These parasites may be of any size up to forms which are segmented and appear ready to rupture. It is not possible to say whether leucocytes containing such parasites are capable of penetrating from the maternal to the foetal circulation, nor is it possible to say whether such parasites, if liberated in the child's circulation, could infect it. All that can be said is that certain of these parasites contained in leucocytes were undoubtedly alive at the time of examination as evidenced by their movements visible

by the dark ground illumination method. These parasites containing leucocytes are, comparatively speaking, rare, and it is unlikely that even if they penetrated into the child's circulation, they would come under observation. In a number of placentas examined the majority of the leucocytes contained pigment (see Plate VI, fig. 2).

If the difference noted by some observers between the total leucocytes present in the umbilical cord vein and arteries is, as stated by Gray (1923), due to the penetration of the maternal leucocytes into the foetal circulation, it is difficult to account for the fact that in spite of the most careful examination we failed to find such pigmented leucocytes in either the cord or the peripheral blood of the child.

In the endeavour to ascertain whether the condition of the arterial and venous cord blood, as regards the proportion of leucocytes present, is as stated, we have in one case made careful enumeration of the leucocytes present in films of each. The result showed that such difference as existed between the leucocyte content of the venous and the arterial cord blood was negligible, but showed a slight preponderance of leucocytes in the arterial blood, in the proportion of arterial 187 and venous 170 to 50,000 erythrocytes.

IV. EXAMINATION OF BLOOD FROM THE PLACENTA

Of 155 placentas of native women examined for malaria 59 were found to be positive, that is 38.0 per cent.

The results of the different blood examinations obtained so far are set out in tabular form arranged according to the ascending percentage of parasitic positives.

Table III.

Showing the parasitic findings of various groups.

	Blood films of	162 Umbilical cords	150 Adult males (peripheral blood)	43 Non-parturient women (peripheral blood)	173 Mothers (peripheral blood)	809 Children (peripheral blood)	150 Mothers' placental blood	155 Placentas
Percentage having	Parasites ...	0·0	2·0	7	6·9	20·9	36·6	38·0
	Crescents ...	0·0	0·0	2·3	0·0	2·8	0·0	0·0

It is interesting to compare the above placental figures with those given in Table IV taken from Clark (1915), which shows the distribution of placental infection among different races in his series of 400 labours.

Table IV.

400 Routine cases of labour.

Race of the women examined	No. examined of each race	No. of positive identifications of malaria	Per cent. of positive cases
North Americans (white)	118	0	0·0
Latin Americans (mestizo) ...	92	3	3·26 +
Europeans (white)	17	1	5·88 +
West Indian Negroes	173	15	8·67 +
Total	400	19	4·75

It is seen that Clark's percentage of positive mothers is low as compared with ours, 4·75 per cent. as compared with 36·6 per cent. A partial explanation of this fact is obtainable from consideration of the groups forming his total. Thus the 118 North Americans (white) give a percentage infected figure of 0·0, whereas the 173 West Indian negroes give the highest figure a percentage infected of 8.67. The difference of incidence is attributed by Clark to the higher hygienic plane of the North Americans, to the greater exposure to infection of the West Indian Negroes on account of their residential surroundings and their much lower hygienic and economic standard. Even taking the figure for West Indian negresses alone, however, the infection rate, i.e., 8·67 per cent. does not approach that seen in West African native women in Freetown, i.e., 36.6 per cent. If placental findings are taken as a criterion of malarial infection, it appears that the West African native women in Freetown are more than four times as frequently infected as the West Indian negresses dealt with by Clark.

Seasonal incidence of infection of the placenta.

The number of mothers whose placenta was examined by blood films varied from 6 to 24 monthly during the year. The total

number examined and the percentage found positive in each month are shown in Table V.

TABLE V.

Showing monthly total of mothers examined and the percentage found positive.

	Total	Percentage positive		Total	Percentage positive
July...	8	62	January	18	39
August	6	33	February	7	43
September	12	42	March	11	18
October	16	19	April	11	18
November	24	38	May	15	53
December	13	31	June	9	56

Type of infection in the placental blood.

Without exception all the cases found positive in the placenta were infected with *P. falciparum ;* in one case a few parasites which resembled quartan were also found. In spite of prolonged examination of the placental films, no crescents were ever found in the placental blood. In writing the account of our first series of twenty-six placentas we drew attention to the absence of crescents in the placental blood, and also in the peripheral blood of such cases as showed infection there. We have obtained no evidence from this larger series that crescents are being formed elsewhere in these infected individuals, as no crescents have been found in any of them in the peripheral blood during, or immediately after, labour. If post-mortem material had been available it might have been possible to determine whether any crescents were present in internal organs. Blacklock (1921) produced evidence from a case of indigenous infection with *P. falciparum* in England that the bone marrow was the most suitable site for the development of crescents, a site which had previously been stated to be favourable by Marchiafava and Bignami and various other observers.

These cases, then, although the infection in the placenta is often very intense, are not producing crescents in this site. Nor does it appear probable that the parasites are migrating from the placenta

to develop elsewhere into crescents, because we do not find crescents in the peripheral blood. The failure of sexual forms to reach the peripheral blood must inevitably result in failure of the parasite to complete its development even when susceptible anophelines bite such women. The rare possibility of parasites of the schizogony cycle being transmitted congenitally to the child must be taken into consideration ; this would doubtless result in the formation later of gametes in the child, and so in a circuitous manner the stages infective for the mosquito would become available. In the meantime we have the fact, proved by abundant evidence, that the mere proliferation of *P. falciparum* on a colossal scale in one organ at least of native adult women does not result in the production of crescents in that organ, nor does it result in their appearance in the peripheral blood.

The anatomy and circulation of the placenta.

Before discussing the distribution of malaria parasites in the placenta, it is necessary to make same reference to the placental circulation. According to the descriptions and diagrammatic representations of the placenta and its circulation contained in many text books of anatomy, the condition is somewhat as shown in the left hand side of diagram No. 1. The arteries and vein of the child are carried from the umbilical cord, and pass subjacent to the amnion into the chorion ; the vessels are carried into the villous processes of the chorion ; the villi lie in the intervillous space and are bathed in maternal blood. As, however, the villi are covered by two layers of trophoblast, the cytotrophoblast layer next the chorionic process and the syncytiotrophoblast layer in contact with the maternal blood, the latter does not come directly in contact with the foetal blood vessels.

The maternal blood gains access to and leaves the intervillous space by arteries and veins which pass through the stratum spongiosum and the basal plate which represents the remains of the stratum compactum. The arteries as they enter the basal plate lose their muscular coat and they and the veins after this point consist of sinuous channels lined only by endothelium ; these channels open into the intervillous space, and at this point they lose their endothelial covering. The intervillous space is lined throughout by the syncytiotrophoblast layer. Therefore the walls of the

intervillous space and the villi which project into it are covered by the same lining structure.

The right-hand half of the figure represents the separated placenta ; according to Gray and others this separation occurs through the stratum spongiosum. On the right half of the diagram have been shown the areas in which infected and uninfected red blood

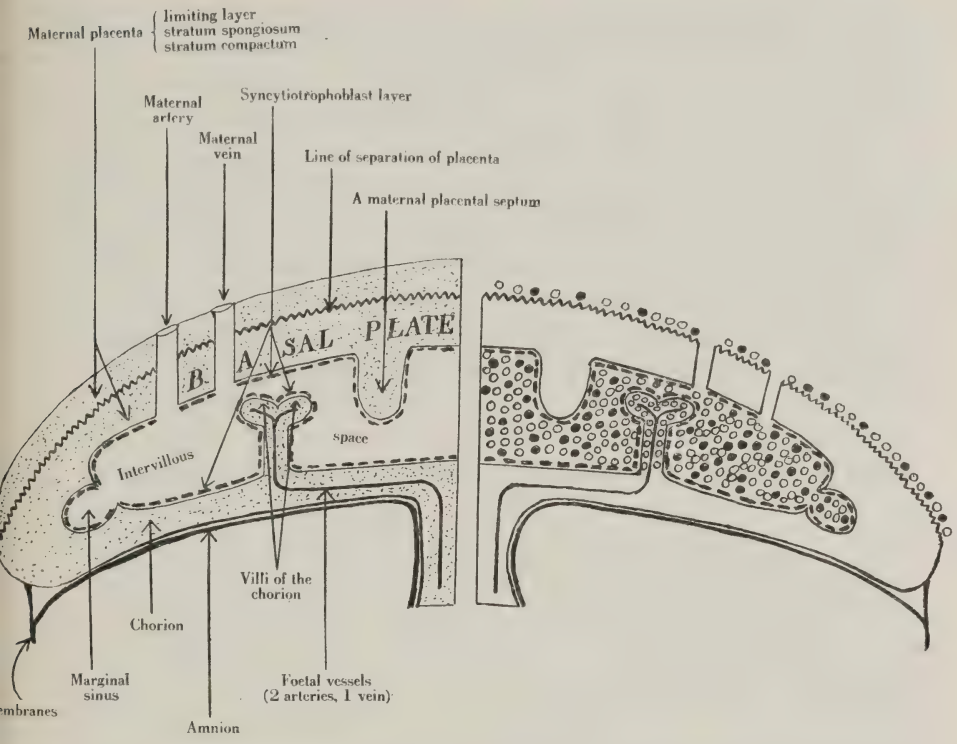


Fig. 1. Diagram of placental circulation (modified from Gray's anatomy). The left-hand figure represents the placenta before birth and the right-hand figure the same after birth. In the right-hand figure infected red cells are marked ● and uninfected red cells marked ○.

cells were found. The methods used were applied not only to the central portions of the placenta but also to the margin, and were :—

(1) The maternal surface of the placenta was carefully washed with normal saline solution ; smears were made from the surface, and also from blood obtained by scraping very lightly with a razor blade ; finally the thinnest possible slice of tissue was taken from the surface and from this thin slice films were spread.

(2) Films of blood from the placenta were examined at various depths from the maternal towards the foetal surface.

(3) The amnion was carefully washed with the normal saline solution and then reflected ; from the surface thus exposed smears were made and then, as in the case of the maternal surface, a thin portion of the tissue was snipped off and smears made from this thin portion.

(4) Wedge-shaped portions of tissue from the margin of the placenta and cylindrical portions from the centre were taken in such a way as to include both the maternal and foetal surfaces and the intervening tissue ; these were embedded and sectioned.

The examination showed that parasites were present in every preparation so made from infected placentas ; some variation occurred as shown in the attached table, in the proportion of infected erythrocytes present in blood films made at different parts of the placenta, from the tissue snipped from the maternal and foetal surface, and from the placental tissue at a point midway between these surfaces ; the results of such examinations are given in Table VI.

TABLE VI.

Showing percentage of erythrocytes infected at different parts and depths of the malarial placenta.

Blood films from	Edge of placenta	Centre of placenta
Tissue snipped from maternal surface ...	11·0	18·2
Tissue midway between maternal and foetal surfaces	9·8	36·2
Tissue snipped from foetal surface subjacent to amnion	8·2	9·4

As is seen in the table the centre of the placenta has a larger proportion of erythrocytes infected than the edge of the placenta ; further, the portion situated midway between the maternal and foetal surfaces in the central portion is the most heavily infected of all.

We were unable to account for this unequal distribution in any way except on the assumption that infected cells tend to accumulate here while uninfected cells pass on. If this area presented a more suitable medium in which the parasites could complete sporulation,

we might expect to find a greater proportion of sporulating forms of parasite in the infected cells of this area. We enumerated the sporulating forms found in each area with the result that in all areas the percentage of sporulating forms was found to be approximately the same ; for example, in one case where 200 parasites were counted in each area by each observer, the percentage of sporulating forms in each area was approximately 14.

Consideration of the distribution of parasites in relation to the anatomy of the placenta.

A point which early attracted our attention was that although the peripheral blood of the mother was free from parasites not only at the time of labour, but also in individual cases which were followed for a month after labour, yet parasites could be found on the maternal surface of the placenta in large numbers. In sections of the placenta thin walled sinuses are found near the maternal surface, some of which, presumably maternal arteries, are free from parasites, while others, presumably maternal veins, contain numerous parasites. Assuming that the condition found after the placenta is delivered were in existence before separation of the placenta, it is difficult to avoid the conclusion that parasites must be present in the maternal veins of the placenta in large numbers. Possibly this is so, and the failure to find these parasites in the peripheral blood may be due solely to the peripheral immunity which we have already postulated.

We believe, however, that the distribution of the parasites on the maternal surface of the placenta found after delivery may not represent the distribution as it occurs in the placenta before separation. The placenta is comparable to a flat sponge on one surface of which is the relatively thick covering membrane composed of chorion internally, and amnion externally, while on the other is the much thinner membrane composed of the remains of the stratum spongiosum externally and the basal plate internally ; internal to these is the lining of trophoblast. The intervillous space with the villi projecting into it occupies the whole area between the maternal and foetal internal surfaces.

The intervillous space everywhere extends up to the maternal surface, as well as to the foetal surface, at the margin as well as in the centre of the placenta. Consequently infected erythrocytes

contained in the intervillous space lie right against the maternal and foetal lining of syncytiotrophoblast. The processes given off into the space from the foetal surface, namely the chorionic villi, have a counterpart in the more scanty septal processes into the space from the maternal basal plate. All these processes are covered

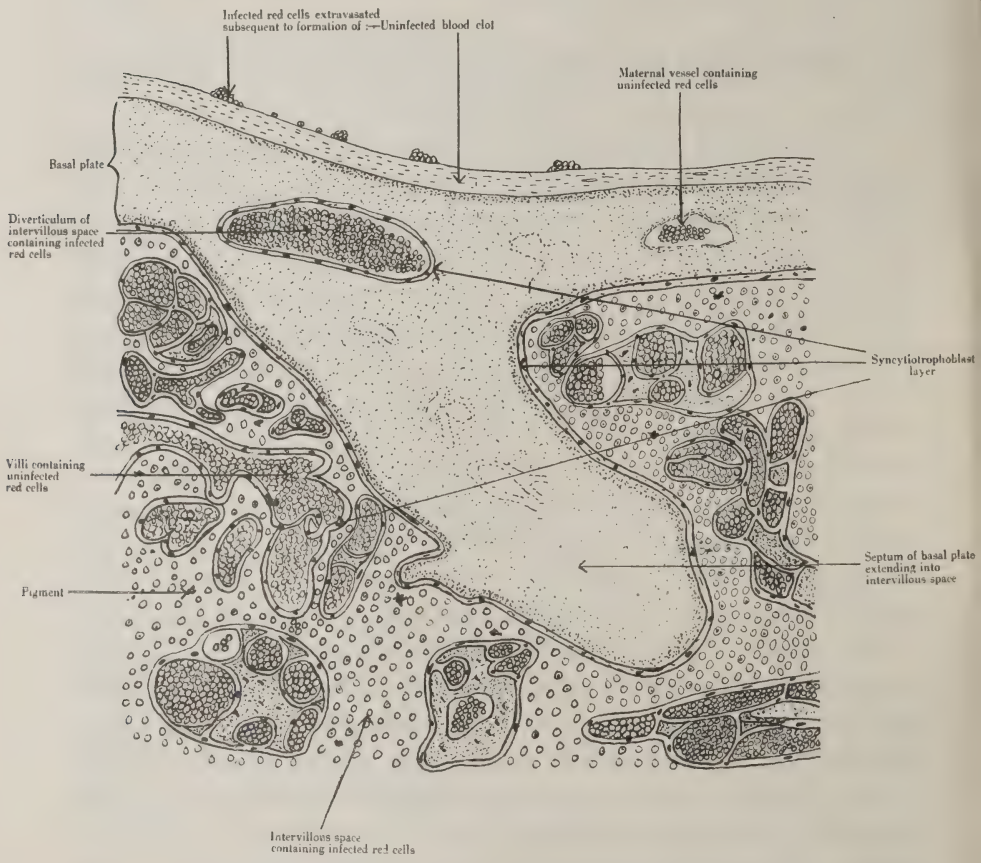


FIG. 2. Section through material surface of placenta [semidiagrammatic]. (Zeiss. Oc. 4. Obj. ½-inch.)

by syncytiotrophoblast and all are bathed in infected blood of the mother. At the origin of these processes are seen what at first appear to be areas of infected cells included in the chorion and basal plate. These are diverticula of the intervillous space cut across and are seen not only to contain infected erythrocytes

ERRATUM

Page 342, Fig. 2. For 'through material' read 'through maternal.'

but also to be lined by syncytiotrophoblast and in some cases to contain a portion of uninfected villous process.

This arrangement in itself would suffice to explain the fact that films made from even the thinnest slice of tissue from the maternal surface or from the foetal surface after reflection of the amnion contain numerous parasites. The presence of infected erythrocytes on the uncut maternal surface of the placenta as seen after its delivery is probably brought about therefore by the aid of two factors, both of which are dependent upon mechanical compression by the uterus upon the placenta after expulsion of the child and separation of the placenta has begun. If the cord has been tied on the maternal side the uterus is contracting upon a mass of tense villi, out of which the blood cannot be expressed through the cord. As the uterine contractions continue and increase, and as the vessels of the villi remain rigidly distended with blood, diminution in volume of the placenta takes place in two ways following the line of least resistance. In the first place the maternal blood lying in the intervillous space is forced back through the remnants of the maternal arteries and veins and emerges on the maternal surface of the placenta. On further pressure the intervillous space ruptures where the membrane is thinnest, that is, through the diverticula on the maternal side, and liberates on to the surface its contained parasites.

We are inclined to believe that in normal circumstances parasites do not extend beyond the limits of the syncytiotrophoblast layer which lines the intervillous space and which invests all processes into it, whether villi of the chorion or septa from the basal plate. Parasites, indeed, as we have shown, may always be found close to the maternal and foetal surfaces and often penetrate into both, but in the latter case they are normally lying in sinuous prolongations of the intervillous space and are still contained within the limiting syncytiotrophoblast layer. We have noted that parasites have never been seen by us in the villi themselves.

Rupture of the diverticula of the intervillous space appears to be attributable primarily to ligation of the cord on the placental side. If the placental side of the cord were not tied there might still be backward oozing from the maternal vessels, but it is clear that this leakage and the leakage consequent upon rupture of the intervillous space diverticula are very much accentuated by the fact that ligation

of the cord keeps the villi which comprise a large proportion of the total volume of the placenta not only engorged with blood but practically incompressible. In some cases it is possible to see in section that the villi themselves are ruptured although this is relatively rare;*

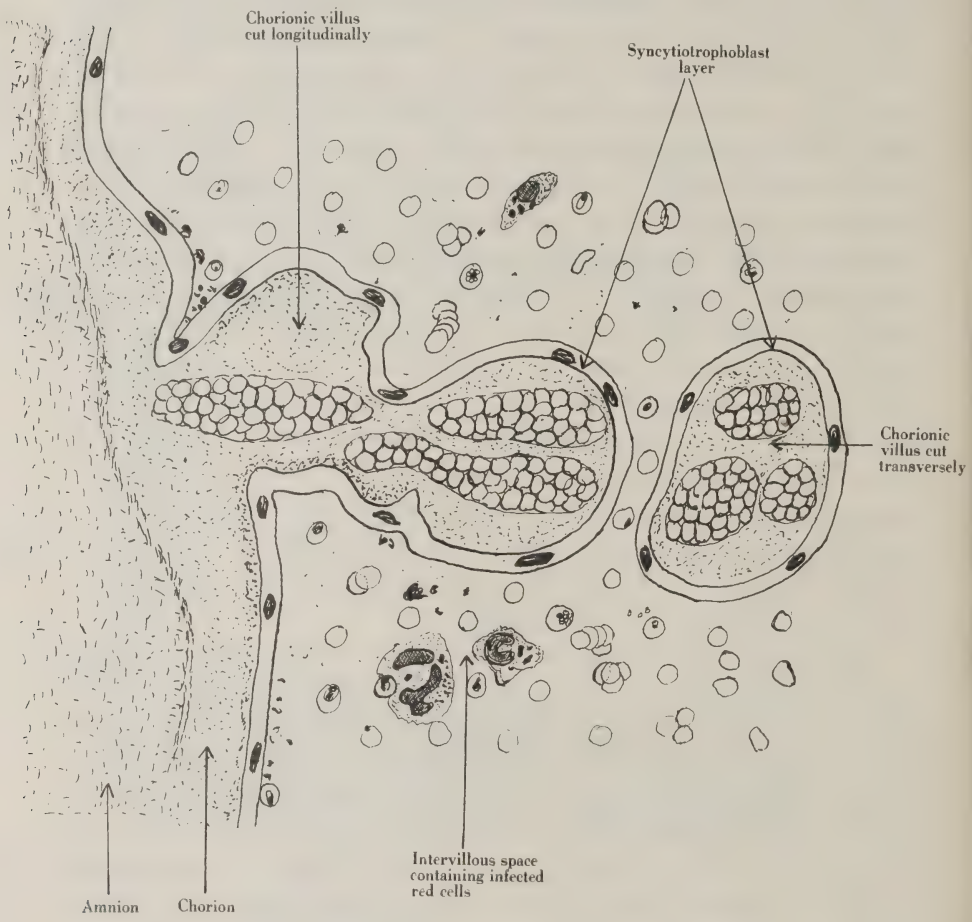


FIG. 3. Section through foetal surface of placenta, chorion and chorionic villi covered by syncytiotrophoblast layer [semidiagrammatic]. (Leitz. Oc. o. Obj. $\frac{1}{12}$. Draw tube down.)

separation and rupture of the syncytiotrophoblast layer over the villus has been observed occasionally and, still more rarely, escape of uninfected red blood cells from the villus.

* Since the above was written, one of us (R.M.G.), has had the opportunity of comparing the sections of malaria-infected placentas with sections of a large number of normal placentas at the Coombe Hospital, Dublin; this comparison showed clearly that the vessels in the villi of infected placentas are greatly dilated, in some cases to such an extent that the chorion, which normally forms the main bulk of the villus, is almost obliterated. Presumably such villi are more liable to rupture. This condition is well shown in several of the villi in Fig. 2.

If the cord were not tied it is conceivable that such rupture of villi might result in infection of the child via the cord vein ; but seeing that rupture is such a rare occurrence even when the cord is tied on the placental side, that is to say, when we have shown the conditions are most favourable to injury to the villi, it seems highly improbable that rupture of the villi could occur unless the cord is tied.

If the cord is tied on the child's side and then cut, leaving the maternal end free to bleed, it is extremely unlikely that any villi could be ruptured. In any case even if they did rupture the infection in these circumstances would obviously not affect the child via the cord. Even when the end of the cord is tied the risk of rupture of villi is extremely small, and here again the child is not exposed to risk via the cord.

There is one aspect of the question, however, which, although it is in the nature of a side issue from our investigation, appears to deserve mention. The facts here brought out have a distinct application to a controversy which has frequently arisen as to the merits or demerits of tying the placental end of the cord. The most recent exponent of the view that such ligation of the cord is injurious, is Vaughan (1925). The procedure is condemned on the ground that ligation of the cord places the uterus at a disadvantage in its efforts to contract.

We have had in this series of malaria infected placentas a unique opportunity of studying this question, and have had a method of distinguishing sharply between the foetal and maternal bloods from the circumstance that the maternal blood contained such a high proportion of infected erythrocytes in many of the cases. The information obtained by us by means of the study of malaria placentas in which the cord has been tied on the placental side enables us to bring forward new evidence to support that school which claims that the uterus when contracting on villi with cord tied is contracting on a mass which for all practical purposes is incompressible. We are, however, far from saying that we believe that this is injurious to the uterus, or that it has any deleterious effect upon its power of expelling the placenta, or upon its own final involution. These matters are outside the scope of this investigation, but we believe that what we have described may stimulate their study on the part of those whose special province it is to deal with pregnancy and the puerperium.

V. CRESCENTS

It has long been recognised that crescents appear relatively rarely in the peripheral blood of adult natives in endemic areas. Recently, Christophers (1924) has confirmed Schüffner's view that crescent formation is not associated with immunization, but that on the contrary, crescent formation is reduced during the process. The negative findings as regards crescents in the peripheral blood and placentas in this series would be in accordance with the idea that the majority of these cases were so far immunized as to prevent the appearance of parasites in the peripheral blood, and that all were so far immunized as to prevent the appearance of crescents either in the peripheral blood or in the placenta. It is of interest in this connection to note that Horowitz-Wlassowa (1924), while noting a specific antibody present in most cases of malaria, has failed to demonstrate its presence in those cases which show many schizonts or gametocytes in the cutaneous blood.

In writing of the peripheral blood of children in endemic areas, Christophers says ' Crescents here are therefore associated with the higher values of parasites, and hence one may judge with the period of acute infestation rather than with that of immune infestation.'

The absence of crescents in the placentas of the native women discussed is clearly not due to lack of parasite proliferation ; it may be that the placenta is in all cases a site in which for some reason crescent formation does not occur ; as suggested in our previous paper, this might be due to some intrinsic and yet unknown character of this organ which renders it unfavourable to the development of mature sexual forms. On the other hand, it is possible that the failure of crescents to develop in the placenta is an indication of a certain degree of immunity having been reached. If this is so, it would indicate that we have in the placental absence of crescents an early sign of the development of immunity. We are then faced with a complex arrangement ; the patient is infected with *P. falciparum ;* there exists a degree of immunity which prevents the development of crescents in the placenta as shown by direct examination of this organ, and probably in other organs as shown by the failure to find crescents in the peripheral blood ; this anti-crescent immunity, then, would appear to be of a general nature affecting the whole circulatory system. Quite a different picture is presented when we examine the immunity against asexual parasites. While the

peripheral circulation appears to be immune with regard to them the placental circulation, far from being immune, offers a most suitable soil for their development on an immense scale.

It is important, first of all, to decide whether the absence of crescent formation which has throughout our series characterised the placental blood is due to an inherent character of all placentas. This could be done by examining the placentas of women who, although infected with malaria, have not resided sufficiently long in an endemic area to acquire any degree of immunity. This investigation could be carried out readily in Europe in the case of pregnant women who return after a short first residence in an endemic area, and who are infected with malaria. If it should prove that such women readily develop crescents in the placenta, it would be necessary to examine also natives of endemic areas who had gone to Europe, and who had infection of the placenta. If the latter cases still showed no crescent formation in the placenta, it would be reasonable to draw two conclusions, firstly, that the absence of crescent formation in the placenta in this series was not due to an inherent character of the placenta, and secondly, that it was due to an acquired immunity. The value of such an examination lies in the fact that if it can be shown that acquired immunity is the cause of crescent non-production, we have in the placenta an accessible internal organ which possesses very obvious advantages for the study of malaria immunity. In view of the difficulty of obtaining material here for the purpose of this study in non-immunized persons, this part of the work must be undertaken elsewhere.

It is frequently stated that crescents are produced more readily after quinine administration. Several of our series of cases had received quinine before labour, some of them for as long a period as ten days, yet in no case, as we have shown, were crescents found.

VI. PATHOLOGICAL EFFECTS OF PLACENTAL INFECTION WITH *P. FALCIPARUM* ON MOTHER OR CHILD

Before discussing any effects which might be attributed to malaria infection, we give an account of the material and examination upon which our conclusions are based. In spite of increasing efforts to obtain permission to examine material post mortem, we have still a vast amount of prejudice to overcome ; this can only be done by gradual education and awakening the interest of those most nearly

concerned, namely, the natives themselves. Our records of post mortem examinations are consequently rather meagre.

The following are the material examined and the methods adopted ; in all cases Leishman's or Giemsa's stain was used.

1. *Maternal peripheral blood.* Thin and thick films were examined from the blood of the ear, at the time of labour.

2. *Placenta.* This was usually examined within 6 hours after labour ; exceptionally as much as 24 hours elapsed before examination was possible. The surface of the placenta was washed and cauterised ; an incision was made through the cauterised area and blood from the bottom of the incision was taken up in a pipette and used for spreading films.

3. *Umbilical cord.* The cord after being washed and cauterised was cut in two places, one as near as possible to the placenta and the other about six inches from the placenta ; films were spread from the blood in the vessels.

4. *Peripheral blood of living children.* Immediately after the birth of the child, thick and thin films were made from the peripheral blood.

5. *Partial examinations of cadaver of dead born children.* In most cases it was impossible to obtain permission for a complete examination ; puncture of certain of the organs by means of a needle was permitted in some cases, in addition to examination of the peripheral blood.

6. *Complete examination of cadaver of dead born children.* Where permission could be obtained, a complete examination was made. This comprised smears of peripheral blood, liver, spleen, kidneys, bone marrow, lungs and other organs.

7. *Examination of cadavers of children who died within seven days.* The examinations carried out in these cases were of the same kind as those in number 5 and 6.

Material from post mortem examination of the children was preserved and material from the placentas was fixed, embedded in paraffin and sectioned.

A. Effect on the mother before and after labour.

In Table VII given below, the main facts concerning the 55 infected mothers and their children are set out, with special reference to the fate of the child.

TABLE VII.

Showing clinical history and peripheral blood findings in 55 mothers infected in the placenta with *P. falciparum*; also the fate of their children.

Case No.	Age	Peripheral blood of mother	Temperature 3 days before or after delivery	Quinine, total amount given before delivery	Child born alive	Child lived 7 days
1	20	+	105° F.	25 gr.	Yes	Yes
3	24	o	100° F.	o	Yes	Yes
4	21	o	105° F.	o	Yes	Yes
5	35	+	100° F.	o	Yes	Yes
6	36	...	100° F.	o	Yes	No
9	18	+	N	o	Yes	Yes
13	18	o	105° F.	30 gr.	Yes	No
16	22	+	103° F.	36 gr.	Yes	Yes
17	36	...	103° F.	5 gr.	No	...
19	30	...	...	o	Yes	Yes
20	26	o	N	...	Yes	Yes
23	22	o	102° F.	o	Yes	Yes
34	37	o	...	...	Yes	Yes
35	22	o	...	...	Yes	Yes
38	20	o	...	...	Yes	Yes
45	36	o	N	o	Yes (2)	No (2)
51	21	o	100° F.	o	Yes	Yes

TABLE VII—*Continued*

Case No.	Age	Peripheral blood of mother	Temperature 3 days before or after delivery	Quinine, total amount given before delivery	Child born alive	Child lived 7 days
52	22	o	N	...	Yes (1) No (1)	Yes ...
53	15	o	N	o	Yes	Yes
58	28	o	100° F.	o	Yes	No
59	22	+	N	o	Yes	Yes
63	29	+	N	o	Yes	Yes
64	24	o	100° F.	o	Yes (1) Yes (1)	Yes (1) No (1)
67	26	o	N	o	Yes	Yes
69	24	o	N	o	Yes	Yes
71	24	+	N	o	Yes	No
75	25	o	N	o	Yes	Yes
81	23	o	100° F.	o	Yes	Yes
86	21	o	N	o	Yes	Yes
92	22	o	...	...	No	...
93	23	o	N	o	Yes	Yes
94	20	o	100° F.	o	Yes	Yes
95	32	o	N	o	No	...
96	36	+	...	o	Yes	Yes
98	20	o	N	50 gr.	Yes	Yes
101	24	...	N	o	Yes	Yes

TABLE VII—*Continued*

Case No.	Age	Peripheral blood of mother	Temperature 3 days before or after delivery	Quinine, total amount given before delivery	Child born alive	Child lived 7 days
102	26	o	N	o	No	...
103	28	o	N	o	Yes	Yes
108	26	o	101·5° F.	o	Yes	No
110	28	...	N	o	No	...
118	30	...	102·5° F.	o	Yes	Yes
128	28	o	N	o	Yes (1) Yes (1)	No (1) No (1)
134	16	o	N	o	Yes	No
136	17	o	103·8° F.	o	Yes	Yes
137	18	o	101° F.	o	Yes	Yes
138	33	o	101° F.	o	Yes	No
140	26	o	104° F.	o	Yes (1) No (1)	No ...
142	20	o	N	o	Yes	Yes
143	22	o	N	o	Yes	Yes
146	34	+	100° F.	o	Yes	Yes
147	26	o	N	30 grs.	Yes	Yes
150	24	o	103° F.	o	No	...
151	25	o	N	10 grs.	Yes	Yes
153	17	+	102° F.	15 grs.	Yes	Yes
155	21	o	...	...	No (1) No (1)	... ...

As is shown in the table 23, that is, nearly fifty per cent. of the infected mothers had fever, and three cases in which fever is not recorded received 50, 30 and 10 grains of quinine. It will be observed that in two cases in which both peripheral and placental blood was infected, namely, cases 59 and 71, there was no fever ; these cases are of special interest as each presented a massive infection of the placenta. It is important to note that certain cases in which malaria was suspected to exist received quinine in varying amounts for different periods before labour without the placenta being cleared of parasites.* Conversely there are seven cases not shown in this table which were diagnosed on clinical grounds as malaria and received quinine in varying doses, and in which the placenta did not contain parasites at the time of birth. Although we know that quinine in doses quoted has failed to eradicate parasites from the placenta in the cases mentioned in the table, we cannot therefore justifiably assume that similar doses will fail in all cases. Some or all of these seven cases, had they not been treated, might have proved infected by placental examination.

In view of the number of cases who received quinine before labour and who still had infection in the placenta, it is possible that the doses were administered too late in pregnancy and in too small quantity owing to the fact that these cases only enter hospital when labour is imminent. It is known (Forchheimer (1915)), that quinine administered to the mother is excreted in the urine of the child. There were three deaths among the 150 mothers of whom the placenta was examined. None of the three who died showed malaria either in placenta nor in the peripheral blood. With regard to the post-partum history of the mothers, we have practically no information, as these cases make a very brief stay in hospital, seldom more than a week. It is therefore not possible to state whether these cases have recrudescences of malaria after leaving hospital. So far we have not had any opportunity of examining the placenta of a woman who at her previous labour had been proved to have an infected placenta.

That infected red blood cells are left behind in the uterus in large numbers when the infected placenta is born there is no doubt, for

* Since the above was written, we have had the opportunity of observing a case whose peripheral blood showed malignant tertian parasites a week before labour, and who subsequently received quinine grains 20 for six consecutive days. The placenta of this case showed a few parasites and many pigmented leucocytes.

we have already shown that parasites abound on the maternal surface of the infected placenta when born. We have also observed parasites in the blood which escapes during delivery of the infected placenta. In the process of delivery of such infected placentas this may be a source of danger where such blood is permitted to reach abrasions on the skin of the child or the attendant.

We must assume that after the expulsion of the main bulk of the parasites with the placenta, one of two events happens ; either the remaining parasites are prevented from entering the maternal circulation owing to closure of the uterine vessels, and they are then thrown out with the remains of the stratum spongiosum, or they are absorbed into the maternal circulation. In the former event the mother's peripheral and general circulation does not become infected from the placental source as a direct result of labour ; in the latter event the result would depend on two factors, i.e., the dose of parasites absorbed, and the degree of the immunity which exists in the peripheral and general circulation of the mother.

B. Effect on the children.

1. *Before birth.* A total number of 164 children were born of the 155 mothers, this figure includes premature children. The single births numbered 146, giving 146 children, and the twin births numbered 9, giving 18 children ; two was the maximum number produced at one birth. There were 148 children born alive and 16 born dead. In the case of children born alive, the only means at our disposal for ascertaining the transmission of malaria parasites or their products to the child *in utero* was the examination of the child's cord and peripheral bloods.

Of the total 148 children born alive 4 are omitted from consideration here because the placentas relating to them were not received or were in such a state of decomposition that they could not be examined satisfactorily. Of the remaining 144 there were 51, i.e., 35·4 per cent. who were born of mothers whose placenta was infected ; while 93, i.e., 64·6 per cent. were born of mothers whose placenta was not infected. Only a short examination period after birth was possible, rarely more than 7 days, but a few cases were observed for a longer period. Of the 51 children born alive, of mothers with infected placentas, 13, i.e., 25·5 per cent. died within 7 days, the

remaining 38 survived the observation period ; of the 93 children born alive of mothers whose placenta was not infected, 5, i.e., 5·4 per cent. died within 7 days, the remaining 88 survived the observation period. Of 18 children born alive, and who died within 7 days, 13, i.e., 72·2 per, cent. were born of mothers whose placenta was infected, while 5, i.e., 27·8 per cent. were born of mothers whose placenta was not infected. Of the 16 born dead, 2 are omitted because the placentas relating to them were in such a state of decomposition that they could not be examined satisfactorily. Of the remaining 14 there were 10, i.e., 71·4 per cent. who were born of mothers whose placenta was infected* and 4, i.e., 28·6 per cent. who were born of mothers whose placenta was not infected.

In none of the children born alive were parasites found at birth, either in the cord or peripheral blood ; nor were pigmented leucocytes seen in any of them.

In 22 cases of dead children, i.e., some of the 10 children born dead, and some of the 18 cases who died within 7 days, we had additional means of diagnosis by post-mortem examination, a partial examination in 12 cases and a complete examination in 10 cases.

None of the 22 children who were examined by one or other of the above methods presented parasitic infection in the peripheral or cord bloods, nor in any organ examined ; nor were pigmented leucocytes found in the cord or peripheral blood of any of them. In three cases, however, pigment was found either free or contained in leucocytes in the internal organs of the child ; the mother's placenta in each of these three cases was infected with malaria. We are not in a position to state definitely what the source and nature of this pigment are ; while it may probably result from red cell destruction in the child, there is no direct evidence to show that this red cell destruction was brought about by the malaria parasite invading the red cells of the child. Our completely negative findings as regards parasites in any of the children are opposed to the idea that the pigment was produced by the parasite itself acting on the child's blood cells. We cannot exclude the possibility of infection having existed in the child and having died out before the time of examination at birth. It appears possible that toxins of malaria absorbed from

* The only case in our whole series in which infection of the placenta was diagnosed by the finding of pigmented leucocytes without parasites being present, is included in this group.

the focus of infection in the placenta will produce red cell changes in the child. In case 64 binovular twins were born with cords attached to adjacent placentas ; these placentas and cords presented remarkable differences in the blood as shown in Table VIII.

Table VIII

Showing the differences in the placentas, cords, and fate of the child in twins (both placentas infected).

	Placenta		Cord		Child	
	A	*B*	*A*	*B*	*A*	*B*
croscopic appearance of placenta, nd fate of child	Anaemic	Normal	Anaemic in first 6 inches only	Normal throughout	Died within 15 hours	Lived 7 days +
pe of parasite ...	30 % of parasites sporulating	No sporulating form seen	...	...	...	...
mber of parasites	1-15 fields	1-25 fields	...	...	...	...
croscopical ppearances	Evidence of great destruction of red cells	Normal except for infected red cells	Same as placenta for first 6 inches, remainder normal	Normal	Normal	Normal

The degree and stage of infection in the two placentas was different ; *A* having a higher degree of infection and a high proportion of sporulating forms. The uninfected red blood cells of placenta *A* presented all stages of lysis ; poikilocytosis and anisocytosis were present. Cabot's rings, pseudospirochaetes and fragmented red cells were very numerous ; on the other hand the uninfected cells of placenta *B* appeared normal.

In cord *A*, apart from the absence of parasites, the changes in the blood were identical with those seen in placenta *A* ; the changes noted were, however, confined to the first six inches of the cord nearest the placenta ; beyond this point cord *A* appeared normal ; cord *B* was normal throughout its length. The peripheral blood of both children appeared to be normal in so far as the non-nucleated red

cells were concerned, and a differential count of the leucocytes, and a comparison of the nucleated red cells showed the following results.

Peripheral Blood Diff. Leuc. Count	P.	LM.	SM.	Eos.	Bas.	Nuc. Red.
Twin *A*	39·0	8·5	40·0	1·5	...	11·0
Twin *B*	51·5	13·0	22·5	4·0	0·5	8·5

The different appearances of the blood in placentas *A* and *B* are illustrated in figures 3 and 4 in the Plate. This case is of interest in that whereas no parasites were found in the child and cord *A*, yet there was evidence in the cord blood of extensive damage to red cells similar to the damage in the placenta *A* blood.

We are compelled to leave unanswered the question what exactly is the pigment found in the organs of the child, in the cases referred to. It is suggestive, however, that in three cases in which pigment was found in the organs of the child, in each case it had died *in utero*, and that there were marked changes in the blood of the placenta belonging to each child; these changes resemble closely the appearances found in the placenta *A* and the first position of cord *A*; in case 64 no pigment was found. The remarkable appearance of the blood in a small portion of cord *A*, i.e., that nearest the placenta, and the similarity of the appearance in the blood of this part of the cord and that of the corresponding placenta suggest strongly that some agency acting in the placenta in causing destruction of red cells had also acted on the blood of the child in the portion nearest the placenta at the time the cord was tied. This agency we suggest is toxin liberated by the parasites sporulating in the placenta. If this child's cord had not been tied for some time after sporulation had occurred in the placenta, it is probable that all trace of this extensive localised destruction of red cells in the cord would have disappeared, being carried away by the circulating blood. The toxin which had in this case begun to pass into the child's blood stream was confined and prevented from circulating by the ligature of the cord, and so was acting in a concentrated form on a limited amount of blood with the results noted and illustrated. The toxic effects were

not observed in the blood of the cord at any point further away than six inches from the placenta.

In Table IX we give a summary of the salient facts concerning the children born dead or who died within 7 days, and in Table X which follows this, we give the figures which would represent the expected results in these cases if we assume that malaria had no part in the production of the mortality.

TABLE IX

Showing the number and percentage of children who were born dead or who died within 7 days, among 61 children born of 55 infected mothers and 97 children born of 95 uninfected mothers.

	Total	Born of 55 infected mothers		Born of 95 uninfected mothers	
		Number	Percentage	Number	Percentage
Children born dead	14	10	71·4	4	28·6
Children who died within 7 days ...	18	13	72·2	5	27·8
Totals	32	23	71·9	9	28·1

In Table X below are given the figures which would be expected provided that malaria infection had no influence.

TABLE X

Showing the totals and percentages in each group in Table IX redistributed in proportion corresponding to the ratio of the infected to the uninfected mothers, i.e., 55 infected to 95 uninfected in a total of 150 cases.

	Total	Born of 55 infected mothers		Born of 95 uninfected mothers	
		Number	Percentage	Number	Percentage
Children born dead	14	5·1	36·4	8·9	63·6
Children who died within 7 days ...	18	6·6	36·7	11·4	63·3
Totals	32	11·7	36·6	20·3	63·4

From a consideration of these two tables we can conclude, if such a small group can be taken as representative, that malaria here has a definite and important effect in the production of a high proportion of infant deaths *in utero* and in the first week of life.

It is difficult to say whether any isolated group of figures is representative of the true facts among a large population, but when it is remembered that these cases here discussed comprise the vast majority of all cases treated in the maternity hospital, and that they include members of every important tribe living in Freetown, we may legitimately assume that they form a fair sample of the urban population in this endemic area.

We believe that almost conclusive evidence is provided by the figures considered above ; but over and above this we have obtained from the study of a large number of infected and uninfected placentas data which convince us that the pathological alterations in the malaria-infected placenta are such that they cannot fail to have a deleterious effect on the child in one or more ways.

(1) *Congenital malaria.* In spite of the enormous infection seen in many placentas we have not seen any parasitic evidence of this condition. We are, therefore, in a position to repeat for this larger series of cases what we said in our previous account of our first 26 cases, namely, that this condition is of great rarity. In view of this it is interesting to note that in other countries very different results have been obtained ; for example, Ziemann (1924) records that Weselko, in 1922, in Albania attributed to congenital malaria the death in the first week of 144 children of mothers infected with *P. falciparum,* while Swellengrebel (1925) records 48 cases of congenital malaria in the near East in each of which a microscopical diagnosis was made at periods varying in time from 1 to 5 days after birth.

(2) *Interference with the nutrition of the child.* We have shown that in some cases as many as 65 per cent. of the red blood cells in the intervillous space are infected. It appears certain that in so far as the red cells are concerned in the nutrition of the child their function must be very seriously interfered with.

(3) *Toxic effects.* It is evident that large amounts of malaria toxins are being produced in heavily infected placentas. It is possible that the toxins produced by the malaria parasite are, as Ziemann (1924)

suggests, anchored in the maternal tissues, or they may be incapable of reaching the foetal circulation ; but the similarity of the blood changes observed in many infected placentas and in the placental portion of the cord in such instances as case 64 quoted above lead us to suppose that the toxins are at least in some cases capable of penetrating into the child's circulation. It appears probable from the above facts that definite effects on the child are brought about by the two last factors, that is to say, interference with nutrition and toxic absorption.

VII. THE AGE INCIDENCE OF MALARIA

(1) *Mothers.* The age incidence of 55 cases of malaria occurring among 148 mothers is shown in Table XI.

TABLE XI

Showing the distribution according to age of 148 maternity cases and of 55 placental malaria infections amongst them.

Years	Total maternity cases	Total malaria cases	Percentage infected
15-20	32	12	37·5
21-25	55	20	36·4
26-30	37	13	35·1
31-35	8	5	62·5
36-40	14	5	35·7
41-45	2	0	...
Totals	148	55	37·2

Excluding the 41-45 age period in which the figures are exceptionally small, the general effect of the table is to show that parturient women at all ages are equally susceptible to malaria infection in the placenta. Taking this table in conjunction with Table VII it will be observed, in so far as clinical manifestations of malaria and effect on the children are concerned, that there is no outstanding difference between the ages groups. It is surprising to observe that some very young mothers, age-group 15-20, e.g., case 53 age 15, and case 9

aged 18 in Table VII with placenta infected can not only pass through labour without clinical manifestations of malaria infection, but can also give birth to apparently perfectly normal children. That such tolerance exists only in some cases is, however, exemplified by case 13 aged 18, and case 153 aged 17.

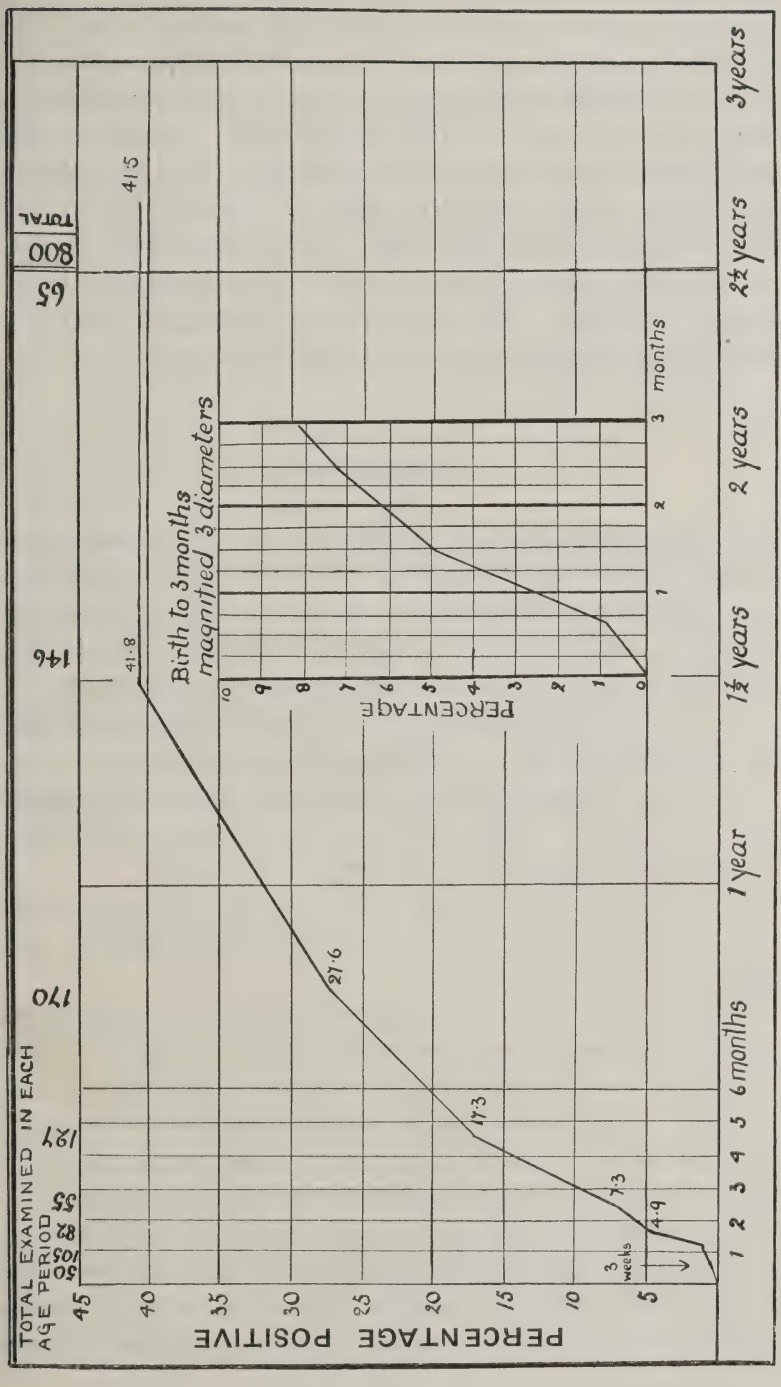
(2) *In adult males and adult non-parturient females.* The figures we have in these groups have already been mentioned, i.e., adult males 150 and adult non-parturient females 43. As the total infection in these groups as judged by examination of the placental blood was only 2 per cent. and 7 per cent., respectively, a curve plotted from them would yield little information as regards the age incidence.

(3) *In children.* The peripheral blood of 158 children born of 150 mothers of whom 55 were infected in the placenta, and 95 were not so infected, proved negative as regards malaria at birth. In addition we have examined with negative results the peripheral blood of 41 new born children of 36 mothers ; the placentas of these mothers were not examined for malaria, but in 35 of them the peripheral blood was examined with the result that two were found positive, diagnosed by the finding of pigmented leucocytes. We were unable to follow the progress of these 199 hospital cases in order to ascertain when they would become infected. The only information to be derived from them in this connection is that at birth and for a period of a week or so after birth no infection was found in any of them. From an infant clinic, however, we are able to provide the figures already dealt with in Table II from which we can show the age distribution of malaria among 800 young children, namely a series of children of ages up to $2\frac{1}{2}$ years.* In each case of this series the peripheral blood was examined once. In Graph II is shown the distribution according to age of infected cases among these 800 children examined, the examinations extending over a period of a complete year.

It is seen that only one case is recorded as positive during the first month of life. From this time on to the age of $1\frac{1}{2}$ years the infection in children shows a regular rise. The character of this curve could be explained in either of two ways. It would be

* Nine children under $2\frac{1}{2}$ years who appeared in the total for seasonal incidence cannot be included here on account of uncertainty as to their exact age.

Page 360. For ‘examination of the placental blood’ read ‘examination of the peripheral blood.’



GRAPH II

Showing distribution according to age of the malaria infected cases among 800 children at their first appearance.

compatible with the idea that the child at birth was endowed with a passive immunity derived from the mother which steadily diminished until the age of $1\frac{1}{2}$. It would also be compatible with the idea that effective exposure to infection increases steadily as the child grows older until it reaches the age of $1\frac{1}{2}$. The flattening of the curve between the ages of $1\frac{1}{2}$ and $2\frac{1}{2}$ (which is as far as our figures go) would, if the passive immunity theory is accepted, almost certainly be due to the gradual acquirement of active immunity by the child after this age, which increases directly in proportion as the passive immunity wears off. The second explanation appears less probable since it fails to explain the flattening of the curve.

CONCLUSIONS

1. Malaria infection of the placenta is very common in native women in Sierra Leone ; it occurs in about 36 per cent. of cases.

2. Infections demonstrable in the peripheral blood are in comparison few, not only in parturient women, but also in non-parturient women, adult males, and even in children.

3. The difference between the placental and peripheral incidence is in all probability due to a peripheral blood immunity.

4. These intensely infected women are practically negligible as a source of danger to the community, so far as gamete production and consequent anopheline infection are concerned.

5. Pathological effects on the mother may be entirely lacking.

6. The children may in some cases be born and survive, and may appear free from any effects.

On the other hand this series contains in other cases proof of a very complete association between maternal infection in the placenta and death of the child *in utero*, or within a 7 day observation period after birth. Our examination of a large number of infected placentas shows that severe destruction of the erythrocytes may occur in the umbilical cord in spite of the absence of congenital malaria.

The effects upon the child, noted by us, are explicable on the grounds that toxic substances are absorbed from the intensely infected placenta of the mother, and that the accumulation of masses of infected red cells interferes seriously with the nutrition of the foetus.

REFERENCES

Blacklock, B. (1921). Notes on a Case of Indigenous Infection with *P. falciparum*. *Ann. Trop. Med. and Parasitol.* Vol. XV, No. 1, p. 59.

Blacklock, B., and Gordon, R. M. (1925). Malaria Parasites in the Placental Blood. *Ann. Trop. Med. and Parasitol.* Vol. XIX, No. 1, p. 37.

Pezepoulos, N., and Cardamatis, J. (1907). Du paludisme congenital. *Centralb. f. Bakt. I.*, Abt. Orig. Vol. XLIII, p. 181.

Christophers, S. R. (1925). The Mechanism of Immunity against Malaria in Communities living under Hyper-Endemic Conditions. *Ind. Jl. Med. Res.* Vol. XII, No. 2, p. 273.

Clark, H. C. (1915). The Diagnostic Value of the Placental Blood Film in Aestivo-Autumnal Malaria. *Jl. Exp. Med.* Vol. XXII, No. 4, p. 427.

Gray, H. (1923). Anatomy descriptive and applied. 22nd ed.

Forchheimer (1915). Therapeusis of Internal Diseases.

Horowitz-Wlassowa, L. (1924). Experimentelle Beitrage zur Frage der Malariaimmunitat (Spezifische Komplementablenkungsreaktion bei Malaria). *Ztschr. f. Immunitätsf. u. Experim. Therap.* Vol. 40, No. 3, p. 268.

Marchiafava, E. and Bignami, A. (1894). The parasites of Malarial Fevers. *New Sydenham Society.* Vol. CL. p. 114.

Swellengrebel, N. H. (1925) De reis der malaria-commissie van den volkenbond naar Oost-Europa en Italië. *Nederl. Tijschr. v. Geneesk.* Jan. 10 and 17. 69th year. 1st Half Nos. 2 and 3, pp. 139-152; 218-228.

Vaughan, K. (1925). The Separation of the Placenta. *Brit. Med. Jl.* April 4, p. 656.

Weselko (1922). In Ziemann, H. (1924). *Malaria und Schwarzwasserfieber.* p. 275.

EXPLANATION OF PLATE VI

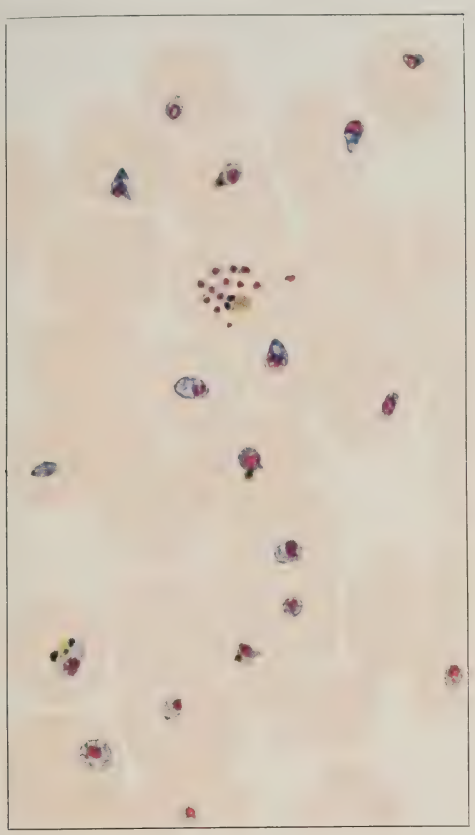
P. falciparum in the placenta.

Fig. 1 Microscopic field from an intensely infected placenta.

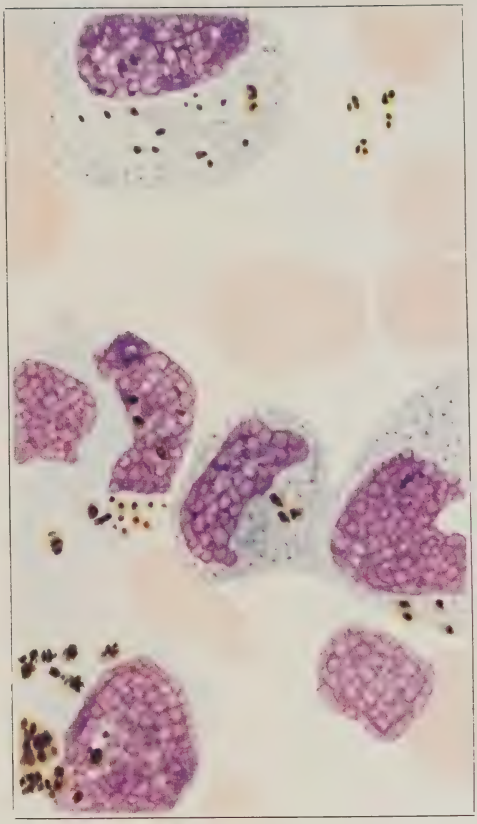
Fig. 2. Intensely pigmented type of placenta. Drawn from the tail of the film.

Fig. 3. Case 64. Placenta A.

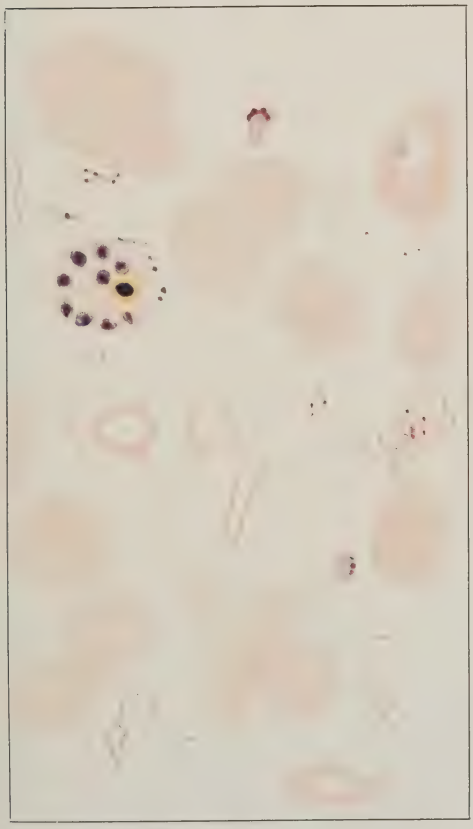
Fig. 4. Case 64. Placenta B.



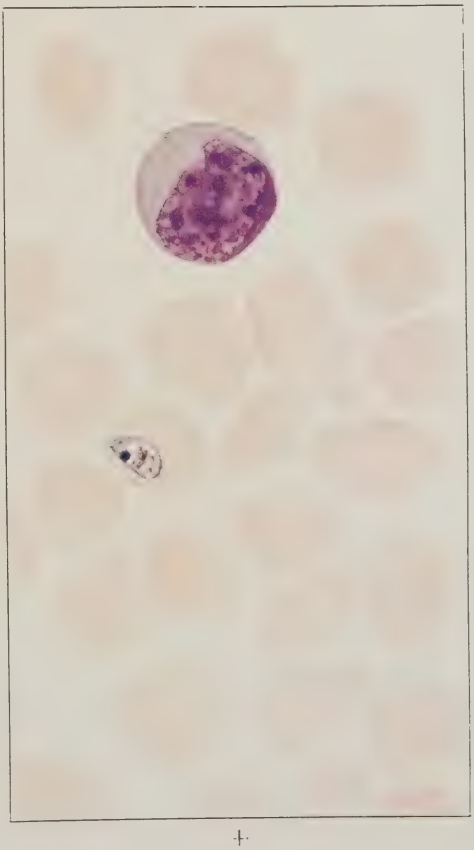
1.



2.



3.



4.

THE EXPERIMENTAL TRANSMISSION OF CUTANEOUS LEISHMANIASIS TO MAN FROM *PHLEBOTOMUS PAPATASII*

BY

S. ADLER

AND

O. THEODOR

MICROBIOLOGICAL INSTITUTE, JERUSALEM

(*Received for publication* 11 *August*, 1925)

Sandflies have been suspected by various authors of being the transmitting agent of oriental sore. The first definite evidence in favour of the *Phlebotomus* theory of cutaneous Leishmaniasis was provided by Wenyon (1912) who found about six per cent. of the sandflies he dissected in Aleppo infected with *Herpetomonas* resembling cultural forms of Leishmania. Wenyon states that all intermediate stages of development between the small non-flagellated bodies and the fully developed flagellates occurred. Later Mackie (1914) found ten per cent. of *Phlebotomus minutus* in Assam infected with *Herpetomonas* but Patton (1922) has pointed out that oriental sore is not endemic in Assam. In Mesopotamia where oriental sore is common and sandflies are a pest, Patton (1919) states that *Herpetomonas* is present in *Phlebotomus papatasii* and *P. minutus*, and in 1922 the same author remarks on the presence of *Herpetomonas* in *Phlebotomus papatasii* and *P. minutus* in Palestine.

Additional evidence for the *Phlebotomus* theory was adduced by Acton (1919) who showed that the distribution of oriental sores on the body corresponds to the distribution of bites of *Phlebotomus*.

In Palestine the epidemological evidence for the *Phlebotomus* theory of transmission of oriental sores (Jericho Boils) is ambiguous but rather favourable to the *Phlebotomus* theory. Canaan (1916) who first demonstrated Leishman-Donovan bodies in oriental sores from Jericho considered that town to be the only endemic centre of cutaneous Leishmaniasis in Palestine. Later however Kligler (1923) reported three cases from Kantara, Dostrowsky (1925) described ten cases from Artuf and also found one case from Bethlehem and one from Mozza, a small village near Jerusalem.

The aetiology of the disease as described by Dostrowsky in Artuf is of great interest. The population of Artuf is one hundred and fourteen and until 1923 sandflies were not observed in Artuf according to the statement of Dr. E. Jaruslawsky, the local physician (sandflies when present in numbers never pass unobserved). In the summer of 1923 the insects had for the first time become a pest to the villagers. This fact raised Dr. Dostrowsky's suspicion that the *Phlebotomus* was the carrier of oriental sores.

In June, 1924, one of us (A.) on Dr. Dostrowsky's suggestion examined the village and found every house infested with *Phlebotomus*. Three species *P. papatasii*, *P. minutus* and *P. perniciosus* were present, *P. papatasii* being by far the commonest. Dr. Dostrowsky then examined the population (ninety-seven) of an Arab village only one hundred metres from Artuf. Material was taken from every suspicious papule and given to one of us (A.). On examination Leishman-Donovan bodies were absent in every case. An examination of this village made by one of us (A.) did not reveal a single specimen of *Phlebotomus*. This may be explained by the fact that this village consists of mud huts without windows and uniformly dark in the interior, while the first village consists of fairly modern houses with whitewashed interiors, the furniture and clothes producing shade and contrast to the whitewashed walls. In Jerusalem, Jericho and Artuf it was noted that *Phlebotomus* prefers the shade produced by contrast to uniform darkness.

In Jericho *Phlebotomus papatasii* and *P. minutus* occur in large numbers, the former species predominating. *P. perniciosus* is absent or very rare. In Mozza *P. papatasii* and *P. perniciosus* are both common. It would seem then that *P. papatasii* is the carrier of cutaneous Leishmaniasis in Palestine, it being the only species common to three localities where cutaneous Leishmaniasis occurs. Schroetter, (1923), incriminated an insect which he called *Phlebotomus el Ghor* as the carrier in Jericho but Martini has shown that this insect is not a *Phlebotomus* and is incapable of biting.

Against the *Phlebotomus* theory is the fact that cutaneous Leishmaniasis is absent from many localities in Palestine where sandflies are a plague, e.g., the village of Rehoboth containing twelve hundred inhabitants where all three Palestinian species

of *Phlebotomus* abound. In Haifa (population about 30,000) which is the place most infested with sandflies in the whole of Palestine, all three Palestinian species being present, oriental sore is hitherto unknown. Still more striking is the fact that up to the present no locally acquired cases of cutaneous Leishmaniasis have been noted in Jerusalem itself. There are always a number of cases of cutaneous Leishmaniasis in Jerusalem from Jericho, Bagdad, Aleppo and Persia. In addition *Phlebotomus papatasii* is very common, *P. perniciosus* also occurs, and *P. minutus* is very rare, i.e., there are, according to the *Phlebotomus* theory, ideal conditions for the spread of the disease. Were even a small number of cases present they would not pass unnoticed for all classes of the population of Jerusalem assiduously attend the numerous clinics of the city for even the most trivial maladies and physicians are on the look-out for a case of locally acquired oriental sore.

It would seem then that on the assumption that a *Phlebotomus* sp. is the carrier of the disease in nature a third and hitherto unknown factor apart from human cases and insect carriers is necessary for the spread of the disease. What this factor is still remains to be investigated.

EXPERIMENTAL TRANSMISSION TO HUMAN BEINGS

Sergent, Parrot, Donatien and Béguet (1921) first described the experimental transmission of oriental sore to a human being. These authors divided five hundred and fifty-nine sandflies into twenty-three batches, crushed them in saline and used the resulting material for inoculation into the arms of twenty-three volunteers. The material was collected at Biskra, an endemic centre of oriental sore, and the experiments were performed at Algiers where oriental sore is unknown.

Only one experiment from a batch of seven specimens of *Phlebotomus papatasii* gave a positive result. The experiment was performed on the 20th August, 1921; two months and twenty days later a papule was noted, and on the following day numerous Leishman-Donovan bodies were found in the papule.

In October, 1924, one of us (A.) commenced an examination of *Phlebotomus* in Jericho. Two hundred and twenty specimens of

Phlebotomus from Jericho, of which one hundred and seventy-four were females, were dissected during October to December, 1924, and three females gorged with mammalian blood were found to contain *Herpetomonas* in their midgut. All stages from non-flagellated forms to long flagellated forms were noted.

The following is a method recommended for the examination of *Phlebotomus* for *Herpetomonas*. If the insect contains no blood, cut off the terminal abdominal segment, stroke the upper surface of the abdomen with a needle to push out the ova, gently pull the head with one needle, holding the other needle against the upper surface of the thorax. In this way the head, oesophagus, salivary glands, oesophageal diverticulum and midgut are removed together, and the individual parts of the alimentary tract can be examined for *Herpetomonas*. If the midgut contains blood gently pull the head away from the thorax. The salivary glands, oesophagus, and oesophageal diverticulum and occasionally the upper part of the midgut will come away together with the head. The rest of the alimentary canal can then be pulled out from the hind end in the usual way.

In December, 1924, sandflies became very rare in Jericho and on the 20th December, 1924, a search through the whole town by a trained assistant yielded only four specimens of *P. papatasii*. Subsequent monthly examinations revealed no sandflies until April 20th, 1925. They were not found on April 4th, 1925, but on April 20th, 1925, they were numerous, *Phlebotomus papatasii* only being present. *Phlebotomus minutus* appeared towards the end of June.

A batch of one hundred and ninety-eight sandflies of which one hundred and ninety-one were *P. papatasii* (one hundred and seventy-five females and sixteen males) and seven *P. minutus* (six females and one male) were collected in Jericho on the 25th June, 1925, and brought to Jerusalem for dissection. Of this batch only one specimen, a female *P. papatasii*, was found to contain *Herpetomonas*. The insect contained no trace of blood and the abdominal cavity was full of ripe or almost ripe eggs. The whole alimentary tract was found to be swarming with *Herpetomonas*. Flagellates were found in the oesophagus, oesophageal diverticulum, midgut and hindgut. They were especially numerous in the upper part of the midgut where swarms of parasites appeared to be attached to the posterior surface of the oesophageal valve, so much so that some parasites appeared at first sight on examination in the fresh preparation to be intracellular. (The oesophageal valve is a well-marked structure in *Phlebotomus*.)

The fact that flagellates were noted in the oesphageal diverticulum is of great interest for in freshly dissected specimens it is frequently seen that waves of peristalsis pass from the posterior end of the oesophageal diverticulum towards the oesophagus, which in *Phlebotomus* is very short, so that the oesophageal diverticulum practically opens into the pharynx. It is thus possible that flagellates may be propelled into the pharynx and buccal cavity and the possibility of a direct infection by the bite of a sandfly must be considered. Hitherto it has been generally held that infection takes place only through the crushing of an infective *Phlebotomus* on the skin, a theory which is very feasible in view of the large number of sandflies crushed on the skin (e.g. Cornwall, 1922). Another point of great interest was observed, i.e. the flagellates were not polymorphic as in the cases observed by Wenyon (1912) and by one of us (A.) in 1924, but they were all elongated and had long flagella. The oesophagus, together with the piece of oesophageal diverticulum and the upper part of the midgut, containing the oesophageal valve, were dissected away together, and another part of the midgut behind the oesophageal valve was dissected off separately; these parts were placed on separate slides, fixed in absolute alcohol, stained overnight with Giemsa, differentiated with a 0·02 per cent. solution of acetic acid and permanently mounted.

The remainder of the material was used for inoculation into the forearm of a volunteer. The volunteer had previously been exposed for three years (1917-1920) to oriental sore in Mesopotamia without contracting the disease. Two points on the skin of the left forearm were scarified and material containing flagellates was rubbed into each on the 26th of June, 1925. On the 31st July, 1925, a small papule which would normally have passed unobserved was noted on one of the inoculated points and on examination Leishman-Donovan bodies were found. The incubation period was thus less than half that noted by the Sergents and their collaborators (1921).

The dissection of individual sandflies and experimenting with material from one infected individual is more satisfactory than crushing large numbers in saline and experimenting with the product, for in the latter case it is impossible to know whether a negative result is due to the fact that none of the sandflies contained

Herpetomonas or whether the *Herpetomonas* was non-infective. From April to June, 1925, a thousand and thirty-seven sandflies collected in Jericho were dissected and found negative for *Herpetomonas ;* on the 9th of June another two hundred sandflies from Jericho were collected and on dissection found negative. Thus the experiment of crushing more than twice the number of sandflies from an endemic centre used by the Sergents and their collaborators and inoculating a single volunteer would have given a negative result.

Nothing was noted on the other site of inoculation; nevertheless the part was scraped and on the 31st of July, 1925, examined for Leishman-Donovan bodies with a negative result. It was again examined on the 4th of August, 1925, with a negative result.

The successful results of the above experiment and the experiment of the Sergents and Parrot, Donatien and Béguet prove that human beings can be infected with oriental sore by inoculation with *Herpetomonas* from *Phlebotomus papatasii,* but the epidemiological evidence that *P. papatasii* is the only carrier of the disease in nature is not yet complete.

In view of the successful infection with Leishmaniform parasites by injection of *Herpetomonas ctenocephali* into mice, rats, and a dog, and by injection of *Crithidia fasciculata* into mice and rats (Laveran and Franchini, 1913), by injection of *Herpetomonas jaculum* from the water scorpion *Nepa cinerea* into mice, by feeding a puppy on dog fleas (Fantham and Porter, 1915), by injecting *Herpetomonas jaculum* and *H. culicis* into birds (Fantham and Porter, 1915) the possibility of other sources of infection apart from *P. papatasii* must be considered in spite of the fact that the distribution of sandflies corresponds more closely than that of any other biting insect to the distribution of oriental sore. It must be pointed out, however, that among others Hoare (1921) working with *Crithidia melophagia, Herpetomonas jaculum* and *H. calliphorae* on mice, sticklebacks, newts and frogs, and Shortt (1923) working with *Herpetomonas ctenocephali* and *H. lucilae,* failed to infect monkeys, dogs, rabbits, rats, mice, pigeons and frogs.

The question of other insect carriers of oriental sore apart from *Phlebotomus papatasii* can only be cleared up in the future by direct experiments on human skin with flagellates from various insects.

REFERENCES

Acton, H. W. (1919). A study of the distribution of Bagdad boils on the body, made with a view to discover the transmitting agent. *Indian Jl. Med. Research*, Vol. VI, No. 3, pp. 262-274.

Canaan, T. (1916). Die Jerichobeule. *Arch. f. Schiffs and Tropenhygiene*, Vol. XX, No. 5, pp. 109-119.

Cornwall, J. W. (1922). Note on Histo-pathology of a non-ulcerated oriental sore. *Indian Jl. Med. Research*, Vol. IX, No. 3, pp. 545-547.

Dostrowsky, A. (1925). Ueber einen neuen endemischen Leishmaniaherd in Palaestina. *Arch. f. Schiffs und Tropenhyg.*, Vol. XXIX, No. 3, pp. 101-111.

Fantham, H. B., and Porter, A. (1915). Some experimental researches on induced Herpetomonasiasis in birds. *Annals Trop. Med. & Parasitol.*, Vol. IX, No. 4, pp. 543-558.

——— ——— (1915). Some insect flagellates introduced into vertebrates. *Proc. Cambridge Philosoph. Soc.*, Vol. XV, Pt. 2, pp. 39-50. *Trop. Dis. Bull.*, Vol. V, pp. 280-282.

Hoare, C. A. (1921). Some observations and experiments on insect flagellates, with special reference to artificial infection of vertebrates. *Parasitology*, Vol. XIII, No. 1, pp. 67-85.

Kligler, I. J. (1923). Oriental Sore in Palestine, with a report on a new epidemic focus. *Transact. Roy. Soc. Trop. Med. & Hyg.*, Vol. XVII, No. 5, pp. 334-336.

Laveran, A., and Franchini, G. (1913). Infections experimentales de Mammifères par des Flagelles du tube digestif de *Ctenocephalus canis et d'Anopheles maculipennis*. *Compt. Rend. Acad. Sciences*, Vol. CLVII, No. 18, pp. 744-747. *Bull. Trop. Dis.*, 1914, Vol. III, pp. 122-123.

Mackie, F. P. (1914). A flagellate infection of sand-flies. *Indian Jl. Med. Res.*, Vol. II, No. 1, pp. 377-379. *Bull. Trop. Dis.*, Vol. V, No. 5, pp. 282-283.

Patton, W. S. (1919). Note on the Aetiology of Oriental Sore in Mesopotamia. *Bull. Soc. Path. Exot.*, Vol. XII, No. 8, pp. 500-504.

——— (1922). Some reflections on the Kala-Azar and Oriental Sore problems. *Indian Jl. Med. Res.*, Vol. IX, No. 3, pp. 496-532.

von Schroetter, H. (1923). Zur Kenntnis der Leishmaniasis cutanea, der sogenannten Orientbeule. *Arch. f. Schiffs. und Trop. Hyg.*, Vol. XXVII, pp. 234-246.

Sergent, Ed. et. Et., Parrot, L., Donatien, H., and Béguet, M. (1921). Transmission du clou du Biskra par le Phlebotome. *C. R. Acad. Sc.*, Vol. CLXXIII, pp. 1030-1032. *Bull. Trop. Dis.*, Vol. XIX, No. 4, pp. 315-316.

Shortt, H. E. (1923). The Pathogenicity of insect flagelattes to vertebrates, with special reference to *Herpetomonas ctenocephali*, F. *Indian Jl. Med. Res.*, Vol. X, No. 4, pp. 908-933.

Wenyon, C. M. (1911). Oriental Sore in Bagdad, together with observations on a gregarine in *Stegomyia fasciata* and the Haemogregarines of dogs and the flagellates of House-flies. *Parasitology*, Vol. IV, No. 3, pp. 273-344.

——— (1911). Report of six months' work of the Expedition to Bagdad, on the subject of Oriental Sore. *Jl. Trop. Med. & Hyg.*, April 1st, 1911.

——— (1912). Note on the occurrence of Herpetomonas in the Phlebotomus of Aleppo. *Jl. Lond. School Trop. Med.*, Vol. I, Pt. 2, p. 98.

——— (1922). Leishmaniasis. A Review of recent literature. *Bull. Trop. Dis.*, Vol. XIX No. I, pp. 1-18, and No. 3, pp. 182-193.

CO-ORDINATION OF EFFORT IN TSETSE-FLY INVESTIGATIONS

(*A paper read at the second Imperial Entomological Conference, London,* 15 *June,* 1925)

BY

WARRINGTON YORKE

(*Received for publication* 9 *September,* 1925)

I have been asked by the Administration of Northern Rhodesia, which I have the honour of representing at this Conference, to put before you the case for co-ordination of effort by the various African Colonies in Tsetse-fly investigation, principally in the experimental determination of the Game-fly relationship. It gives me considerable satisfaction to open a discussion on this subject, as I have long felt the necessity for co-operation of effort if any definite advance of knowledge is to be achieved. In fact, it will be within the recollection of certain here present that I pressed this point of view at the first Imperial Entomological Conference, five years ago, and at a subsequent meeting of the Royal Society of Tropical Medicine, when certain recommendations of the Glossina Sub-Committee of the Bureau of Entomology came up for review.

Now let me make it clear at once that nothing which I shall say to-day is in anyway directed against entomological research. As I said five years ago, I do not wish to place difficulties in its way, but I desire most sincerely to encourage it; and have the greatest admiration for the work which is being done by the various entomologists scattered throughout Tropical Africa, who, at considerable risk to themselves, are doing their utmost to advance knowledge. But I have long held, and I see no reason to change my views, that the problem is not a purely entomological question and that, in devising any plan of research, we must bear this in mind. The real problem is, of course, Trypanosomiasis of man and his domestic animals, and it comprises four factors: (1) the

pathogenic virus or trypanosomes; (2) the population and the domestic stock; (3) the transmitting agent or tsetse-fly, and (4) the reservoir of the virus or big game. In my judgment substantial advance in knowledge can only be achieved by research carefully devised and adequately co-ordinated with the object of taking into consideration, at the same time and in the same locality, all of the above factors. In short, I advocate centralization of effort.

What has been done in the way of research into the Trypanosomiasis problem since the Glossina Sub-Committee of the Imperial Bureau of Entomology published its report five years ago? Probably only those who, like myself, have to read and summarise the innumerable papers dealing directly and indirectly with this subject are in a position to realise the enormous amount of human energy which is being devoted to it. There are the entomological papers of Lloyd, Swynnerton, Carpenter, Fiske, Schwetz and others; the very able and suggestive epidemiological papers of Duke; the dozens of papers dealing with the action of various drugs on infected man and stock, not to mention equally numerous papers of a purely academic nature. These reports are scattered throughout a vast range of journals and periodicals. I can assure you the mere task of reading and summarising them for the *Tropical Diseases Bulletin* is no small undertaking. While the reviewer is filled with admiration for the energy and enthusiasm shown by these reports, he is only too conscious of the fact that the energy is often misdirected, that the reports, although often very long, frequently, owing to the omission of some essential information, do not permit of any inference, that even work admirably conceived and executed is often brought to nought by the fact that those who are conducting it go on leave without arrangements being made to carry it on. As an illustration of this one cannot do better than cite his own experience in attempting to summarise the position of knowledge regarding the therapeutic action of certain drugs in trypanosomiasis. Time and again one finds a record of most carefully conducted observations on long series of patients; then at periods varying from a few months to a year after the commencement of the work the observer goes on leave, or is placed on some other duty, and nothing more is heard of the patients. Now we can only judge of the result of treatment by ascertaining what has happened after the lapse of a

number of years, and as this information is in the vast majority of instances not forthcoming, the initial excellent work is thus rendered useless and knowledge or, shall I say, ignorance, remains *in statu quo.*

I had a letter the other day from Dr. Lloyd, who, as you know, has wide experience of tsetse work and is at present investigating the subject in Nigeria. He tells me that he has fenced round a small area with the object of making a game exclusion experiment. He writes :—

'The fence was less trouble to construct than I anticipated, and is strong enough to keep out small stuff, but would not stop a roan or buffalo. In construction, when it was about three-parts done, a herd of some thirty roan got in and were there when we went to work. The labourers started to chase them and did capture a small one, but the great beasts crashed and leaped through in twenty places and the noise frightened some pig at the pool and they drove the wire out pig-shape in a dozen spots. After it was closed it took some clearing, although the area is only a half square mile. Duiker were the worst trouble as they got into the thickets and would not be flushed. However, it is clear of antelope now. The results are promising to be of interest but I fear they will not be convincing. *Tachinoides* does not seem to be affected but *morsitans* has become very scarce compared to the control. As an anomaly the infection has gone up considerably. This shows that most of, if not all, the *morsitans* in the place are emigrants from the neighbouring belts of fly. The main point of interest is the very emaciated condition of the female flies.'

Well, gentlemen, I know Dr. Lloyd intimately, and was associated with him on the Luangwa Commission. He is an extremely able and conscientious worker and, if his foreboding should unfortunately turn out to be true and the results prove unconvincing, it will, I am satisfied, not be through any fault of his, but merely of the system under which he is working. As most of you know I have always been an ardent advocate of a large and carefully controlled experiment of game destruction in a localised area and believe that from it we should obtain information of the greatest possible value. Some of you will be relieved to hear that it is not my intention to enlarge upon this much-debated subject on the present occasion. I do not like to assume the rôle of a prophet, but I am afraid that we shall not learn much from Dr. Lloyd's experiment. He is evidently too short of funds to carry it out efficiently and on a sufficiently large scale ; he is working almost single-handed and it is very doubtful to me whether, if unassisted, he will be able to make all the observations that such an experiment demands. Finally, in order to obtain information of real value from an experiment of this sort, not only

must it be preceded by a thorough and scientific investigation of the conditions, both in respect of fly and of the trypanosomiasis of man, stock and game, but it must be followed by an equally careful investigation extended over a sufficient length of time—probably running into a number of years—or no precise information regarding the results of game elimination can be expected. In due course Dr. Lloyd will, doubtless, go on leave and then, if we can be guided by what usually happens on such occasions, the work will either come to an end, or, which amounts to the same thing, someone who has other interests, or no interests at all, will take over.

In connection with this game exclusion experiment, you will perhaps pardon me if I refer to a passage in the extremely interesting Report of the East Africa Commission which I had the pleasure of reading a few weeks ago. The passage runs as follows :—

> ' The question of game destruction is a very thorny one and has aroused much feeling. In this connection the opinion of Mr. Walter, now Lord, Rothschild, is worth recording : " To prove to the utilitarians the absolute uselessness of this proceeding, I should like to point out that the extermination of the game animals in any large area would be a task of several years' duration and the following would take place. As, year by year, the large animals grew scarcer, the tsetse flies *Glossina palpalis* and *morsitans*, which are the means of spreading sleeping-sickness in man and nagana in animals, would be driven to bite monkeys, carnivora, rats, mice, and the numerous small animals of those regions ; these would be infected and the trypanosomes of the disease would gaily survive. This would not only mean the continuance of the disease in its present degree, but would also cause a sharp increase of both diseases." '

Now, sir, I must confess that personally I should have experienced some difficulty in finding anything less worth recording. In the first place, Lord Rothschild ventures to prejudge in the most categorical manner, and without the slightest evidence, what would happen as the result of an experiment—to my mind a most dangerous and unwarrantable procedure—and in the second place, even assuming his premise, for which of course we have similarly no support, namely, that tsetse flies in the absence of large animals would be forced to feed on monkeys, carnivora, rodents and mice, the inference that ' these would be infected and the trypanosomes of the disease would gaily survive, and that this would not only mean the continuance of the disease in its present degree, but would also cause a sharp increase of both diseases,' indicates complete

ignorance of what is known regarding the effect of the pathogenic trypanosomes of man and stock on these small animals.

I mention this because it illustrates in such an admirable manner my point that the Trypanosomiasis problem is not one which can be fully investigated by entomologists alone or, for that matter, by any other class of worker.

It is not my intention to refer in detail to the most valuable work which Dr. Duke is carrying out in Uganda on the protozoology and epidemiology of the disease. All who are familiar with his work realize its great value, but here again I feel that the work is suffering because Dr. Duke's other duties make great demands upon his time and because he lacks sufficient expert assistance and adequate financial resources to put to the crucial test the theories which he has built up at the cost of years of patient research. I am glad to learn that one of the recommendations of the International Conference on Sleeping Sickness which met in London last month under the auspices of the 'League of Nations' is that the International Commission, which it is suggested to form, should be placed under the presidence and control of Dr. Duke, and I am especially glad to see that they have coupled the recommendation with the suggestion that Dr. Duke's staff should be increased by the services of a biochemist and entomologist. Apart from this, I do not hope for much from the labour of the International Conference. Such a Conference seems to me to be premature. I cannot believe that its efforts are likely to advance knowledge and we hardly know enough at the present time to formulate regulations governing the International Frontiers in Tropical Africa. In my judgment, much more is to be hoped from an inter-Colonial Conference and from the co-ordinated and sustained effort which it would be in the power of such a Conference to ensure.

The case for co-ordination appears to me to be overwhelmingly strong, for the following reasons :—

Many investigators are at present working in more or less isolation at the different aspects—entomological, epidemiological, pathological, and therapeutic—of trypanosomiasis. The cost of this work is divided amongst many Colonies and therefore probably does not fall unduly heavily upon any one Colony, but in the aggregate the total annual expenditure must be very large. Unfortunately, as the

individual workers are isolated, insufficiently supplied with funds and assistance, and compelled to leave their work at more or less stated intervals, and sometimes at periods which are not stated, it is not surprising that much that is done is unsatisfactory and incomplete owing to want of organisation to ensure continuity, and consequently much time and money is wasted. Such a process has continued long enough; it is uneconomical and although the expense to each Colony may be relatively slight, in the aggregate it is large, and knowledge, if it advances at all, does so slowly and uncertainly.

Many of the problems which demand solution are very large, as, for example, the relationship of game to trypanosomiasis and the tsetse or the various problems which, five years ago, the Glossina Sub-Committee proposed should be investigated. Such problems cannot, with any hope of success, be investigated by isolated workers, whether entomologists or pathologists, but only by large and well-equipped Commissions having at command large funds.

I would therefore urge, as I did five years ago, (1) that in future, effort should be concentrated instead of dissipated; (2) that the work of the entomological and medical and veterinary research into the Trypanosomiasis problem be combined under one central organisation, and such organisation be supported by pooled contributions of all the African Colonies interested; (3) that the personnel of the investigating commission or commissions be large enough to ensure continuity of work in all directions, thus obviating interruptions due to such exigencies as illness or leave and preventing staleness and inertia, which is so likely to result from isolation; (4) that sufficient funds be placed at the disposal of the investigating commissions to allow of the employment of adequate native labour, so that experimental work can be undertaken on a sufficiently large scale, thus enabling the investigation of the relationship of fly and trypanosomiasis to game, and of the various problems enumerated in the Report of the Glossina Sub-Committee of the Imperial Bureau of Entomology, to be carried out in a satisfactory manner and with some reasonable prospect of success.

Whether such a scheme would cost more than is at present being spent individually by the different Colonies, I do not know. Certainly a large sum would be needed, and if this were not forthcoming the whole plan would collapse. Whether the problem is sufficiently

grave to warrant a large expenditure in a serious endeavour to find a solution, I will not attempt to discuss, as I am neither a politician nor a student of political economy, but judging from the valuable Report of the East Africa Commission to which I have already referred I gather that certain politicians and economists are abundantly satisfied of the gravity of the situation.

A NOTE ON MICROFILARIAE IN TANGANYIKA TERRITORY

BY

J. F. CORSON

(*Received for publication*, 15 *August*, 1925)

PREVIOUS OBSERVATIONS

Geographical distribution and incidence. Little appears to be known of endemic areas. Feldmann (1904), Marshall (1909) and Grothusen (1910) noted the prevalence of *Mf. perstans* in the district of Bukoba. It had been observed previously by Zupitsa, in 1897-98. Feldmann examined over 6,000 persons and found from 24 to 86 per cent. of the population, in various parts of the district, infected, the northern parts showing a greater incidence than the southern. In the south-east of the territory the district around Liwale appears also to be an endemic area as *Mf. perstans* was found by Mr. Irvine, sub-assistant surgeon, in 1924, in 31·5 per cent. of 402 adults examined. In regard to *Filaria bancrofti* even less is recorded. Elephantiasis was stated by Grothusen (1909), and previously by others, to be endemic in the Ubena-Ebene area, to the north-west of Lake Nyassa. Recent reports point to the prevalence of this disease in districts near the great lakes, as Mwanza and Bukoba in the north, and near Tukuyu (Neu Langenburg) in the south. In the region of the sea coast, there appear to be many cases of elephantiasis in Mafia Island, though no figures are yet available, while a considerable number are operated upon in the larger towns. Engeland (1920) found 32.32 per cent. of 297 native soldiers at Dar-es-Salaam infected with *Mf. bancrofti*. They, however, like the population of the coast towns, include people from many different parts.

Incidence in Dar-es-Salaam. In the years 1908-09 and 1909-10, microfilariae were found in slightly over 2 per cent. of nearly 40,000 thick blood films, examined chiefly for malaria and taken, no doubt, in the day-time. During the years 1922, 1923 and 1924, in the course of routine examination of a large number of thick blood films at the bacteriological laboratory at Dar-es-Salaam, microfilariae,

sheathed and unsheathed, grouped together, were found, as recorded in the annual reports, in 2.5 per cent., 2.2 per cent., and 3.6 per cent., respectively. The much greater incidence of *Mf. bancrofti* than of *Mf. perstans* in such examinations, was noted by Engeland and Manteufel (1911).

Species of microfilaria found. *Mf. bancrofti* and *Mf. perstans* only were found by Engeland and Manteufel. Fülleborn also (1908 and 1913a) found only these two species, with one possible exception, in blood slides received from Bukoba, Usumbura and Shirati, near the great lakes in the north, and from Dar-es-Salaam. The possible exception was a very small sheathed microfilaria, found by Manteufel (1911) in the blood of a soldier's boy in Dar-es-Salaam. The slide sent to Fülleborn, broken on the way, contained only about 10 microfilariae, stained with haemalum. Fülleborn admitted, with all reserve, that it might belong to a new species or be *Mf. powelli*, but that its possible identity with *Mf. bancrofti* could not be excluded. *Mf. loa* has not been found. Neave (1912) gives a list of flies found in German East Africa, which includes three species of Chrysops, viz., *C. bicolor* Cordier, *C. longicornis* Macq, and *C. magnifica* Austen. Limited reference only to subsequent literature has been available and this may not represent present knowledge. It is of interest that a considerable number of natives of West Africa were brought to this country during the late war.

Periodicity of Mf. bancrofti. That well-marked periodicity occurs in at least a great majority of cases was shown by Engeland. He found the microfilaria, as stated above, in 32.32 per cent. of soldiers, in the night blood, but in only 2.06 per cent. were they present also in the day time. Whether or not forms without periodicity also occur, as in the Southern Pacific and, perhaps, in West Africa, has not apparently been shown.

PRESENT OBSERVATIONS

From 300 to 400 thick blood films, chiefly of Africans, but including some Indians, taken at various unstated hours of the day, are examined monthly in ordinary routine work at the bacteriological laboratory at Dar-es-Salaam. In 768 such films, examined recently, microfilariae were found in 6.7 per cent. The latter figure refers to different individuals so the percentage given is a minimum.

Similar films were taken between 10 and 11 a.m., from 140 school pupils and from 140 prisoners. In the former, *Mf. bancrofti* was found in eight cases (5·7 per cent.) and *Mf. perstans* in one. In the latter *Mf. bancrofti* was present in seven cases (5 per cent.) and *Mf. perstans* in five. Examination in detail, including measurements with a camera lucida, was made in 30 cases, and observations as to periodicity in 21 cases. In a few cases only were living specimens examined and 'vital' staining with neutral red and azur II used. Staining with weak methylene blue, as described by Foley (1913) and by Sharp (1923) was not employed. Giemsa's stain and haemalum were chiefly used. The results confirm those of German observers. Microfilariae indistinguishable from *Mf. bancrofti* and *Mf. perstans* were the only kinds observed. Brief mention of a few details only is therefore made.

Mf. bancrofti. Morphology. The *sheath,* in nearly all the specimens, appeared to be unstriated. In one specimen the free anterior part of the sheath, in length about equal to one-fourth that of the worm, showed very clear, regular cross striation. If, as has been suggested by Fülleborn (1913b), it may be simply an impression of the striation of the body of the worm, in this case it was remarkably well-defined. Striation of the sheath has been observed by Brumpt (1922), who suggested that it might indicate a larval skin, and by Foley. It is referred to in a review of a paper by Biglieri (1923). The *anterior end* of the worm, in specimens stained with Giemsa's solution, showed various appearances, corresponding more or less with the descriptions of various authors. An interpretation of them could not confidently be made. Neither in living nor in stained specimens could a 'fang' or 'prepuce' be recognised. *Striation of the body,* in deeply-stained specimens, was observed to extend throughout the whole length of the worm, from the extremity of the anterior end to the tip of the tail. With ordinary staining it was not seen in front of the first nuclei. The *nuclei* were counted in a few specimens. The results did not agree with the figures given by Sharp. The number in front of the 'nerve ring,' for example, was from 60 to 70 or more. The appearances of the '*excretory cell,*' the '*central viscus*' or '*Innenkörper*' and the '*G*^{1} *cell*' of Rodenwaldt agreed with Fülleborn's description and illustrations of *Mf. bancrofti.* The *tail* was not infrequently folded upon itself.

Measurements. 114 specimens from 26 cases. As the tip of the tail could not be seen clearly in many specimens, the *last tail nucleus* is assumed to be at 95 per cent. of the total length. The average position in 17 specimens measured was at 94.6 per cent.

The terms denoting the ' fixed points ' of Fülleborn are used.

N.	Ex-P.	Ex-C.	G^1-C.	L. Tail-C.	Total length.
19	29	29.5	69.7	272.8	(287.1)

In over 83 per cent. of 114 specimens the ' *nerve ring* ' was situated at a point between 18 per cent. and 20 per cent. of the length. The following are the figures of Fülleborn's measurements of 28 examples.

Microfilariae from German East Africa ; ordinary thick dry preparations from 5 different slides ; haemalum staining.

	N.	Ex-P.	G^1-C.	A-P.	L. Tail-C.	Total length
Average ...	19.9	30.1	70.3	82.6	95.3	263.7
Minimum ...	18.3	27	67	79 ?	94.4	245.5
Maximum ...	21.1	33	73.3	88.4?	96	291

Periodicity. Only persons in whose blood microfilariae were found in the daytime were examined. Measured quantities, viz., 20 c.mm. of blood were taken in 13 cases. In eight other cases, by the kindness of the staff of the native hospital, two thick films at midnight and two by day were taken on two or three successive days. The day time was usually 9 a.m. In no case was the number of microfilariae in the day blood equal to that in the night and in nearly all cases the disproportion was greater than could be accounted for by ordinary fluctuations, at the same hour on successive occasions, of, say, 300 per cent. Fluctuations are known to be considerable. In one case, for example, the number in 20 c.mm. of blood taken at the same hour of the night on several occasions, varied between 235 and 604. This case showed 17 at 5 p.m. and one only at noon, on two occasions. The greatest number found in daytime blood was 62, in 20 c.mm. of blood at 9 a.m. In this case, at 11 p.m., the number was 211. One case showed seven at noon and 13 at 11 p.m. So far as any conclusion can be drawn from these few observations, it would appear that a form of *Mf. bancrofti* without periodicity, if existing at all in this country, must be rare.

Mf. perstans. Citrated blood was centrifuged and dehaemoglobinised, for examination, especially of the anterior end.

In living specimens a small spot, usually terminal, occasionally apparently lateral, was seen. ' Vital staining ' with neutral red showed a small conical structure, base to the front, just behind the outline of the anterior end. In some specimens the anterior margin appeared to show minute papillae. No constant feature, however, could be seen in the examination of about 20 specimens. No ' fang ' was seen.

Stained specimens. These showed the morphological characters of *Mf. perstans.* The nuclear ' break ' at 84 per cent. was a fairly constant character. Deep staining failed to show more than very slightly-marked cross striation.

Measurements. 47 specimens were measured. There is some lack of uniformity in measurements given by different authors even in the position of such a constant and sharply-defined feature as the ' nerve ring.' The following examples are given for comparison.

Brumpt (1922). Fülleborn's terms are used.

N.		Ex-P.		A-P.
26.4	...	36	...	83

These figures are quoted by Stephens and Yorke (1921).

Rousseau (1919).

N.		Ex-P.		G^1-C.		A-P.
25	...	32	...	62.6	...	83.5

Macfie and Corson (1922).

N.		Ex-P.		G^1-C.		A-P.
22.5	...	32.7	...	62.3	...	81.1

In the present cases the average figures were as follows :—

N.		Ex-P.		A-P.		Total length.
22.8	...	31.9	...	84.2	...	189.2

In over 90 per cent. of the specimens the position of the ' nerve ring ' was between 20 per cent. and 24 per cent. of the length.

The small form of *Mf. perstans* was not observed.

SUMMARY

1. Little is known of endemic areas of filarial infection in Tanganyika Territory. In addition to districts near the great lakes in the north-west, *A. perstans* is probably endemic in the south-east, around Liwale.

2. *Mf. bancrofti* and *Mf. perstans* are the only forms known to occur.

3. *Mf. bancrofti* was found to have a well-marked periodicity in all cases in which sufficient numbers were present, though occurring, in a small proportion of cases, in the blood in the daytime.

REFERENCES

Biglieri, R. (1923). Nouvelles observations sur les microfilaires de Tucumana. *C. R. Soc. Biol.*, Vol. LXXXVIII, p. 362. Rev. in *Trop. Dis. Bull.*, Vol. XX, p. 631.

Brumpt, E. (1922). Précis de Parasitologie, 3me ed., 1922.

Engeland, O. (1920). Beobachtungen ueber den Turnus und das prosentuale Vorkommen der *Mikrofilaria bancrofti* in Deutsch Ostafrica. *Arch. f. Sch. u. Trop. Hyg.*, Band XXIV, p. 51. Rev. in *Trop. Dis. Bull.*, Vol. XVII, p. 83.

Engeland, O., and Manteufel, P. (1911). Ergebnisse einiger Untersuchungen ueber Mikrofilarien bei Menschen. *Arch. f. Sch. u. Trop. Hyg.*, 1911, Band XV, No. 12, p. 721.

Feldmann (1904). Ueber *Filaria perstans* im Bezirk Bukoba. *Arch. f. Sch. u. Trop. Hyg.*, Band VIII, p. 285.

Foley, H. (1913). Études morphologiques sur les microfilaires à gaine, (*Mf. bancrofti* et *Mf. diurna*). Observations faites chez les tirailleurs sénégalais d'Algérie. *Annales de l'Institut Pasteur*, Tome 27, p. 50.

Fülleborn, F. (1908). Untersuchungen an menschlichen Filarien und deren Uebertragung auf Stechmücken. *Beihefte z. Arch. f. Sch. u. Trop. Hyg.*, 1908, Band XII, Beiheft 9, p. 353.

——— (1913a). Beiträge zur Morphologie und Differentialdiagnose der Mikrofilarien. *Beihefte z. Arch. u. Trop. Hyg.*, 1913, Band 17, Beiheft 1.

——— (1913b). Die Filarien des Menschen. In *Handbuch der pathogenen Mikroorganismen*, von Dr. W. Kolle u. Dr. A. von Wassermann. 2 Auflage. Band VIII.

Grothusen (1909). In *Medizinalberichte ueber die Deutschen Schutzgebiete*, 1908/09.

——— (1910). *Idem.* 1909/10.

Macfie, W. Scott, and Corson, J. F. (1922). A new species of filarial larva found in the skin of natives in the Gold Coast. *Annals of Trop. Med. & Parasitol.*, Vol. XVI, p. 407.

Manteufel, P. (1911). In *Medizinalberichte ueber die Deutschen Schutzgebiete*, 1910/11.

Marshall (1909). In *Medizinalberichte ueber die Deutschen Schutzgebiete*, 1908/09.

Neave, S. A. (1912). Notes on the bloodsucking insects of Eastern Tropical Africa. *Bull. Ent. Res.*, Vol. III, p. 275.

Rousseau, L. (1919). Filariose au Cameroun. *Bull. Soc. Path. Exot.* Tome XII, p. 35.

Sharp, N. A. Dyce (1923). *Filaria bancrofti* and *Loa loa*. A Note on some Methods of Differentiation of their Embryos. *Trans. Roy. Soc. Trop. Med. & Hyg.*, Vol. XVII, p. 177.

Stephens, J. W. W., and Yorke, W. (1921). In *The Practice of Medicine in the Tropics.* Ed. by Byam and Archibald, 1921. Vol. III, Sect. XI.

SPRING RELAPSES IN BENIGN TERTIAN MALARIA

BY

ADAM PATRICK, M.D., M.R.C.P.

PROFESSOR OF MEDICINE, UNIVERSITY OF ST. ANDREWS.

(*Received for publication* 21 *August*, 1925)

While engaged, between 1916 and 1918, in the treatment of soldiers who suffered from malaria and who had been invalided from the Salonika army to the hospital base at Malta, I was struck by two clinical facts. The first was that patients who had been transferred to convalescent camp after recovery from attacks of benign tertian malaria, showed an extraordinary tendency to have relapses in the early spring, although the weather was genial; the second, that several patients whom I had treated (1919) in the late autumn, for severe subtertian malaria, remained well for two months or longer, and then relapsed about February, but this time with a benign tertian infection. It is possible that the men referred to in the latter case had previously experienced attacks of this form of malaria, although, in some instances at least, there was no history of it. It seemed possible that the tertian infection remained latent for some months after inoculation of the subject, and showed itself for the first time some months later. Indigenous malaria is extremely rare in Malta, and one could be all but certain that the tertian infection had not been acquired on the island, but there was no certainty that men coming from such a highly malarious country as Macedonia had not previously suffered from a benign tertian attack.

Acton, Curjel and Dewey (1921) record a similar experience. Of a series of 102 malignant tertian infections received for treatment at Dagshai, in India, in 1918, only seven were diagnosed as mixed parasitic infections due to the benign and malignant tertian parasites; yet in this series there were 64 benign tertian relapses, indicating that the majority of these mixed parasitic infections had been overlooked.

Some time ago a patient of my own developed benign tertian malaria in a manner which leaves little doubt that the first attack of the disease occurred after an incubation period of several months.

W.A., aged 37, a ship's officer, consulted me on 2nd December, 1921, about an illness which had been diagnosed as malaria. His ship had been in Bombay for a fortnight, from 1st October, 1921, and just before she left port he fell ill with malaise and headache, but without rigor or vomiting. This illness ceased in three or four days. A fortnight later, on the voyage to Europe, he had a second attack, of four days' duration, with similar symptoms. A third attack occurred about 7th November, while his ship was lying at Antwerp, and then for the first time he had shivering and some vomiting. When I saw him four weeks later, he felt out of sorts, but had 'on days and off days.' The spleen was considerably enlarged, and subtertian parasites, both rings and crescents, were present in the blood.

He was given a thorough course of quinine, beginning with a daily intravenous injection of bihydrochloride for four days, the first of 10 grains, and the three others of 15 grains each. From 7th till 26th December he took, by the mouth, 30 grains of quinine sulphate daily in solution ; thereafter, for a month, 20 grains a day ; and then 12 grains daily. A few crescents were present in the blood on 9th December. On seven subsequent examinations, up to 10th February, no parasites were found. By the middle of January the patient felt very well, and remained well, still taking 12 grains of quinine sulphate daily, until 8th March, when he vomited in the evening, complained of headache, and shivered a little. Two days later he had a more severe attack, and a two-day periodicity was established when I was called by his doctor to see him on 15th March. On that date the spleen was two inches below the ribs, and parasites, now benign tertian, were numerous in the blood. Subtertian forms were not observed, and the patient said that the symptoms were quite different from those which he had previously experienced, the shivering and headache being severe, and the attacks periodic. He quickly improved with a further course of treatment, but relapsed on 15th June, when benign tertian parasites were again found in the blood. There was a further relapse at the end of September, after

which he remained well until he passed out of my observation in January, 1923.

In this case there was no history of previous benign tertian malaria, and as the period from December till March was spent in the west of Scotland, infection must have taken place at least five months before the first attack. His previous visit, before October, 1921, to a malarious country was in March of the same year, when his ship had called at Bombay. But his first malarial attack of any kind did not occur till October, 1921. The subtertian parasites were apparently killed off by the course of quinine, but the benign tertian resisted it and caused an attack, even although the patient was taking 12 grains of quinine sulphate a day. It is to be noted that this tertian attack came on in the spring, as in the cases I referred to in the opening paragraph.

Although no reference is made to this peculiarity of tertian malaria in most text-books, the point is not a new one, for Dr. J. G. Jack has drawn my attention to a passage in Shakespeare's *Henry V*, Part 1, Act 4, Scene 1, lines 111-112, where Hotspur says :

> ' No more, no more ; worse than the sun in March,
> This praise doth nourish agues.'

REFERENCES

Acton, H. W., Curjel, D. T., and Dewey, J. O. (1921). The Diagnosis and Treatment of Benign Tertian and Malignant Tertian Fevers, Section II. *Indian Journal of Medical Research*, Vol. VIII, No. 4, p. 763.

Patrick, A. (1919). Experiences with Intravenous Injections of Quinine and Antimony in the Treatment of Malaria. *Journal of the Royal Army Medical Corps*, Vol. XXXII, pp. 407-429.

ANTI-RABIC PROCEDURE IN PALESTINE WITH SPECIAL REFERENCE TO DECENTRALIZATION OF TREATMENT

BY

G. STUART, M.A., M.B., Director

AND

K. S. KRIKORIAN, M.D., Assistant Senior Medical Officer

Laboratory Section, Department of Health, Government of Palestine

(*Received for publication* 3 *September*, 1925)

I. INTRODUCTION

Rabies has been known to be endemic in Palestine for very many years. The country, in virtue of its geographical position, is one peculiarly liable to the disease in that it is bounded on three sides by countries in which rabies is prevalent while its general configuration favours the continued existence of the common ' carriers ' of the disease—the jackal and the pariah dog.

In the decade immediately prior to 1923 in Palestine, the most convenient course open to persons bitten by animals suspected of rabies was to proceed to Jerusalem to attend for treatment at a private institute where Pasteur's original dried cord method was practised.

On the establishment of railway communications between Palestine and Egypt in 1918, however, the Army Authorities made the decision that all military personnel affected should be sent to the Anti-Rabic Institute in Cairo.

This somewhat unsatisfactory state of affairs continued until it became increasingly apparent that the necessity for the provision in Palestine itself of a mode of treatment, recognized as adequate both by Military and Civil Authorities alike, was absolute, if numbers of lives were not to be sacrificed every year. Such loss of life was ascribed to two causes : to lack of proper facilities for treatment in this country, and to ineffective treatment in Cairo consequent upon the lateness of arrival of the patients.

Early in 1923 the serious attention of the Department of Health was given to coping with the situation ; there was no doubt that persons in ever-increasing numbers were being bitten by rabid dogs and jackals all over Palestine ; the result was that an undertaking to supply an anti-rabic vaccine of undoubted reputation and of proved reliability,—one able to fulfil all army and civilian requirements—was given by the Laboratory Section of the Department.

Table I shows the number of untreated cases reported on as having died in hospitals during 1922 from Hydrophobia, and well illustrates the gravity of the situation at that time.

Table I.

No. of cases	Sex	Age	Wounds (all inflicted through naked skin and not cauterized)			Biting animal	In days	
			No.	Location	Gravity		Period of incubation of hydrophobia	Duration of symptoms
1	M	13	1	Outer surface lower leg	Slight	Dog	38	4
2	M	13	1	Finger	Slight	Dog	22	2
3	M	78	3	Dorsum of hand	Severe	Dog	44	4
4	M	47	3	Eyebrow, Nose, Upper Lip	Severe	Jackal	16	3
5	M	22	2	Hand	Slight	Dog	30	3
6	M	12	2	Ala and tip of nose	Medium	Dog	17	1
7	M	8	1	Tip of nose	Medium	Dog	44	2
8	M	45	2	Nose	Medium	Jackal	32	4

Additional considerations, however, emphasised the necessity for the undertaking, not the least amongst which was the fact that all expenses in connection with indigent patients presenting

themselves for treatment at the private institute in Jerusalem had to be defrayed by the Government at very considerable cost, while again, the Military Authorities were involved in much inconvenience and expenditure by having to send all bitten soldiers to Egypt.

It will be appreciated, therefore, that the Department's decision was based on strictly utilitarian and economic grounds.

II. SELECTION OF VACCINE

As the result of a lengthy and comprehensive survey of the various recognized modes of anti-rabic treatment, we finally resolved that the vaccine most suitable for Palestine was undoubtedly that originally introduced by Fermi of Sassari, modified by Semple, and elaborated by Harvey and McKendrick at the Central Institute, Kasauli.

The valuable reports issued by the Kasauli Institute, along with the published memoirs of Semple, on the one hand, and the close study, on the other, of the economic conditions of Palestine determined our selection.

The relative values of the three chief methods of treatment—all of which had received fair and full trial at Kasauli—are clearly enunciated by Harvey and Acton in their critical ' Examination into the degree of efficacy of anti-rabic treatment,' while the reasons which led up to the final adoption by that Institute of a carbolized vaccine in preference to the living viruses of Pasteur and Hoegyes are stated in convincing fashion.

After the inception of the Institute in 1900 the dried cord method of Pasteur was employed for seven years. While, admittedly, this treatment was highly successful, yet the occurrence, though rare, of ' accidents paralytiques ' both during and after treatment could not be regarded otherwise than with disfavour. The possibility, however remote, of such accidents—believed by Harvey and McKendrick to be probably of an anaphylactic nature and due to the unavoidable introduction by this method of an excess of nerve protein during the course of injections—was responsible for a change being made to the dilution method of Hoegyes in 1907. This alteration in policy was in every way justified and the results of its adoption afforded striking confirmation of the theory advanced

by Harvey and McKendrick, for with the use of a comparatively small amount of foreign nerve protein coincided the complete cessation of cases of paralysis.

A further change of policy, however, took place in 1912 when, as a result of brilliant researches by Fermi, Semple and others, it was conclusively proved that the employment of a dead vaccine afforded a protection at least equal to that given by the living viruses of the previous methods.

Since that time the accumulated evidence of twelve years' experience has upheld beyond all question the opinion first put forward by Fermi as to the value of treatment by a carbolized vaccine.

Again, the stimulating example set by Col. Hamerton who, faced with an exceedingly difficult problem in Irak, most successfully coped with the situation there, proved a great encouragement to us, and so it was with every hope of similar success in Palestine that we established in Jerusalem, on May 1st, 1923, the Central Anti-Rabic Institute. In order, however, that the ' greatest good of the greatest number ' of inhabitants likely to be at risk might be served, ten subsidiary treatment centres in the districts, supplied with the vaccine prepared at the Central Institute, were opened on the same day.

III. PROCEDURE

It will be immediately obvious that if decentralization is to be successful, medical officers in charge of subsidiary centres must be completely conversant with all matters relating to anti-rabic procedure.

Each officer appointed to carry out such work here reports first to the Central Institute where he undergoes a full course of instruction.

During this course he has to give evidence that he has familiarized himself with the various considerations governing the policy adopted, with the whole subject of rabies and its prevention, and with every detail of procedure touching on the preparation, distribution, storage, and administration of the vaccine.

To ensure that each centre is conducted in strict accordance with instructions laid down, one or other of us carries out routine inspections.

A. General Considerations.

(1) *Was the biting animal rabid ?*

An animal should be considered rabid

(*a*) if it dies from an undiagnosed disease
(*b*) if it has been killed.
(*c*) if it has disappeared

} within ten days of its biting the patient.

(*d*) if it shows marked alteration in behaviour ; if, for example, it makes unprovoked assault on human beings or other animals, especially if such results in many persons or animals being bitten.

(*e*) if a jackal makes an unprovoked attack on a human being.

(2) *Has the patient been exposed to the risk of infection ?*

(*a*) A person cannot be infected by (the saliva of) an animal except when that animal is actually suffering from rabies and during the ten days immediately prior to that animal's developing symptoms (two to five days before the appearance of symptoms, Roux and Nocard, Nicholas).

(*b*) The contagion, no matter what its virulence or concentration, cannot penetrate uninjured skin or mucous membrane.

In this connection, wounds which have been granulating twenty-four hours or more are considered impervious to the virus.

A very definite risk of infection is run when blood or serum oozes from cut or abrasion.

B. Procedure to be followed.

(1) *In respect of the biting animal.*

(*a*) On no account should the animal be destroyed (there are, of course, necessary exceptions to this dictum). It should be kept under observation in close confinement, when it will, if infected and infective, have developed symptoms within ten days.

If, on the other hand, the animal remains healthy at the end of ten days, it can be regarded as having been free from the possibility of causing infection at the time of biting.

(*b*) If the animal dies during the period of observation (ten days) without showing the classical signs of rabies, it should, nevertheless, be considered as having been rabid in so far as the treatment of bitten persons is concerned.

(2) *In respect of the bitten person.*

(*a*) After giving suitable local treatment to the wound (*vide* below), one must pronounce upon the biting animal. If the animal appears to be normal it should ordinarily be kept under observation for ten days, at the end of which period, if it still shows no signs of disease, it can be considered to have been non-infective and the bitten person therefore free from risk. But treatment should be begun immediately (and to this there must be no exception made), in the following circumstances :—

(*a*) When the animal develops symptoms of ill-health, dies, is killed, or escapes, during its ten days' observation period.

(*b*) When the patient has been bitten on the face, on the hand, or very severely (even through clothing) in other parts of the body.

(*c*) If the patient has been bitten by an unknown animal in the dark or while asleep in the fields (common incident enough during the summer among the *Fellaheen*).

It is, however, of first importance to note that when an animal dies during its observation period, vaccine treatment must be begun at once, and on no account whatsoever should the result of the laboratory examination of the dead animal's brain be awaited. Apart from the loss of valuable time incurred by such a wait, it must be remembered that failure to find Negri-bodies by the microscopist does not always signify that the animal was free from rabies.

(3) *In respect of the bitten animal.*

See Appendix II, paragraphs 5 and 6.

C. Diagnosis of Canine Rabies.

(1) *Clinical.*

There exists at present no better description than that given by Mohler and Eichorn in their translation of Hutyra and Marek's textbook on 'Spezielle Pathologie und Therapie der Haustiere,' published by Ballière, Tindall and Cox, and to this the reader is referred.

(2) *Laboratory.*

The brain of a dog or other animal which has died of suspected rabies or which has been shot or otherwise killed as a result of its

having bitten persons or other animals should be extracted and forwarded to the Central Institute (here part of the Central Laboratories). A simple method of extraction is to saw the head sagittally and remove the two halves of the brain intact.

When the brain has to be sent from a distance for laboratory examination, the two halves, so extracted, are placed in a wide-mouthed can and packed about with well-powdered salt. The salt must completely fill the receptacle. The lid is now to be replaced and soldered by a tinsmith prior to despatch.

We advocate this procedure of forwarding in salt in preference to that usually recommended, viz. : in separate tins containing glycerine and alcohol respectively, because the brain thus arriving in a fresh state allows of full examination.

The brain is now freed from salt by washing in sterile normal saline solution.

Laboratory investigation consists of the search for Negri-bodies and of the performance of the biological test by rabbit inoculation.

For the detection of Negri-bodies, preliminary smears from the hippocampus major or cerebellum are made and coloured with Giemsa's stain. The results are controlled by examination of sections of these parts of the brain fixed in Zenker's fluid and coloured with haematoxylin-fuchsin or Mann's stain. With the latter we have found difficulty in obtaining uniformity of results, but with haematoxylin-fuchsin we have been very successful in accordance with the following procedure :—

(*a*) Stain with haematoxylin for seven to ten minutes.

(*b*) Blue well in tap water.

(*c*) Counterstain with acid fuchsin for three minutes.

(*d*) Differentiate in tap water.

(*e*) Pass rapidly through alcohol, clarify with oil of cloves, and mount in balsam.

In regard to the Biological Test : it is performed by injecting an anaesthetized rabbit sub-durally with 0.2 c.c. of a 1 per cent. emulsion previously prepared from the medulla of a suspected animal.

The emulsion is made by rubbing-up in sterile normal saline solution a small piece of the medulla and thereafter filtering the suspension through gauze. It is then introduced sub-durally by

means of a hypodermic syringe (the needle of which is bent at a right-angle) into an opening made by a small (Eyre's intracranial) trephine in one of the parietal bones of the rabbit. It need hardly be pointed out that the strictest aseptic precautions must be observed throughout the operation. After a variable length of time in positive tests symptoms of paresis occur, and death takes place usually in from fifteen to twenty-five days.

D. Treatment.

When it has been decided that a patient requires treatment certain curative methods are employed.

(1) *Local treatment.*

Every endeavour must be made to get rid of as much of the virus deposited in the wound as possible and that without delay. Such common expedients as ligaturing, when possible, the affected part, encouraging bleeding, and freely opening up and thoroughly cleansing the wound are to be resorted to.

Cauterization of every part of the wound must now be performed with such caustics as pure carbolic or fuming nitric acid. Here we employ fuming nitric acid and consider it likely to be effective only if applied within half-an-hour of the time of biting. (Adherence to this time limit enables us to state that in our series of cases, of 1,920 persons treated, only 40 were efficiently cauterized.)

(2) *General treatment.*

The patient must, during treatment and for ten days thereafter, follow a quiet well-ordered existence. Chill, fatigue, and excitement must be avoided, while alcohol should be especially restricted.

(3) *Specific treatment* (vaccine treatment).

E. The Vaccine.

(1) *Nature and Preparation.*

The vaccine prepared by the Central Institute consists of a 2 per cent. suspension of the brain of a rabbit killed with fixed virus, in a solution of 1 per cent. phenol in distilled water.

Before being bottled and issued to the ten treatment centres, however, it is diluted with an equal quantity of normal saline

solution. Rabbits of about 1,400 grammes are inoculated sub-durally with fixed virus emulsion, and this operation is performed only on such numbers of rabbits as will presumably supply all demands for vaccine and will keep the ' strain ' going.

Attention to the technique of the trephining and inoculating operations results in a minimal number of rabbits being required, and the practically 100 per cent. success obtained here is due to the operators' observing various essentials :

(*a*) Absolute asepsis throughout.

(*b*) Rapidity in carrying out the trephine portion of the operation.

(*c*) Slowness in injecting the emulsion of fixed virus along the needle, held parallel to the dura.

(*d*) Covering the trephine opening with a pad of sterile cotton wool and maintaining slight pressure during the process of inoculation and during the slow drawing-out of the needle on completion of the operation. This procedure precludes regurgitation of the virus emulsion.

(*e*) Care in the choice of the fixed virus used for passage.

The virus in use here was originally obtained from Cairo and produces symptoms usually on the fifth, though occasionally on the sixth day after sub-dural inoculation. Whereas, for the production of vaccine, the brain of any animal showing symptoms on the fifth or sixth day is used, here ' passage virus ' is invariably selected from the brains of those rabbits that have developed symptoms on the fifth day.

This we find most important in keeping up the fixity of the virus and in obtaining uniform results of virulence.

On the eighth or ninth day, then, after inoculation, the rabbits, now moribund, are killed by chloroform, dipped in a weak solution of cresol, and dissected.

The brain, after naked-eye inspection and after cultures have been taken from it to ensure sterility, is ' extracted ' and weighed in an accurate balance. Brains showing excessive haemorrhage or other abnormality are discarded. The brain is now pounded in a sterile mortar and during this process the carbolic solution is added little by little. On an average this operation should take from fifteen to twenty minutes to complete, and when the brain has been well-emulsified into a thick sticky paste, the remainder of the

carbolic solution is slowly mixed in, until a suspension of 1 in 50 of brain substance has been obtained.

We now have a 2 per cent. brain emulsion in a 1 per cent. carbolic solution and this is filtered through two la ers of gauze to get rid of the connective and vascular tissues, and then placed in an incubator at 37° C. for twenty-four hours.

Although all workers whose methods we have studied use a carbolic solution made up with normal saline, we, after repeated observation, have preferred to employ distilled water, as this we find gives a better suspension, while precipitation of the brain matter does not occur so readily as with saline.

After the emulsion has been in the incubator for twenty-four hours, it is taken out and mixed with an equal volume of normal saline so that the vaccine now consists of a 1 per cent. brain emulsion in 0.5 per cent. carbolic solution. After passing aerobic and anaerobic cultivation tests, the vaccine is run into sterile bottles of 30 c.c. capacity in which it is distributed. Of this vaccine each person, irrespective of age, sex, or severity of bite, receives intracutaneously 5 c.c. daily, 2.5 c.c. on each side of the abdomen.

In the first year of work here, we diluted the above suspension further with an equal quantity of normal saline just before its administration, thus following the exact procedure (apart from the substitution of distilled water) described by Harvey and McKendrick and Col. Hamerton, and giving a 0.5 per cent. suspension of brain substance in 0.25 per cent. carbolic solution. In our second year, for considerations elsewhere discussed, it was decided to inject directly, without previous dilution, a 1 per cent. emulsion—and this has been in practice here since May, 1924.

(2) *Distribution, and instructions for use.*

Before despatch from the Central Institute the vaccine bottles are properly capped, paraffined, and labelled. On each label is given the following information : nature of the contents of the bottle ; the dosage ; the mode of administration ; and directions relating to storage. In addition to this, by means of a rubber stamp are affixed further particulars :—viz., the serial number of the vaccine, the date of manufacture, and the date on which the vaccine bottle—irrespective as to whether its contents have been used or

not—is to be returned as 'out of date' to the Central Institute. This date of return is invariably three months after the date of manufacture shown on the label of each bottle.

The medical officers in charge of the various treatment centres make known their requirements fortnightly, and these indents, when met, maintain the stock held by each centre at a constant level.

The vaccine is administered in accordance with the printed instructions wrapped round each bottle :—

(*a*) Sterilize 5 c.c. Record syringe.

(*b*) Shake the bottle well.

(*c*) Withdraw 5 c.c. of vaccine by pushing the needle of the syringe through the rubber cap (previously sterilized by dipping in alcohol).

(*d*) Inject 2.5 c.c. of the vaccine on one side of the abdomen and 2.5 c.c. on the other, by inserting the point of the needle at an acute angle between the superficial and deep layers of the skin (intracutaneously).

(*e*) The complete course of treatment consists of fourteen such injections of 5 c.c. on successive days.

(*f*) The same dose is given to children as to adults, and bites of all severities are treated alike.

F. Advantages of adopting this Method of Treatment.

(1) The employment of this carbolized anti-rabic vaccine precludes the possibility of its producing *per se* the disease its object is to prevent.

Cases recorded in literature show that the injection of living or merely somewhat attenuated virus is not entirely free from danger. Babes in this connection states : 'It is very probable that fixed virus in certain cases can produce hydrophobia after subcutaneous injections. The employment of this virus, therefore, must be prohibited in the treatment of human subjects unless the organism has been first prepared with a virus sufficiently attenuated.'

Surprising, indeed, is the number of persons requiring assurance that treatment itself involves no risk, and here one is frequently asked whether, should events prove that treatment was unnecessary, any harm can possibly ensue from the administration of vaccine.

With the use of carbolized vaccine, we are fortunately able to reassure patients completely in these respects.

(2) Its use is not followed by any harmful effects.

Occasionally local hyper-reaction occurs, due to individual idiosyncrasy. Here there has been no sign of abscess formation although over 60,000 intracutaneous inoculations have been made. Further, the vaccine contains the smallest amount of nervous tissue commensurate with efficient treatment, and thereby are avoided the so-called 'post-treatment paralyses' which occasionally follow certain other methods of treatment.

(3) *Accuracy of dosage.* That uniformly successful results have been established can be claimed for no method unless dosage of vaccine can be accurately determined.

In regard to cord methods two factors must be taken into account, which militate against an accurate estimation of the number of immunizing units injected into a patient.

(*a*) Cords differ much in size, varying largely in proportion to the size of rabbit employed. A very appreciable difference must exist between the numbers of immunizing units contained in 1 c.c. of thick and in 1 c.c. of thin cord, respectively.

(*b*) Where the dried cord method is used, it will be obvious that a ten days' dried cord, from a large rabbit, will contain, normally, more living virus than a ten days' cord from a small one because desiccation proceeds more rapidly in the latter. Such difficulties do not obtain with carbolized vaccine. If care be taken to pound the brain, during manufacture, for a fixed period, say twenty minutes, and to filter the resultant suspension through gauze of uniform thickness, emulsions of unvarying strength are produced.

(4) Its dosage over fourteen consecutive days (the complete period of treatment) remains constant for all bitten persons, irrespective of age, sex, severity of bite, location and multiplicity of wounding, interposition of clothing, and different conditions requiring consideration when other methods of treatment are employed.

The reason for the application of a universal dosage lies in the fact that each case for which treatment is prescribed is regarded as being sufficiently serious to warrant the full and intensive dosage given by this method.

(5) *Economy and rapidity of production.* The use of carbolized vaccine permits of great economy both in respect of animals and of time. From a rabbit of average weight (1,400 grammes) can be produced vaccine sufficient for twelve complete courses of treatment. Further, one does not require to inoculate rabbits daily, but only as occasion demands and just often enough to maintain the ' strain.'

The actual material cost of treatment of nearly 2,000 bitten persons, here, during the past two years, has been little more than the purchase price of 150 rabbits. The time taken to produce the vaccine is short and we have clearly shown that any well-equipped laboratory can, in addition to its routine work, undertake the successful manufacture of the vaccine without additional expert staff.

An illustration of the rapidity of production is afforded by a recent occurrence when, on a farm over 100 miles from the Central Institute, 75 valuable animals were bitten by a rabid dog.

The issue of a quantity of vaccine (5,250 c.c.) sufficient for the treatment of these 75 animals was made without delay, and within nine days our reserve stock was at its normal level.

(6) Carbolized vaccine retains its maximal potency and powers of immunization for a period of at least three months if preserved under requisite conditions—away from light and in an ice-box.

It is, consequently, suitable for use in small countries where rabies is present, but too rare to justify the expenditure involved in the creation of Anti-Rabic Institutes. Such countries can purchase, from time to time, quantities of vaccine sufficient for estimated maximal requirements and their stock can be renewed quarterly at small expense. Transjordania—a country adjoining and obtaining its vaccine supplies from Palestine—affords an excellent example.

On the other hand, when living virus is employed for treatment, vaccine cannot be sent to a distance without a diminution of efficacy and without risk of its becoming infected. It is for this reason that, in our belief, carbolized vaccine is the only one of practical value in the prophylactic and curative treatment of animals.

(7) The vaccine is manufactured in a Central Institute and can be issued therefrom to any number of treatment centres where its administration to bitten persons forms part of the routine duties of the Government medical officers there. The advantages to the bitten persons of this are as follows :—

(*a*) Treatment can be begun without loss of time—an incalculable advantage in a condition where the importance of the time factor is paramount.

(*b*) The patient can be treated at or near his own home, and thereby is avoided the necessity for his undertaking long fatiguing journeys.

(*c*) The bitten person may move from town to town in accordance with the dictates of his business and be assured of an uninterrupted course of treatment.

In this connection, and to demonstrate the exceptional applicability of this form of treatment to unexpected circumstances, we would refer to an episode which occurred during 1923. In a military camp at Sarafand, two British and five Indian soldiers were severely bitten by a jackal. The jackal was shot, its brain extracted and forwarded to the Central Institute, where the finding of Negri-bodies and a positive biological test proved the animal to have been rabid at the time of biting. The bitten persons were treated at Ramleh Anti-Rabic Centre for seven consecutive days, at the end of which time the Indian regiment was ordered to proceed abroad. The Regimental Medical Officer was supplied with vaccine sufficient for seven further injections to each patient, and the remaining half of the course was administered on board ship. Reports forwarded later showed that treatment had been successful in each case.

Probably one of the most satisfactory results of our procedure, however, is that no case of hydrophobia has occurred during the past year for lack of facilities for treatment. In marked contrast to this state of affairs were the conditions (summarised in Table I) obtaining in 1922—in the year previous to the adoption of the present system of decentralisation of treatment.

(8) *Efficacy.* A.—*Theoretical considerations.* It has to be admitted, however, that the only factor determining ideal treatment is its ' Efficacy.'

If a treatment is to be universally successful it has to be able not only to prevent the onset of hydrophobia in the case of average severity, but also to ward off the disease in grave cases of short incubation periods.

The greatest advantage claimed for carbolized vaccine and its

period of administration is that it is an attempt to deal with such cases as may have short incubation periods. There can be no possibility of cure once the virus has reached the brain ; now, in a certain percentage of cases the virus does actually attach itself to the brain in or in about fifteen days ; it is obvious, therefore, that any treatment calculated to prevent the disease in these cases must aim not only at producing in the system a sufficiency of antibodies, but at producing them *within fifteen days*. Under such circumstances the time limit is of first importance, and the logic of ' intensifying ' treatments of head and face bites by prolonging them beyond the minimal period sufficient to excite and promote antibody formation in as great an area as possible is by no means clear. Surely here the thing to do is to increase the dosage administered within the minimum period of time at one's disposal. The importance of realising this cannot be over-estimated, and a glance at this modified table of Bauer (II) will show short incubation periods to be by no means rare. Further, in Table I, are two such cases.

Table II (after Bauer).

Number of days incubation	Percentage of total
1–19	8·24
20–39	28·34

A short intensive course of treatment might likewise prove of extreme value in those cases where bitten persons report at an institute late. Here again time is all-important and the maximum dosage bearable should be administered in the shortest possible period.

Moreover, the use of brain matter instead of cord doubtless contributes towards the efficacy of carbolized vaccine and its superiority over cord methods.

Brain matter is said by Nitsch to be ten times more virulent

than spinal cord. In using brain, therefore, we are giving a larger proportion of specific antibody-producing substance and a smaller one of the useless, probably harmful, nervous tissue than is practised in methods of cord immunization.

B.—*Practical results*. We shall first record the experimental and then the practical evidence on which our assertions as to the efficacy of this method are based.

(1) *Experimental.*

The subjoining Tables III and IV are self-explanatory.

Table III

Immunizing Experiments.

No. of experiment	Animal	Duration of Treatment	Quantity of 1% carbolized vaccine injected subcutaneously	Test	Result
1	Rabbit	14 days	28 c.c.	0·2 c.c. of 1% fixed virus emulsion introduced subdurally 15 days after last injection.	Lived
2	Rabbit	14 days	28 c.c.		Lived
3	Rabbit	14 days	28 c.c.		Died in 15 days
4	Rabbit	14 days	28 c.c.		Lived.
5	Rabbit	14 days	28 c.c.		Died in 18 days
6	Rabbit	Not treated	None		Died in 8 days
7	Rabbit	Not treated	None		Died in 8 days
8	Rabbit	Not treated	None		Died on 9th day

The temporary nature of the immunity conferred is well illustrated in regard to rabbit No. 2. Without prior immunization this animal was innoculated with 0.2 c.c of 1 per cent. emulsion of fixed virus, fourteen months later, and died in eight days.

To show the evidence of immunity in the serum of rabbits treated with 1 per cent. carbolized vaccine, we have, guided by examples from existing literature, performed the experiments recorded in the next table.

Table IV.

Animal immunized	Vaccine used for immunizing	Time after completion of treatment when serum was tested	Proportion of serum and 1% fixed virus emulsion tested	Tests applied to mixture of serum and virus	Result
Rabbit	1% carbolized anti-rabic	15 days	Serum 1 c.c. + fixed virus 1 c.c. incubated 2 hours at 37°	Subdurally into rabbit	Remained well
Rabbit	do.	do.	do.	do.	do.
Rabbit	do.	do.	do.	do.	Died 12th day
Rabbit	do.	do.	Only fixed virus 1% emulsion	do.	Died after 8th day
Rabbit	do.	do.	do.	do.	do.
Rabbit	Not immunized	Directly	do.	do.	Died in 8 days
Rabbit	do.	do .	do.	do.	do.

(2) *Deductions from treatment of bitten persons.*

In the various records given below it has been considered advisable to include in the first part of each table, mostly for purposes of interest and comparison, the total number of bitten persons attending the various anti-rabic treatment centres ; the number whose treatment was considered to have been unnecessary as reckoned by the biting animals having remained alive and well after ten days' observation, and the number of cases where a regular and complete course of treatment was carried out. On the last number alone have the death and so-called ' failure ' rates been estimated.

The only fair and accurate means, however, of arriving at the actual success or failure of any line of treatment is to base the final calculations only on the number of bitten persons treated who had been definitely or presumably exposed to risk of infection.

The second part of each table, therefore, consists of an enumeration of :

(1) Those persons definitely-at-risk as proved by :—

(*a*) Laboratory investigation.

(*b*) Veterinary officer's certificate obtained after observation of the biting animal.

(2) Those persons presumably-at-risk, i.e., who had been bitten by animals fulfilling the conditions laid down under A. General Considerations, paragraph 1, items (*d*) and (*e*).

(3) Death and ' failure ' rates calculated on a total of (1) + (2).

Statistics from May 1, 1923, to April 30, 1925.

Part 1.

Number of persons treated at various Government anti-rabic treatment centres in Palestine	1,920
Number of treatments interrupted or unnecessary	470
Number of regular and complete treatments administered ...	1,450
Total Deaths	12
Death rate	0·82%
Deaths occurring later than 15 days after completion of Treatment	4
'Failure' rate	0·27%

Part 2.

Number of persons definitely-at-risk—	
(*a*) Proved by laboratory investigation	270
(*b*) Proved by veterinary officers' certificates	123
Number of persons presumably-at-risk	420
Death rate	1·47%
'Failure' rate	0·49%

The above statistics, however, represent the results obtained from the combination of two definite work periods differentiated by alteration in daily dosage; thus, whereas in the period 1923-24, treatment consisted of the daily administration of 2 c.c. carbolized vaccine over fourteen consecutive days, during 1924-25 a dosage of 5 c.c. daily over the same number of days was substituted.

The results achieved during the two periods afford interesting comparison.

Part 1.	Period 1923-24 Dosage 2 c.c. of 1% emulsion	Period 1924-25 Dosage 5 c.c. of 1% emulsion
Number of persons treated	886	1,034
Unnecessary treatments	138	332
Regular and complete treatments	748	702
Total deaths	9	3
Death rate	1·2%	0·4%
'Failures'	4	0
'Failure' rate	0·5%	0
Part 2.		
Number of persons definitely-at-risk—		
(*a*) Proved by laboratory investigation	180	80
(*b*) Proved by veterinary officers' certificates	48	75
Number of persons presumably-at-risk ...	213	207
Death rate	2%	0·8%
'Failure' rate	0·9%	0

It will be observed that 307 and 340 persons belonging respectively to the 1923-24 and 1924-25 periods are not included in Part II, although they have been bitten by animals fulfilling the conditions laid down in A. General Considerations, paragraph 1, items (*a*), (*b*) and (*c*)—conditions which demand the immediate treatment of the bitten persons from the point of view that the biting animals are to be considered rabid. Such number, if included, would obviously lessen both the death and 'failure' rate, thereby elevating the degree of efficacy of this form of treatment.

It is generally admitted that a certain percentage of cases cannot be benefited by any form of anti-rabic treatment. In such cases the incubation period is very short and the virus reaches the brain before a sufficient degree of immunity can be conferred. Excellent examples are afforded by numbers 2, 4, and 6, in Table I, and by the first line statistics of Table II.

On the other hand it must be admitted that when a patient dies of hydrophobia, say one, two, or three months after completing a full and regular course of treatment, this case cannot be included in the above category but must be considered as a failure of treatment in that either the effect of treatment was nil or that it sufficed merely to delay the arrival of the virus in the brain, thereby prolonging the incubation period of the disease, which latter alternative is exemplified in Table III, number 3 and 5, of our immunizing experiments.

Now in view of the results obtained in the 1923-24 period, with the administration of 2 c.c. daily for fourteen days, it may be a matter for surprise that we resolved to make an alteration in dosage to 5 c.c. daily over a similar period.

However, a study of the following four cases—our only failures of treatment as judged by the conventional standards, viz., when hydrophobia supervenes later than fifteen days after completion of a regular course of treatment—was mainly instrumental in bringing about our decision.

Case 1. Male, aged 10 years, severely bitten on forehead, hand and leg through naked skin by a jackal, reported one day after bite for treatment, the wound not having been cauterized before arrival. Treatment was regular over 14 days. Symptoms developed 59 days after the date of bite, and 44 days after completion of treatment.

Case 2. Male, aged 35 years, severely bitten on face, eyelid, and thumb by jackal through naked skin, reported three days after bite, the wound not having been cauterized, and attended regularly for treatment. Symptoms appeared 81 days after the bite and 65 days after completion of treatment.

Case 3. Male, aged 8 years, bitten slightly on upper extremity through naked skin by a dog, arrived two days after bite with wound not cauterized. Symptoms occurred 36 days after the bite and 21 days after completion of treatment.

Case 4. Male, aged 30 years, bitten severely through clothing on lower extremity by a dog, arrived immediately after for treatment and had wound cauterized with fuming nitric acid. Treatment was administered regularly over 14 days. Hydrophobia supervened 39 days after the bite and 26 days after completion of treatment.

We felt convinced that these four cases died, not as the result of any special exaltation of a virus (virus de rue renforcé) or shortness of incubation period, but because the treatment administered had not been sufficiently intensive : with this conclusion Professor Fermi, of Sassari, to whom we submitted the facts, was in complete agreement. (It is, however, worthy of record that thirteen other persons, bitten by the animals inflicting bites on the four persons cited, received full and regular courses of vaccine also, and all thirteen are to-day alive and well, one-and-a-half years after completion of treatment.)

As a result an alteration in dosage was effected for all cases, on May 1st, 1924, after a month's preliminary trial had demonstrated beyond question the ability of children and adults alike to tolerate the daily increase from 2 to 5 c.c.

It was resolved by us to make an increase in daily dosage rather than in the period of vaccine administration, so that the method of treatment might be made as widely applicable as possible and especially with regard to such cases as might have short incubation periods.

Although we consider that results in human beings have justified the alteration, yet Table V will demonstrate our inability to adduce experimental proof of the value of increased dosage in laboratory animals.

Further, from this table it would appear that :—

(*a*) Better results in rabbits are obtained by the giving of small doses over a number of days (14) than of larger doses over a shorter period.

(*b*) It is impossible to reduce below a certain limit the time over which the total quantity of vaccine, ordinarily sufficient for complete immunization, can be usefully administered.

(*c*) As large a dose as 30 c.c. of a 0.5 per cent. emulsion in 0.25 per cent. carbolic may be given without the production of toxic symptoms.

Table V

Experimental animal used	Dose and Period for Immunization					Whether or not animal survived treatment	Subdural Test
	2 c.c. daily for 14 days	4 c.c. daily for 14 days	6 c.c. daily for 10 days	15 c.c. daily for 3 days	One injection of 30 c.c.		2 weeks after treatment tested with 0·2 c.c. of 1% emulsion of fixed virus
Rabbit	1	...	...	...	...	Survived	Survived
Rabbit	1	...	...	...	...	Survived	Died
Rabbit	1	...	...	...	...	Survived	Died
Rabbit	1	...	...	...	...	Survived	Survived
Rabbit	...	1	...	...	...	Survived	Survived
Rabbit	...	1	...	...	...	Survived	Died
Rabbit	...	1	...	...	...	Died	...
Rabbit	...	1	...	...	...	Survived	Died
Rabbit	...	...	1	...	...	Survived	Survived
Rabbit	...	...	1	...	...	Survived	Died
Rabbit	...	...	1	...	...	Survived	Died
Rabbit	...	...	1	...	...	Survived	Died
Rabbit	...	...	...	1	...	Survived	Died
Rabbit	...	...	...	1	...	Survived	Died
Rabbit	...	...	...	1	...	Survived	Died
Rabbit	...	...	...	1	...	Survived	Died
Rabbit	...	...	...	...	1	Survived	Died
Rabbit	...	...	...	...	1	Survived	Died
Rabbit	...	...	...	...	1	Died	...
Rabbit	...	...	...	...	1	Survived	Died

We would now draw attention to the fact that in the records of statistics published above have been included figures for total deaths and for the so-called failures of treatment as well as their respective percentages.

'Failure,' in accordance with convention, is applied only to

those cases which, in spite of treatment, develop hydrophobia later than fifteen days after a complete anti-rabic course has been administered. By the same convention are excluded from ' failures ' all bitten persons dying during treatment or within fifteen days of its completion.

(Remlinger, e.g., has shown that during 1901-1908, in Constantinople, where the original Pasteur method is utilised, of persons dying despite treatment no fewer than 80 per cent. could be so excluded.)

Statistics based on this mode of computation will obviously vary with regard to :—

(*a*) Length of time taken to adminster a course of treatment.

(*b*) Lateness of arrival of the bitten person for treatment.

The longer a person defers reporting for treatment, and the longer the period of treatment—the lower the ' failure rate ' of an institute.

It is felt that such statistics tend unduly to emphasise the value of certain forms of anti-rabic treatment and especially of such as normally require a lengthy period for adminstration.

We realise that this method of recording deaths and failures would be ideal, if all institutes were to employ the same method whereby the same dosage of a similarly prepared vaccine was administered during the same period of time. As matters stand at present, however, when anti-rabic methods are many and diverse, it is our opinion that the only way to present statistics which can admit of real comparison is a simple enumeration of the deaths occurring at an institute and the percentage such bears to the total attending population proved or believed to be at-risk.

Deaths from hydrophobia should then be reported on in full, with especial reference to the duration of treatment, to the number of days intervening between completion of treatment and onset of symptoms, to the lateness of arrival for treatment, and to the irregularity of attendances during the course.

This information, in addition to that supplied in Appendix I, will generally enable an impartial pronouncement to be made upon such cases in their relation to treatment.

G.—Records. It was at once recognised that one of the criticisms most likely to be levelled against decentralisation of treatment would be the presumed unreliability of the records of available information.

With the view, therefore, of meeting just such a charge we evolved the present procedure of keeping records of the particulars of all patients treated in the various Government Centres.

Appendix I gives the form which enables a complete register of cases undergoing anti-rabic treatment to be kept at each treatment centre by the medical officer in charge. Immediately a patient has completed his course, the original form is submitted to the office of the Central Institute for filing, while the duplicate is retained at the treatment centre concerned. Three months after the last day of completion of treatment, a final report on the patient is submitted to the Central Institute by the responsible medical officer and this report certifies to the patient being in good health (or otherwise) on that date. This procedure, we consider, allows of the ready compilation of exact statistics.

IV. SUMMARY

1. Carbolized Anti-rabic Vaccine is an efficient and safe treatment for persons bitten by rabid animals.

2. It can be manufactured without additional staff in any well-equipped laboratory.

3. It can be distributed to any number of treatment centres where as good results attend its use as at the place of its production.

4. Better results have followed the employment here of a dosage of 5 c.c. daily of a 1 per cent. emulsion, over fourteen days, than of 2 c.c. of the same emulsion over the same period.

5. Carbolized vaccine is most practicable in the curative and prophylactic treatment of farm animals.

It can be easily administered in rural districts by veterinary officers.

6. It is a great advance on older methods of treatment in the wideness of applicability and economy of production of the vaccine. It is at once the most economical and utilitarian mode of treatment.

7. Bitten persons can be treated at or near their own homes, and thus—the all-important consideration of immediate treatment aside—they are spared the expenses connected with travel to, and residence in, a strange town.

ACKNOWLEDGMENTS

Our thanks are due to the Director of Health, Col. G. W. Heron, D.S.O., O.B.E., for his unfailing encouragement and for his affording us facilities which rendered the whole scheme of decentralisation possible. We are indebted also to Dr. Miftah, Director of the Pasteur Institute, Cairo, for many favours, but especially for his ungrudging assistance in the training of our personnel.

For the sake of completeness, we have considered it advisable to add three appendices :—

I. The form employed to register cases undergoing anti-rabic treatment.

II. Regulations for the control of rabies (in which is incorporated the procedure to be adopted in the case of the bitten animal). These regulations made under Art. 43 of the Ottoman Law concerning Diseases of Animals, and drawn up by Col. E. R. Sawer, Director of Agriculture, have been in force since December, 1924.

III. Further measures adopted to free Palestine from rabies and hydrophobia.

REFERENCES

Babes, V. (1912). Traité de la Rage, Chap. 28, p. 482.

Bauer (1886). Inkubationsdauer der Tollwut beim Menschen. *Münchener Medizinische Wochenschrift*, Nos. 37 and 39.

Fermi, C. (1906). Contr. sp. allo stud. della rabia. *Giorn. della S. d'hygiene.*

Ferrans (1907). Supplementary article on Lyssa in Kolle and Wasserman's *Handbuch der path. Mikr.*, p. 646.

Hamerton, A. E. (1922). Rabies in Irak and its treatment by Carbolized Vaccine. *Jl. of the R.A.M.C.* Vol. XXXIX, No. 6, p. 403.

Harvey, W. F., and Acton, H. W. (1922-1923). The Degree of Efficacy of Anti-Rabic Treatment. *Indian Jl. of Med. Research.* Vol. IX, No. 4, and Vol. X, No. 4, p. 1,020.

Harvey, W. F., and McKendrick, A. (1921). 'Rabies' in *Practice of Medicine in the Tropics*, Vol. III, Chap. 100. Byam and Archibald.

——— ——— (1907). Theory and Practice of Antirabic Immunization. *Scientific Memoirs—Government of India, No.* 30.

Nitsch (1906). Remarques sur la methode pasteurienne du traitement préventif de la rage. *Bulletin de l'Institute Pasteur*, Vol. IV, p. 1,057.

——— (quoted). Supplementary Article on Lyssa, p. 646 in Kolle and Wassermann's *Handbuch der path. Mikr.*

APPENDIX I

Local Form O. M. 186

Department of Health

REGISTER OF CASES UNDERGOING ANTI-RABIC TREATMENT.

No. Date District

Information about patient :

Name Age Profession
Residence and Address
Nationality Sent by
Date of bite Animal inflicting bite
Station where bitten

Wounds

	Number	Gravity	Bitten on naked skin or through Clothing
Head and neck			
Upper extremity			
Lower extremity			
Body			

Have wounds been previously treated ? (cauterized) and when ?

Information about the animal.

Owner of animal Address
What has become of the animal ?
Other persons bitten, with names and addresses
Other information (e.g. " dog bit unprovoked ")

Diagnosis.

1. Condition of animal from enquiry
2. Microscopical Researches (Negri bodies)
3. Experimental inoculation ? Result Date

Treatment.

1. When started 2. Vaccine and Dosage 3. Serial No. of Vaccine

4. Attendances

Month															
Dates															

5. Conduct of patient during treatment
6. Accidents, if any, during treatment
7. Other remarks

Signature of M. O.

Final Report on Patient.

No.
On being three months after the last day of the completion of treatment, the patient is alive in good health (or otherwise) and is living at (address)
District Signature of M. O.
Date

APPENDIX II

REGULATIONS FOR THE CONTROL OF RABIES.

1. Every person having had in his possession or under his charge an animal affected with or suspected of Rabies shall give notice of the fact with all practicable speed to the Mukhtar, President of the Municipality or Police as the case may be. Failure to give such notice renders the person liable to a fine of £1 to £5 or to imprisonment not exceeding one month.

2. It is the duty of Mukhtars, Presidents of Municipalities and Police, on receiving such notice, to destroy the affected animal or to place it in strict isolation, and to transmit the information immediately to the District Governor or District Officer who will delegate the Veterinary Inspector to institute inquiries.

3. On confirmation of the disease and receipt of the report of the Veterinary Inspector, the District Governor or District Officer shall form a sanitary commission in accordance with the provisions of the said Ottoman Law, to execute the measures necessary for the control and suppression of the disease.

The commission shall be composed of the District Officer as president, and the Veterinary Officer, a representative of the Public Health Department, the Local Commandant of Police, and a member of the Municipal Council as members.

4. Every animal affected with Rabies shall be destroyed. Any animal whose behaviour leaves no doubt as to its being rabid shall be destroyed on the spot, and its body, in the case of dogs, cats and small animals, taken to the nearest District Veterinary Officer for disposal.

5. Animals bitten by rabid animals shall be dealt with as follows :—

(*a*) Donkeys, dogs, cats, monkeys, etc., shall be destroyed.

(*b*) Local camels ; bulls, cows, calves, oxen, sheep and goats shall be slaughtered ; but provided such animals are slaughtered within seven days of the date when first bitten, their carcases, if free from other diseases, may be exposed for sale as food.

(*c*) Valuable horses, mules, bulls, cows and calves shall be destroyed, or (1) strictly isolated for four months under the observation of Government Veterinary Officers and on premises approved by the Department of Agriculture, and (2) vaccinated with Anti-rabic Vaccine at the owner's risk and cost.

It is prohibited to sell such animals for any purposes whatsoever, during the period of observation.

6. Every animal bitten by a suspectedly rabid animal, and any dog which has been in contact with a suspectedly rabid dog shall either be destroyed or shall be strictly isolated :—

(*a*) Under the observation of Government Veterinary Officers, and

(*b*) In special cages, kennels, or stables, or on premises approved by the Department of Agriculture ; and

(*c*) At the entire risk and expense of the owner ; and

(*d*) For a period of six months in the case of dogs, or four months in the case of herbivorous animals or in both cases for such a period as will allow the diagnosis to be confirmed by the District Veterinary Inspector.

7. Every animal which has bitten a human being shall be placed in strict isolation and under observation for a period of at least ten days at the owner's risk and expense.

8. In no case may such animals be detained for purposes of observation by any private person or institution.

9. Animals will be destroyed by order of the District Officer or the District Veterinary Inspector, and no compensation will be paid in respect of such animals when they are :—

(*a*) Rabid or suspectedly rabid, or bitten by such animals ;

(*b*) In contact with a rabid or suspectedly rabid dog or other carnivorous animals.

10. The carcases of rabid or suspectedly rabid animals will be burned or deeply buried unskinned, but only after the examination by a District Veterinary Inspector or on the authority of the District Officer, in places selected by them.

11. The District Governor or President of a Sanitary Commission formed under the Law, shall in any locality where a case of rabies has occurred, issue a notice proclaiming the measures to be taken to control and suppress the disease, and the owners or persons in charge of animals shall observe and comply with such regulations.

12. The Administrative Authority, after notifying the public in the town or area, may proceed at any time to poison or destroy in any manner vagrant, stray, ownerless or collarless dogs and dogs not carrying the municipal tally.

13. Any person who fails to comply with any of the foregoing regulations or orders issued by the District Governor or Sanitary Commission for the Suppression of Rabies, or who does not assist in execution of such orders, shall be liable to prosecution before a magistrate under Art. 39 of the Ottoman Diseases of Animals Law of the 5th December, 1910 (1329 Moslem year), and on conviction to imprisonment not exceeding three months or to a fine not exceeding £20.

APPENDIX III

Apart from the preparation of a suitable vaccine for the treatment of persons bitten by rabid animals further measures were adopted to deal with the menace.

(*a*) The extermination of jackals and stray dogs—the common transmitters of the disease to human beings ;

(*b*) The education of the public in town, district and village regarding the nature of the disease, its method of transmission and the action to be taken by an individual who has been exposed to risk of infection from being bitten by a dog or other animal.

In regard to (*a*), the following action was taken by the Departments of Agriculture, Police and Prisons, and Health acting together :—

(1) The organisation of a constant campaign conducted by the Gendarmerie in country districts to lay bait poisoned with strychnine capsules in places frequented by jackals and pariah dogs. The campaign is carried out

along lines carefully worked out by the Department of Agriculture and during the present year has resulted in the finding of over 1,000 dogs and 750 jackals. The number of animals killed is known to exceed those actually found dead, as many which have swallowed poisoned baits travel some distance before the poison takes effect ;

(2) In town areas the first step towards reducing the numbers of ownerless and pariah dogs was to regulate the registration and licensing of dogs kept by householders as guards or domestic pets. This registration is effected by the staff of the Municipalities under model regulations drafted by the government Departments concerned and adopted by all towns. Municipal employees are authorised to apprehend and destroy all dogs found at large not bearing on their collars a numbered tally indicating that they have been duly licensed.

When the number of ownerless dogs is found to be increasing in any given area of a town in spite of these measures, the assistance of the Police is called upon and the animals are destroyed by shooting.

From 1st January, 1923, until 30th June, 1925, over 20,000 ownerless dogs were destroyed.

Municipalities furthermore are required to make arrangements for the safe custody of dogs whose observation by a veterinary officer is necessary on account of their suspicious behaviour or unwarranted attacks on human beings. This is effected by the Municipalities providing 'Observation Kennels' constructed in accordance with plans prepared by the Department of Health and approved by the Chief Veterinary Officer.

In regard to (*b*), articles have been written for publication in the newspapers of the country drawing attention to the dangers of hydrophobia and informing the pub'ic where to present themselves for anti-rabic treatment if they chance to have been bitten by a dog or other animal. Pamphlets have been written in all the official languages for distribution in schools. Through the medium of the Department of Education these pamphlets reach a very large number of homes throughout the country and opportunity is taken by the teachers when giving these papers to the children to explain to them in short and simple language the essential facts concerning the disease and its prevention.

This educative work is of great value, for in hydrophobia no less than in other communicable disease the intelligent co-operation of the public is essential to secure the success of preventive measures undertaken by the Government.

ERYTHROCYTOSIS IN ARTIFICIALLY-INOCULATED MALARIA

BY

G. de M. RUDOLF

AND

J. C. RAMSAY

(*Claybury Mental Hospital*)

(*Received for publication* 12 *September*, 1925)

In eleven cases of general paralysis inoculated subcutaneously with benign tertian malarial blood, Pijper and Russell (1925) found that an erythrocytosis occurred before the anaemia developed. In the graph showing the mean of the daily observations on these cases, the increase of the red cells occurs before the onset of the fever and is to the extent of about 750,000 cells per c.mm. In the charts of two cases, however, the erythrocytosis is shown to persist until the eleventh and twelfth days of the fever. The greatest increase recorded by these observers was 2,000,000 cells per c.mm.

On the other hand, R. M. Gordon (1925) found no increase of the red cells in three general paralytics inoculated with benign tertian malaria by means of anopheline mosquitos. Ben-Harel (1923) found in a series of 23 blood inoculations of *Proteosoma praecox*, in canaries, that the red cells diminished in numbers before the parasites were found in the blood stream.

The observations described in this paper were made upon cases of general paralysis inoculated with benign tertian malaria at Claybury Mental Hospital. Case No. 1 was inoculated by means of anopheline mosquitos by Lieut.-Col. S. P. James. The other cases were inoculated with defibrinated malarial blood by means of the method described by one of us elsewhere (1925). Cases Nos. 1 and 2 were females, 62 and 42 years of age ; cases Nos. 3 and 4 were males, 40 and 50 years of age.

The red cell estimations were made with the Thoma-Zeiss counting apparatus and the haemoglobin estimations by means of Oliver's haemoglobinometer. The blood was collected between 9.30 a.m. and 10 a.m. every day from cases Nos. 1 and 2, and at 5.30 p.m. from

cases Nos. 3 and 4. Three red cell counts were performed in cases Nos. 3 and 4, the averages being taken as the correct readings. In cases Nos. 1 and 2 the red cell count was invariably repeated several times if there was much variation from the previous day's count. The average was taken of the two counts which approached each other most nearly. These methods of counting the red cells were used on account of the great error there is in the usual red cell count, Gordon (1925) placing it at 500,000 cells per c.mm. The temperature of each patient was recorded every four hours unless it was above normal; it was then recorded each hour until normal was regained. No drugs, other than those mentioned on the charts, were given.

Red Cells. The results obtained from the red cell counts are shown on the charts. All four cases show very clearly an erythrocytosis preceding the anaemia. The relation of this increase of the red cells to the onset of the fever was very variable. In case No. 1 it occurred before and during the onset, in case No. 2 it coincided with the onset, in case No. 3 the greatest increase occurred after the fifth rise of temperature and in case No. 4 after the eighth. As the count was not commenced until six days after the onset of the fever in this case, it is possible that there had been an erythrocytosis previous to the one recorded.

This erythrocytosis is in agreement with the findings of Pijper and Russell, but not with those of R. M. Gordon or Ben-Harel. As neither of the two latter workers record observations made every day, it is possible that an erythrocytosis occurred between the observations. Cases Nos. 1 and 2 of the present series show that the erythrocytosis may persist for only a few days. Gordon does, however, describe one case on whom red cell counts were performed every day, but the estimations were not commenced until the ninth day of infection. There were then 6,000,000 red cells per c.mm. As this is a comparatively high figure for an untreated general paralytic in England, perhaps the count represents an erythrocytosis. In case No. 1 of the present series, 6,000,000 red cells per c.mm. were found on the tenth day of infection.

The duration of the anaemia is of interest. In cases Nos. 1 and 3 it persisted for at least 11 and 13 days after the commencement of the quinine. In case No. 4 it persisted for at least six days after the course of quinine had been started, becoming more

profound although no further febrile paroxysms had occurred. This case corresponds with one described by Gordon. In this patient, a female aged 13 years, the red cells continued to fall although the parasites had disappeared from the peripheral blood. In case No. 4 of the present series the same condition occurred. The lowest red cell count found in the present series was 1,300,000 cells per c.mm.

Recovery from the anaemia took place in a comparatively short time. In cases Nos. 1, 2 and 3, the red cells reached 5,000,000 per c.mm. in about three weeks. During this period the cells increased by 1,700,000 per c.mm. in case No. 1, by 2,600,000 per c.mm. in case No. 3, and by 3,300,000 per c.mm. in case No. 2. In these cases the degree of the anaemia did not influence the time required for normal to be regained. James (1920) gives a chart, after Ziemann, showing regeneration of the red cells after naturally-acquired malaria. In this case the normal was regained 16 days after the anaemia. Case No. 4 shows a more rapid recovery, 5,000,000 red cells per c.mm. being reached in about a week after the anaemia. In cases Nos. 1 and 2, both of whom received neo-kharsivan in addition to quinine, the regeneration of the red cells was no more rapid than in case No. 3, and less rapid than in case No. 4. Neither of the two latter cases were given this preparation. As neosalvarsan has a definite parasiticidal action on *Plasmodium vivax*, both in the naturally-acquired infection (D'Esterre, 1920; Nieuwenhuyse, 1921; Johnson, Gilchrist and Hay-Michel, 1921) and in the artificially-inoculated form (Pijper and Russell, 1924), it might be expected that the parasites would be destroyed more rapidly in cases Nos. 1 and 2, than in cases Nos. 3 and 4 and, consequently, the red cells would be regenerated more rapidly. This did not occur.

In cases Nos. 3 and 4, red cell counts were continued after the normal had been regained. In each case an erythrocytosis was found. The highest count obtained in case No. 3 was 5,920,000 cells per c.mm., and in case No. 4, 6,280,000 per c.mm. This post-anaemia increase of red cells has been observed, according to Ben-Harel, in the naturally-acquired infection, and this worker noted it in canaries who had suffered from infection with *Proteosoma praecox*. In two of the birds the erythrocytosis persisted for a little over six

months. Of the two cases under review, No. 3 showed a count of 4,990,000 cells per c.mm., with a haemoglobin percentage of 65 on July 10th, nearly six months after the last rigor, while No. 4 gave a count of 5,870,000 cells per c.mm. and haemoglobin at 90 per cent. on July 22nd, exactly six months after the last rigor. Although the highest point reached in these two cases was not permanent, in neither case was the count lower than it had been before the malarial anaemia. In case No. 4 it was about one million higher.

Haemoglobin and Colour-Index. In cases Nos. 3 and 4 estimations were made of the haemoglobin. The results, with the colour-indices, are shown on the charts. The haemoglobin does not vary to the same extent as do the red cells and the colour-index remains low. The colour-index in case No. 4 is of the secondary anaemia type throughout. This corresponds with the two cases reported by Gordon. This agreement is not seen in case No. 3, for the index exceeds unity on two occasions in this patient. In both cases it falls very low at certain periods.

Number of Parasites. In cases Nos. 1 and 2 the relative number of parasites was found by counting the total number of asexual forms in 100 fields of the microscope, using 1/12th in. oil-immersion objective and thin blood-films. Although the actual number of asexual parasites was very different in the two cases, both patients show that there is a tendency for the temperature to vary with the number of parasites in each patient. The temperatures of the two cases were recorded every hour when above normal, at other periods every four hours. In the cases investigated by Pijper and Russell the temperatures were recorded every four hours throughout. In their cases there is no clear relationship between the number of parasites and the degree of fever.

Previous Malaria. Three of Pijper and Russell's nine cases were known to have had previous attacks of malaria, whilst the remainder had a doubtful history with regard to this point. Of the present series, cases Nos. 1 and 2 are known not to have suffered from the infection previously. It is clear, therefore, that the increase of the red cells preceding the anaemia does not necessarily bear any relation to previous malaria, for the increase was found both in patients who had suffered from previous malaria and in those who had not.

Sex. As the increase of the red cells preceding the anaemia was found in both female (Nos. 1 and 2) and male (Nos. 3 and 4) cases, the erythrocytosis is not dependent upon the sex of the patient.

Mode of Inoculation. Case No. 1 was inoculated by means of anopheline mosquitos, the remaining patients by means of subcutaneous injection of malarial blood. Pijper and Russell's cases were inoculated by the latter method. The pre-anaemia erythrocytosis was found in all the cases. The increase of the red cells is not dependent upon the method of inoculation, therefore, so far as these two methods are concerned.

SUMMARY

(1) In four cases of general paralysis inoculated with benign tertian malaria an erythrocytosis was found to precede the anaemia. The erythrocytosis occurred whether the inoculation was performed by means of mosquitos or by subcutaneous injection of malarial blood. It was independent of the sex of the patient and of a history of previous malaria. The succeeding anaemia occurred, or persisted for several days after the cessation of the fever. Regeneration of the red cells was complete within three weeks, although the degree of anaemia was very different in the four cases. An erythrocytosis was found to follow the anaemia.

(2) The haemoglobin was found, in two cases, to vary with the red cells, but regeneration was less rapid. The colour-index was, as a rule, of the secondary anaemia type. At certain periods it became as low as ·5.

(3) In two cases the number of parasites was found to vary approximately with the degree of fever, although the number of parasites was very different in the two cases.

Our thanks are due to Dr. G. F. Barham for permission to publish the above observations from the records of Claybury Mental Hospital, and to Dr. F. Paine for his kind assistance.

We are again indebted to Lieut.-Col. S. P. James for his kind advice.

REFERENCES

Ben-Harel, S. (1923). Studies on Bird Malaria in relation to the Mechanism of Relapse. *Amer. Journ. Hygiene*, Vol. III, p. 652.

D'Esterre, J. N. (1920). Note on the Treatment of Recurrent Malaria by Novarsenobenzol. *Lancet*, Vol. II, p. 552.

Gordon, R. M. (1925). Changes in the Blood in Primary Malaria. *Ann. Trop. Med. & Parasitol.*, Vol. XVIII, p. 595.

James, S. P. (1920). Malaria at Home and Abroad. London.

Johnson, J. P., Gilchrist, K., and Hay-Michel (1921). On the action of certain special preparations on Malarial Parasites and their employment in the Treatment of Malaria. *Brit. Med. Journ.*, 1921, Vol. I, p. 80.

Nieuwenhuyse, A. (1921). Neosalvarsan in Malaria. *Nederl. Tijdschr. v. Geneesk.*, Vol. I, p. 3349.

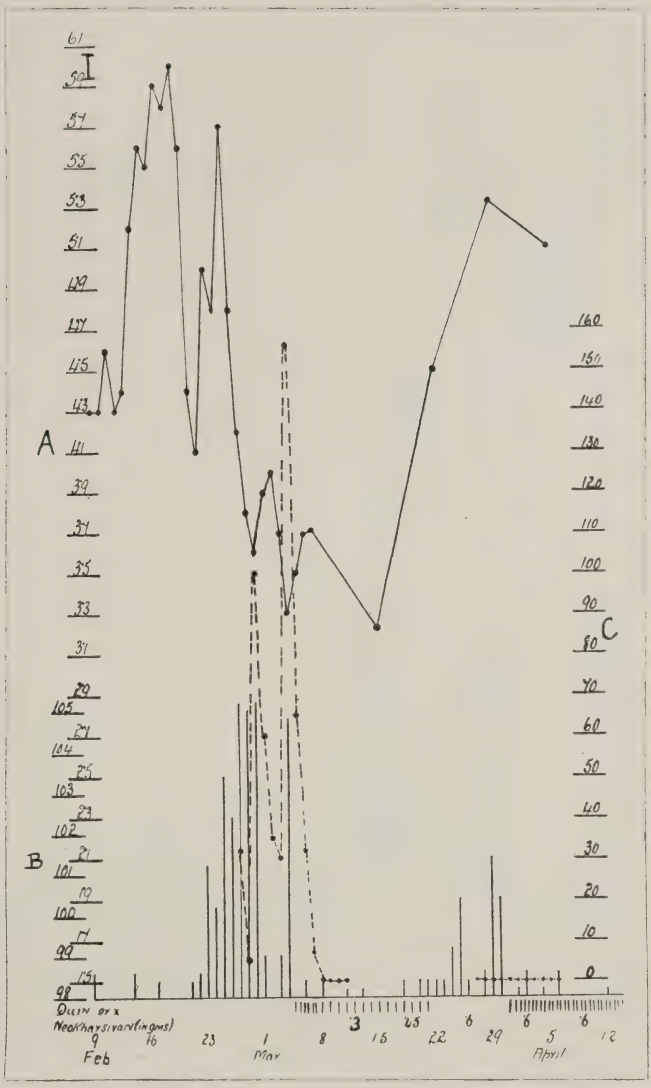
Pijper, A., and Russell, E. D. (1925). Observations on Inoculated Malaria. *S. African Med. Rec.*, Vol. XXIII, p. 178. Also *Brit. Med. Journ.*, 1924, Vol. II, p. 620.

Rudolf, G. de M. (1925). Malarial Treatment of General Paralysis of the Insane. *Lancet*, 1925, Vol. I, p. 793.

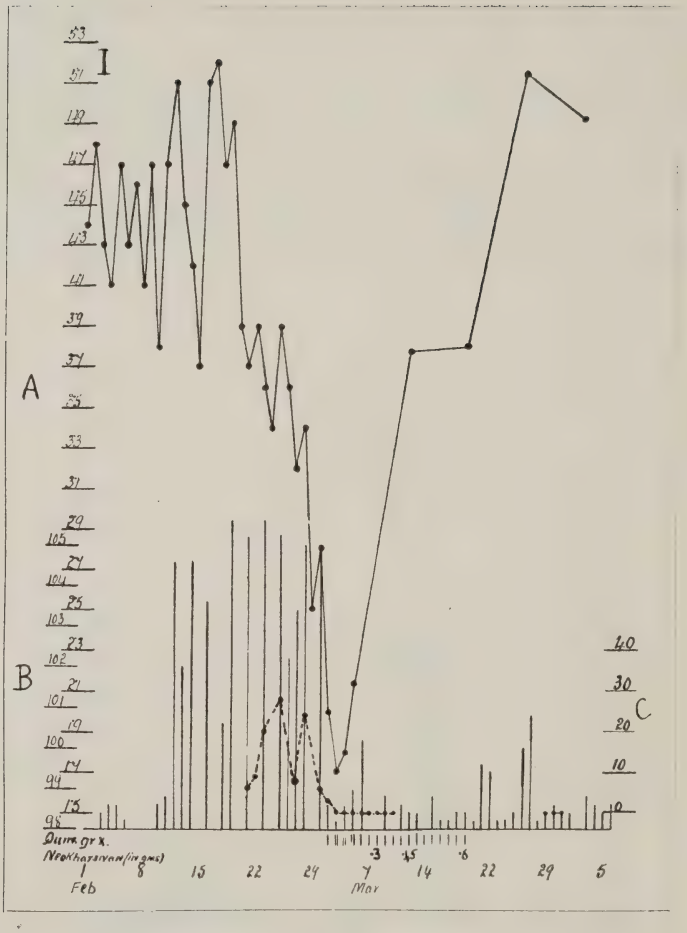
EXPLANATION OF CHARTS

A. Number of red cells in millions per c.mm.
B. Temperature in degrees, Fahrenheit.
C. Number of parasites in 100 fields.
D. Haemoglobin percentage.
E. Colour-Index.
I. Day of Inoculation.

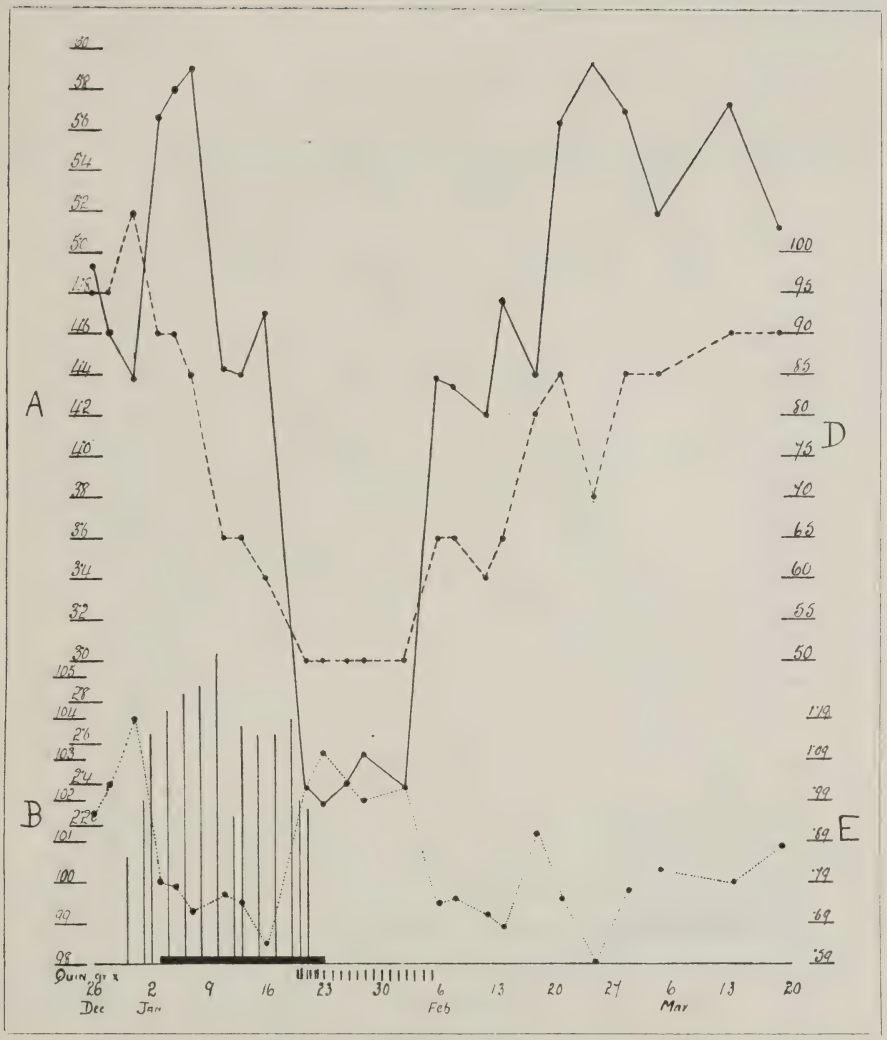
Red cells ———
Parasites – – – – – – and ▬▬▬
Haemoglobin – – – – – – –
Colour-index .....
Temperature |



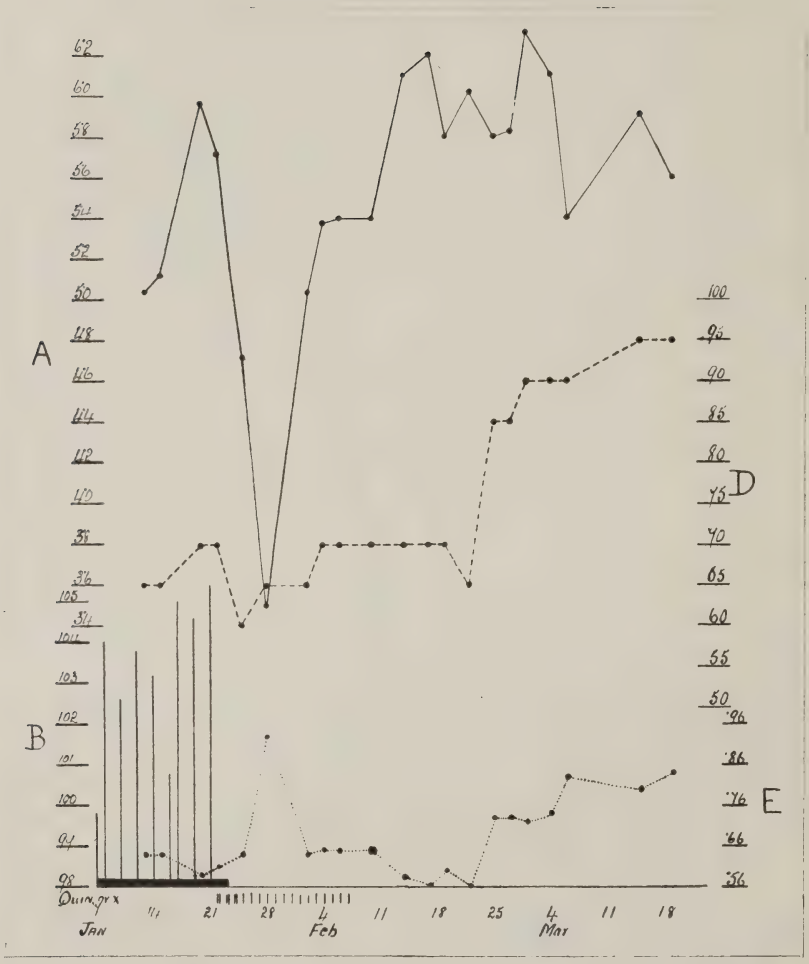
Case No. 1



Case No. 2



Case No. 3


A
B
D
E
62
60
58
56
54
52
50
48
46
44
42
40
38
36
105
104
103
102
101
100
99
98
100
95
90
85
80
75
70
65
60
55
50
96
86
76
66
56
Quinquina
JAN
11
21
28
4
11
18
25
FEB
4
11
18
MAR

CASE No. 4

THE EFFECT OF ANCYLOSTOME, ASCARIS, AND TRICHURIS INFECTIONS ON THE HEALTH OF THE WEST AFRICAN NATIVE

BY

R. M. GORDON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication* 28 *October*, 1925)

PLATE VII

TABLE OF CONTENTS

I. INTRODUCTION

The limitations of the work must first of all be made clear.

1. It deals solely with the West African male native, as studied at Freetown, and any conclusions drawn apply only to this race.

2. It is concerned only with the effects of the infection on the individual; it is not concerned with the importance of the infected individual as a propagator of the disease.

3. The lesions and symptoms produced by migrating larvae are considered to be a separate subject and are not discussed.

4. The effect of treatment is not considered; whereas this subject is obviously one of great importance, it appears to the writer that the first and most important consideration is the effect, if any, of ancylostome infection on different races of mankind.

5. No distinction is drawn between infection with *A. duodenale* and *N. americanus*. Darling and others (1920) and Darling (1922) show that *A. duodenale* is more important as a producer of anaemia than an equal infection with *N. americanus*. Adler (1925) gives the proportion of *A. duodenale* to *N. americanus* in Freetown as 1 : 10.

Malaria and ankylostomiasis are usually accepted as the most important diseases affecting natives in West Africa, and certainly entail a greater expenditure of money than any other two diseases. The effects of Malaria are definite, and the pathological changes which it produces in the individual can be demonstrated and classified, while the value of its eradication is too obvious to need argument. The position as regards ankylostomiasis appears to be entirely different ; the causal organism and its life cycle are established, and the value of its eradication appears generally accepted, but the effect of the worm on its host and the pathological lesions produced by it seem to be subjects evoking the widest differences of opinion, ranging from those who regard ankylostomiasis in general as having little effect on the human host, to the other extreme, which considers that any infection, however light, is responsible for illness of the individual, and calls for immediate treatment ; between these two extremes are to be found numerous observers who consider that a certain concentration of worms must be present in the gut before any symptoms appear. Unless it is conceded therefore, that any infection, however small, is pathogenic, it is obvious that any attempt to define the pathogenicity of ankylostomiasis must include a statement showing the degree of infection of the individuals considered ; it is doubtful if any value can be attached to mere comparisons of infected and uninfected individuals, such comparisons being frequently made and often illustrated with photographs showing poorly developed individuals suffering from ancylostome infection as diagnosed by the finding of ova (the number not being stated) in the stool, and well-developed, athletic-looking individuals free from infection. The two photographs accompanying the present article represent, in the one case, six boys selected at random from amongst the heaviest infections, and in the other case, six boys selected at random from amongst the negative or lightly infected group ; if anything, it is the heavily infected group which appears to show the best physique. In the enormous bibliography of hook-worm

disease definite figures of the degree of infection of the cases considered are surprisingly few, this lack of figures being, of course, largely due to the fact that until the publication of Stoll's (1923) method of estimating the number of ova in a given sample of faeces, there was little uniformity of opinion as to what constituted a ' light ' or ' heavy ' infection, and it is probably this lack of uniformity of opinion that has led to the surprising diversity of statements concerning the pathogenicity of ankylostomiasis.

Another difficulty encountered is that the literature dealing with ankylostomiasis appears almost entirely to ignore concomitant infections with other gut helminths, even when the pathogenicity of such helminths is admitted by the authors of the publication.

During the first part of the present work, which consisted in examining prison cases, the writer was impressed with the high proportion of Ancylostome cases which were also infected with Ascaris or Trichuris or both ; in subsequent examinations, therefore, a count was kept of these ova, and a classification made of the cases on the same lines as in the ancylostome work. Infection with the larvae of *Strongyloides stercoralis* was also common in Freetown, as noted by Maplestone (1924) ; they are not recorded here owing to the difficulty of estimating the degree of infection, which varied enormously with the consistency of the stool. Ova of a cestode (probably *T. saginata*) and on one occasion those of *S. mansoni* were also noted ; both infections were rare and when seen the ova were too few in number to be worth estimating.

Granted that ankylostomiasis is pathogenic, there still remain great differences of opinion as to the manner in which this pathogenicity manifests itself in the individual and what lesions, if any, the worm produces in the gut. Thus a perusal of the ' Rockefeller Bibliography of Hookworm Disease ' (1922) shows that it tabulates articles on almost every conceivable sign and symptom ranging from arthritis to night-blindness. The present writer attempted to compile a table summarising the views of modern authorities on the subject, but it was found that such a table became hopelessly unwieldy, as it had to include columns for almost every system and organ of the body. Most, though not all, authorities are, however, agreed *that ancylostome infection adversely affects the host by producing* (1) *Anaemia,* (2) *Poor physique,* (3) *Mental dullness,* (4) *Lack of*

energy. It is with these four points that the present paper, which deals with 137 natives, of whom 114 (83 per cent.) were infected, is concerned.

The number of cases dealt with is small and it may at first seem unnecessary to add them to the already overburdened literature on the pathogenicity of ankylostomiasis, but the information concerning these cases has been made as comprehensive and as exact as possible, whereas, as already stated, by far the greater proportion of published literature on this subject lacks figures showing the degree of infection of the cases under consideration and abounds in statements associating this or that symptom with ancylostome infection, such statements being unsupported by any proof except the finding of an unestimated number of ancylostome ova in the stool of the patient. It is interesting in this connection to consider the following statements. Stephens (1916), quoting Löbker and Bruns (1906), writes ' Whilst up to modern times it has been generally maintained that the great majority of worm diseases cause more or less marked symptoms, the exact investigations of the last few years have made it plain that the great majority of people with worms are not only perfectly healthy, but the most careful clinical observations show no single sign of any ill-effect of the intestinal parasites on the health of the host (Löbker and Bruns).' Clayton Lane (1917) points out that the reference date of Löbker and Bruns is 1906 and dismisses the whole statement as being out of date ; referring to the Rockefeller Sanitary Commission for the Eradication of Hookworm Disease and the Rockefeller International Health Commission, Lane continues as follows : ' It is obvious that the opinions based on this enormous experience, which in the five years of the Sanitary Commission's existence, covered over 1,300,000 persons, carries a weight borne by those of no other person or scientific body in the world ; and that should any individual elect to differ, the onus of fully justifying his own attitude must lie with himself.' Such a statement as this has the natural effect of deterring the individual observer from adding his small quota to so vast an array of figures ; no one who has studied the literature of the Rockefeller Commission can but be impressed with the magnificent work published and the splendid results obtained by this body of investigators. Yet at the time of Lane's paper (1917) the present

writer is unaware of any paper published by the Rockefeller Commission which dealt with the degree of infection of the persons considered, except in a few instances where a rough comparison is made by the general appearance of the number of ova in the stool ; the whole of the 1,300,000 cases referred to are only considered as a comparison of infected and uninfected individuals ; thus Strong (1916) investigated the effects of ankylostomiasis on the physique and mentality of 115 school children and drew the following conclusions.

' (1) *Our figures show that hookworm disease interferes with physical development. Treatment alleviates this condition to a considerable extent. Apparently young children can regain most of the physical conditions, if not all, which they have lost due to the infection.* But the data do also very strongly suggest that the severer the infection and the longer it persists, the less likely it is that the child will ever reach his normal physical development.' He draws the following conclusions as a result of the mental tests. ' The figures show, then, that hookworm disease unmistakably affects mental development. *Treatment alleviates this condition to some extent but it does not, immediately, at least, permit the child to gain as he would if he had not had the disease. And the figures apparently further show that prolonged infection may produce prolonged effects upon mentality—effects from which the individual may never entirely recover.*' No estimation was made in these cases of the concentration of ova in the stools or the number of worms in the intestines of the children. The consideration of these cases, therefore, resolves itself essentially into a comparison of infected and uninfected cases.

Our present knowledge shows that such a comparison is liable to very wide error. To quote one instance only : Hill (1923) records 282 cases, of whom 142 with 1 to 2,099 ova per gm. showed no symptoms, while 57 with 2,100 to 5,099 only showed very slight and indefinite symptoms. A few months prior to the publication of this paper, Lane (1923b) wrote as follows : ' It is at least certain that there is a growing mass of evidence that the so-called carrier is improved in health and working power by disinfestation, and I know of no published evidence suggesting that there is any limit below which infestation is immaterial. Statements of personal belief on this matter appear misplaced. The fact seems to be that there is

no satisfactory evidence, either for or against the belief that the lightest infestations are immaterial to their host.' This statement would appear to the present writer to be a very fair summary of the state of affairs at the time the paper was published, except that the latter portion of the statement seems to negative the value of the remarks as regards the so-called carrier being improved in health and working power by disinfestation, and would also appear to indicate that Lane has considerably modified his previous views, as in 1919 he states : ' Each year adds to the accumulated facts indicating that even light infections are a definite handicap to growth in wisdom and stature, and to the full possession of that modicum of health and wealth which makes life worth living.'

A search of the literature has shown that the following are the more important papers dealing with the effects of ankylostomiasis on the host when the degree of infection is approximately known. (1) Darling, Barber and Hacker (1920) ; (2) Darling and Smillie (1921) ; (3) Smillie (1922) ; (4) Hill (1923) ; (5) Cort, Payne and Riley (1923) ; (6) Mhaskar and Kendrick (1923) ; (7) Cort (1924) ; (8) Mhaskar (1924) ; (9) Chandler (1925) ; (10) Stoll and Tseng (1925). The work of these writers on the pathogenicity of ankylostomiasis is almost entirely concerned with the question of anaemia, and there appears to be a great need of further investigation as to its effects on the health and mentality of different native races.

It will be noted that the above summary refers only to ankylostomiasis ; the writer is unaware of any publication dealing with the pathology of Ascaris or Trichuris infection based on a knowledge of the intensity of the infection in the individuals concerned.

II. CASES AVAILABLE, CLASSIFICATION OF CASES, COLLECTION OF MATERIAL

Only cases which were under constant supervision and discipline were selected. They were chosen from amongst three sections of the native male community in Freetown. (1) 49 youths aged 10 to 22 (average age 18) attending school, the majority as boarders ; (2) 40 City Police of all ages from 23 to 50 ; (3) 48 gaol prisoners of all ages from 17 to 49. One hundred and thirty-seven cases

were thus examined, the work occupying about four hours daily for a period of eight months.

In every case the examination, as regards physique, mentality and energy, was carried out by the officer in charge of the institution concerned, according to a fixed scheme previously carefully discussed and agreed upon between the officer and the Laboratory. In order to avoid any bias that might result from any previous knowledge, the officers in charge of the institutions did not know the degree of infection of the inmates, and the Laboratory was unaware of the classification of the cases it was examining. The haemoglobin percentage, as shown by a Talquist scale, was estimated by the writer who was not aware of the identity of the particular case he was at the time investigating. In addition to the haemoglobin estimation, each case was examined as regards three other categories : (1) Physique and general fitness, (2) Mentality, and (3) Energy, and placed in order of merit, in an *A*, *B* or *C* class in each of these three categories.

The physical examination requires no special comment ; it was not necessarily a medical examination (though the doctor's report was usually available) but consisted in placing the native in class *A*, *B*, or *C* according to his general physique and fitness when seen stripped.

The mental examination was not directed to ascertain how much the individual knew, but rather to discover his mental alertness and ability to learn ; thus a boy at the head of his class might be placed in category *C* because he had gained his position at the head of the class by remaining behind when brighter boys had been moved on. The mental classification was comparatively simple in the case of the boys and police who were being regularly taught and questioned, but in the case of the gaol prisoners, it had to consist of an examination of the man's mental ability as judged by the answers he gave to a series of simple questions.

Energy was defined as the keenness with which an individual attempted any mental or physical task allotted to him ; it was frequently found that this classification gave very different results from the other two ; thus a native might be classed as physically poor (*C*) and mentally dull (*C*), but as regards energy very good (*A*) because, though his ability to perform any task, whether physical

or mental, allotted to him, was bad, yet the energy he showed in trying to perform the task was excellent.

Strong (1916) has published a long and very carefully detailed account of his investigations on the effects of ankylostomiasis on the physique and mentality of 115 school children living in a ' hookworm infected county.' He divided the children into five groups according to whether ' (1) They were not infected (Group A) ; (2) They were infected but not treated (Group B) ; (3) They were infected and later cured (Group C) ; (4) They were infected and treated but not completely cured (Group D) ; or (5) They were infected and treated but the final condition of their infection could not be determined (Group E).'

The tests applied to these groups were extremely ingenious and interesting, but appeared too complex for use in a native community such as Freetown. The question of Strong's conclusions from these tests has already been dealt with (see Introduction).

The classification having been completed, the case was issued with a faeces container marked with his number and, as a rule, the specimen was passed under the observation of an individual appointed for the purpose ; the specimen was then dispatched to the Laboratory and examined according to the technique described later. The information regarding the case was therefore tabulated on two forms, one form being filled in by the Laboratory and the other by the person in charge of the school, barracks, or gaol, the two portions being compared together only when the work was completed. In the case of the 48 gaol prisoners only ancylostome ova were counted and facilities for haemoglobin estimation were not available ; in the case of the 40 city police and the 49 youths attending school, Ascaris and Trichuris ova were counted in every case, and in 84 of the 89 cases the haemoglobin percentage was also estimated.

III. TECHNIQUE OF ESTIMATING THE NUMBER OF OVA IN THE FAECES

Stoll (1923) published a technique for counting hookworm eggs in faeces, and in 1924, he published a further paper in the conclusions of which he states ' A relationship of approximately 1 : 2 : 4 is found to exist in general between the weighed amounts of formed, mushy (unformed), and diarrhoea faeces, passed per day. This affords an

easy interpolation by which to bring counts made on faeces of any of the three categories to a similar plane, the basis of formed faeces, so that they can be compared *inter se*.' The reader is referred to these two papers for details of the technique, which was exactly adhered to except for the following trifling modifications and additions. (*a*) Stoll balances the container and its faeces on the scales and removes 3 gm. into a large test-tube containing three glass beads and 45 c.c. of $\frac{N}{10}$ NaOH. The writer found it simpler and less messy to stir thoroughly the specimen of faeces and weigh out 3 gm. into a small metal container previously balanced on the scales, and then slide the metal dish, containing the 3 gm. of faeces, into the large test-tube and add 45 c.c. of $\frac{N}{10}$ NaOH and three glass beads. The metal dish aids greatly in rapid emulsification of the faeces when shaking the tube, and is also very convenient for dealing with liquid faeces. The dish referred to is made of the thin tin used in sealing boxes of cigarettes sent to the tropics ; the tin should be cut into a square and the four corners inbent so as to form a rectangular water-tight trough measuring about 2 in. $\times$ $\frac{3}{4}$ in. $\times$ $\frac{1}{2}$ in. (*b*) Stoll says the diluted faeces ' was immediately sampled with a pipette graduated at 0.15 c.c.' It will be found in practice that in some cases faecal debris adheres to the outside of the pipette and interferes with accuracy by draining into the fluid which is being discharged on to the counting glass ; in order to avoid this error it is advisable to draw up fluid past the 0.15 c.c. mark, rapidly wipe the outside of the pipette with a wisp of wool, discard the excess of fluid and discharge the remaining 0.15 c.c. on to the counting glass. It is necessary to perform this operation very rapidly in order to avoid sedimentation occurring. (*c*) Stoll measures 0.15 c.c. of the diluted faeces on to a large slide and covers this with a single 22 $\times$ 40 mm. coverslip. The writer found it more convenient to use three amounts of 0.05 c.c. and count each separately. (*d*) A small point, but one which, if neglected, interferes with accuracy, is that the surplus uncovered fluid lying along the edge of the coverslip should be first examined, otherwise the rapid drying up which occurs in the tropics will render the counting of ova difficult. (*e*) At the commencement of the work it was found that certain bodies, probably derived from some

vegetable in the native dietary, imitated unfertilised Ascaris ova with such extreme fidelity, both as regards size and morphology, that they necessitated careful examination with the high power in order to differentiate them from ova ; it was therefore decided to include only ' fertile ' Ascaris, Ancylostome or Trichuris ova in the counts. (*f*) Chandler (1925) advocates examining uncovered preparations, as by this method one can blow aside obscuring flocculi of undissolved faecal debris, while doubtful egg-like objects can be verified by blowing on the fluid and causing them to roll about. The writer tried this method prior to the publication of Chandler's paper and abandoned it because it was found that any current of air occurring in the laboratory caused the ova in the fluid to move about and lose their position in the field which was being counted.

IV. ACCURACY OF STOLL'S METHOD AND ITS VALUE IN COMPARING THE DEGREE OF INFECTION IN DIFFERENT INDIVIDUALS

Stoll (1923), when describing his method of estimating the number of hookworm eggs in faeces, claimed that this technique was ' accurate to within 10 per cent.', while Maplestone (1924), who tested the accuracy of the method by control counts of ova made with saturated salt solution, and cultures of larvae made from the same faeces sample, came to the conclusion that the method was ' not accurate to within 10 per cent.'

In order to compare together the ovum content of stools of different consistencies, Stoll (1924) advocates the taking of a formed stool as a standard and multiplying the ovum content of a mushy stool by two and a liquid stool by four (this being the method adopted in the present work) ; Chandler (1925) regards mushy stools as normal for the Indian native, and therefore divides the results obtained from formed stools by two and multiplies those of liquid stools by two ; whatever the accuracy, therefore, of any technique for estimating the number of ova in a single given sample of faeces, it is obviously absurd to discuss the finer points of accuracy of such a technique when applied to the estimation of the average number of ova in a series of stools varying in consistency, for the definitions ' formed ' ' mushy ' (or ' semi-solid ') and ' liquid ' are not fixed

definitions, and an examination of even as few as fifty specimens will convince any worker on this subject that every variation between these standards is to be found, where one observer will define a stool as mushy and multiply his result by two, another will call it liquid and multiply his result by four. A clear-cut distinction must, therefore, be drawn between estimating the number of ova in a given sample of faeces, and the comparing together of the average number of ova passed by different individuals on different occasions ; it is obvious that the first can be performed to any degree of accuracy if sufficient time and care are expended ; thus Stoll's method admittedly does not detect the presence of ova in the stool if less than 100 per gm. be present. Therefore all single worm infections will be missed. Now Lane's (1923-1925) method will detect less than this concentration and clearly, therefore, his method is more accurate and, therefore, more suitable for such an estimation. The present work, however, is not concerned with such an estimation, but is concerned with the comparison of the ovum content of the stools of different individuals on different occasions ; now the ovum content of such stools may vary according to the quantity of faeces passed (presumably the less food taken the smaller the quantity of faeces and the greater the concentration of the ova), the consistency of the faeces, and the fecundity of the worms. When such a number of uncontrolled factors exist the more minute points of accuracy are of little importance ; what is required is a method whereby negative (by negative is meant less than 100 ova per gm.), moderate, and heavy infections can be compared together, and for this purpose Stoll's technique seems well adapted. That such comparative accuracy is obtainable by Stoll's method appears to be proved by the figures given in Table I. It is of interest to note that the counts of Ascaris and Trichuris infections do not correspond nearly as closely as do those of anclystome infections, a possible explanation being that fewer worms are present in the two former infections and that the variations in the fecundity of the worms are, therefore, more clearly noticeable.

For further particulars regarding the comparative accuracy of Stoll's (1923) and Clayton Lane's (1923-1925) method, the reader is referred to articles by Sweet (1924) and Chandler (1925b).

Tables Ia, Ib, Ic.

Showing the number of ova actually counted in 0·01 gm. of faeces in the same individual on three occasions, amongst a series of 114 positive Ancylostome cases, 16 positive Ascaris cases, and 39 positive Trichuris cases. Solid specimens are shown by black figures. When a specimen was 'mushy' or 'semi-solid' the result has been × 2, and when liquid × 4. For the purpose of reference the numbers given to the cases have been adhered to throughout the tables and text.

Table Ia.—One hundred and fourteen Positive Ancylostome Cases.

Note.—The occurrence of a decimal point in a few of the cases is due to the figure being the average of a series of counts on the same specimen.

Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1st examination...	32	**0**	**3**	**24**	**50**	**38**	**2**	**6**	0	**3**	**0**	**13**	**4**	26	**13**	**3**	2	**3**	**9**	**7**	**10**	**8**	**9**	**17**	**54**	**2**	**11**	**3**	**5**
2nd examination	32	**2**	**1**	**44**	**67**	**38**	**1**	**3**	**1**	**4**	**4**	**11**	**1**	**12**	8	2	0	**1**	**8**	**8**	**7**	**6**	**29**	**23**	**50**	**4**	**9**	**8**	**4**
3rd examination	28	**0**	**2**	**49**	**49**	**30**	**1**	**4**	**1**	**3**	**2**	**9**	**6**	28	**35**	**0**	6	2	**9**	6	12	**6**	**4**	**18**	**53**	**5**	**9**	**17**	**5**

Case	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58
1st examination...	**4**	**6**	**26**	**203**	**41**	128	0	**122**	**154**	**21**	**16**	**12**	**15**	**304**	**272**	**50**	**10**	**196**	**53**	**38**	**102**	**75**	**30**	**97**	**122**	**92**	**15**	**65**	**27**
2nd examination	2	**9**	**19**	**122**	**48**	**140**	**0**	**110**	**127**	**27**	**17**	**18**	**22**	**313**	**295**	**42**	**4**	**62**	**48**	**42**	**70**	**111**	**43**	**81**	**122**	**114**	**13**	76	**53**
3rd examination	0	**5**	**18**	**143**	**43**	**144**	**4**	**61**	**117**	**37**	**22**	**17**	**20**	**488**	**126**	**26**	**13**	**135**	**50**	**77**	**109**	**130**	**40**	**70**	**136**	**130**	**11**	**70**	**31**

Case	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87
1st examination...	**1**	**175**	**16**	**40**	2	**5**	**21**	**3**	**5**	8	**0**	**5**	**271**	**1**	**43**	**4**	**29**	128	**66**	13	**2**	23	0.5	5	**4.5**	**46**	**9.5**	**4.5**	**53**
2nd examination	**4**	**155**	**17**	**25**	**3**	2	**11**	**3**	**7**	**13**	**0**	**2**	**188**	**7**	**42**	2	**37**	178	56	**15**	**8**	7	0	**6**	4	**51**	32	6	**39**
3rd examination	10	**79**	**13**	**29**	**0**	**3**	**19**	**0**	**4**	**12**	**1**	3	**174**	**5**	**57**	**4**	**34**	96	**55**	12	**3**	22	3	16	**5**	**88**	32	**5**	**50**

Case	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114
1st examination...	**23**	**70**	5	**0**	22	**0**	**2**	10	**0.5**	0	4	**23**	**92**	**1**	**27**	**6**	**79**	**14**	19	**2**	**3**	**4**	30	1	**34**	1412	**141**
2nd examination	20	**80**	**15**	**0**	16	0	2	4	**1**	**2**	**13**	**43**	**165**	**2**	**76**	8	**77**	**13**	**33**	1	**12**	**7**	39	**2**	20	1070	**210**
3rd examination	**29**	**31**	12	16	**54**	**16**	**1**	4	**0**	**0**	32	**25**	128	**1**	...	0	**65**	**19**	...	2	**5**	8	...	4	18	...	**182**

Table Ib.—Sixteen Positive Ascaris Cases.

Case	3	115	116	30	38	43	51	57	117	64	65	66	72	74	119	75
1st examination	**44**	**115**	48	**164**	**507**	**96**	**31**	**102**	**61**	**5**	**34**	**192**	**63**	**218**	**21**	**16**
2nd examination	**12**	**103**	**53**	214	**317**	**103**	**42**	48	88	2	**37**	**126**	**134**	92	**31**	**16**
3rd examination	**30**	**88**	**27**	144	**455**	**291**	**44**	**124**	**134**	**4**	**54**	**214**	**64**	**275**	**30**	**47**

Table Ic.—Thirty-nine Positive Trichuris Cases.

Case	1	120	6	7	8	11	12	14	15	17	19	20	21	22	23	24	121	26	122	31
1st examination	0	**1**	2	**1**	**1**	**0**	**0**	8	**10**	**1**	**1**	**0**	**3**	**4**	**0**	**0**	0	**6**	**2**	**5**
2nd examination	0	**0**	**2**	**0**	**1**	**2**	**3**	**1**	4	**1**	**0**	**19**	**1**	**2**	**1**	**2**	**2**	**0**	**0**	**0**
3rd examination	2	**0**	**2**	**2**	0	**0**	**1**	2	**16**	0	**1**	48	4	**3**	0	**0**	**1**	**1**	**2**	**3**

Case	35	38	43	49	123	60	117	62	63	64	68	124	125	69	73	74	119	75	76
1st examination	0	**0**	**52**	**1**	**3**	**1**	**3**	**0**	**0**	**3**	0	**1**	**3**	**1**	**5**	**14**	**5**	**0**	2
2nd examination	**2**	**1**	**48**	**4**	**2**	**6**	0	**0**	**0**	0	**0**	**3**	**2**	**0**	**6**	12	**4**	**0**	2
3rd examination	**0**	**0**	**134**	**5**	...	**7**	**0**	**2**	**1**	**1**	**1**	4	0	**2**	**17**	**10**	**3**	**3**	2

V. EFFECTS OF ANCYLOSTOME, ASCARIS, AND TRICHURIS INFECTIONS ON THE (*A*) HAEMOGLOBIN PERCENTAGE, (*B*) PHYSIQUE AND GENERAL FITNESS, (*C*) MENTALITY, (*D*) ENERGY, AND (*E*) URINE, OF WEST AFRICAN NATIVES

In the results which follow, the degree of infection is expressed as the average number of ova per gm. of faeces, this figure being the mean of three Stoll counts on each individual case, except that five cases amongst the 137 examined for ancylostome infection, and two each amongst the eighty-nine Ascaris and eighty-nine Trichuris series were only examined on two occasions, the natives concerned having left the Institution before the third examination was completed. The counts were usually made at intervals of four to seven days, but occasionally longer periods intervened. The expression ' average number of ova per gm. of faeces,' when applied to a number of cases constituting a group or class, includes negative cases, that is to say, it is the figure arrived at by adding together the average number of ova per gm. of faeces of each member of the group and dividing the result by the total number of individuals in that group ; the same rule holds good for the heading ' average number of ancylostomes per individual.' The figures for the number of ancylostomes are, of course, only roughly approximate, but they are included in the tables as they would appear to give a more concrete idea of the degree of infection ; the estimation of the number of ancylostomes is based on the supposition that every forty ova per gm. of faeces represents one adult female worm ; this relation between ova per gm. and parent worm is given by Stoll (1923b) as 44 to 1, by Darling (1922) as 22 to 1, by Lane (1923) as 33 to 1, and by Davis (1924) as 85 to 1. To this figure must be added the proportion of male worms which is here estimated as two males for every three females (see Darling, Barber and Hacker (1920), Stoll (1923b), Adler (1925)). The final formula is, therefore, $\frac{x}{40} + \frac{2}{3}\left(\frac{x}{40}\right)$ where x is the number of ova per gm. of faeces. Figures of the number of Ascaris and Trichuris present in the gut are omitted, as there appears to be no authoritative statement as regards the average daily egg production of these two species, except those of Davis (1924) who, as the result of the examination, and subsequent treatment, of

sixteen positive Ascaris cases computes the average number of eggs per female, per gm. of faeces, to be 3,466, and Moosbruger, as quoted by Brumpt (1922), who, as the result of an autopsy, gives the Trichuris figure as seven ova per female per gm. of faeces.

The influence of Ancylostome, Ascaris and Trichuris infections will be considered as regards their effects on five conditions. (A) Haemoglobin percentage. (B) General physique and fitness. (C) Mentality. (D) Energy. (E) Urine.

(A) *Haemoglobin percentage.* It will be seen that Table II lends no support to the commonly accepted view that Ancylostome infection tends to lower the haemoglobin reading ; nor do Ascaris or Trichuris infections appear to have any influence, for it can be seen that the group with the higher haemoglobin reading actually contains a slightly higher average degree of infection than the group with the lower haemoglobin reading. It is perhaps unfortunate that the haemoglobin readings in the two groups approximate so closely, but this was unavoidable as the haemoglobin readings in all the West Africans examined fell between 90 and 70 per cent.

Table II.

Showing the percentage of individuals infected with Ancylostome, Ascaris, and Trichuris, and the average degree of infection, in each of two groups of West African natives classified according to the haemoglobin reading.

Groups based on Haemoglobin per cent.	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostomes per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A 81–90% Hb	57	86	19	47	3,670	1,878	125	21,100	42,630	2,230	150
B 71–80% Hb	25	84	16	36	2,890	1,947	118	23,100	19,500	933	120

The question now arises whether intense infection produces any marked change ; with the object of investigating this point the ten heaviest infections in the case of each worm are set forth in Table III.

Table III.

Showing the ten heaviest Ancylostome, Ascaris, and Trichuris infections observed amongst West African natives, and the haemoglobin reading for each case.

Ancylostome				Ascaris			Trichuris		
Case	Haemoglobin per cent	Number of ova per gm. of faeces	Computed number of Ancylostomes	Case	Haemoglobin per cent.	Number of ova per gm. of facees	Case	Haemoglobin per cent.	Number of ova per gm. of faeces
44	70	23,100	962	38	80	42,630	20	80	2,230
71	80	21,100	879	74	75	19,500	15	80	1,000
33	80	15,600	650	66	75	17,733	73	75	933
35	85	13,700	570	30	85	17,400	74	75	900
60	90	13,600	567	115	80	10,300	60	90	460
76	85	13,400	558	117	80	9,430	119	75	400
38	80	13,200	550	57	85	9,130	14	80	360
47	70	13,100	546	72	75	8,700	49	90	330
54	90	12,660	527	116	80	4,260	22	85	300
55	70	11,200	466	65	85	4,160	124	85	266

It will be seen from Table III that, broadly speaking, two-thirds of the heaviest infections in the case of each worm fall into the higher haemoglobin group; moreover, if we consider the haemoglobin content of those natives who were uninfected, we find that of the eighty-two cases in which haemoglobin readings were made, twelve were negative as regards ancylostomes, and of these eight fell into the higher haemoglobin group and four into the lower. Sixty-seven were negative as regards Ascaris, and of these forty-six fell into the higher haemoglobin group and twenty-one into the lower. Forty-six were negative as regards Trichuris, and of these thirty fell into the higher haemoglobin group and sixteen into the lower. Thus it appears that roughly two-thirds of the heaviest infections and two-thirds of the negative cases fell into the higher haemoglobin group, and this figure corresponds with the relative size of high and low haemoglobin groups amongst the total eighty-two natives examined, that is, 57 to 25.

From these facts it seems clear that there is no correlation between intensity of infection in the individual and the haemoglobin reading. It might, of course, be argued that all the natives under consideration exhibited some degree of anaemia ; this may be so but it must be borne in mind that in none of the eighty-two West African natives—whether infected or uninfected—chosen at random, was the haemoglobin reading more than ninety, so that in any case, if the readings in this series were less than normal, this anaemia cannot be due to any of the three worms under consideration.

Conclusions regarding the influence of Ancylostome, Ascaris, and Trichuris infections on the haemoglobin percentage of eighty-four West African Natives.

1. A group of individuals with a low haemoglobin percentage does not show a greater percentage of infected cases than a group with a higher haemoglobin percentage.

2. A group of individuals with a low haemoglobin percentage does not show a greater average degree of infection than a group with a higher haemoglobin percentage.

3. Individuals with a high degree of infection do not necessarily show a low haemoglobin percentage.

This tolerance, so far as ankylostomiasis is concerned, would appear to be shared by some, at any rate, of the Indian races. Thus Mhaskar and Kendrick (1923), working in the tea estates of Madras, report as follows :—' There is no correlation between the haemoglobin average and the number of hookworms harboured ; the presence of anaemia is not necessarily a sign of heavy infection.' Chandler (1925), using Stoll's technique, writes : ' In a study of 100 individuals in the Alipore Central Jail, Calcutta, sixty-seven of whom were infected with hookworm, but only six of whom had more than 1,000 eggs per gm. of faeces, no differences in haemoglobin percentage between the infected and uninfected individuals could be found.'

On the other hand, Stoll and Tseng (1925), working with Chinese cases, trace a definite connection between the number of ancylostomes harboured and the degree of anaemia ; it is important to note, however, that the haemoglobin percentage of sixty-four ancylostome-free cases only averaged 66.7 per cent. and concerning these they write : ' The malaria is held to account for the low average haemoglobin of the hookworm negatives, and probably also influenced the degree of anaemia in the hookworm positives.'

(B) *Physique and General Fitness.* From a consideration of the figures in Table IV it is clear that the percentage of positive ancylostome cases is approximately equal in all three groups; on the other hand, there appears at first sight to be a very definite relationship between the average degree of infection and the physical standard of the group in which the cases occur; on consulting the column of maximum infections, however, it will be seen that the maximum infections occurring in Group B and Group C are much higher than that in Group A.

Table IV.

Showing the percentage of individuals infected with Ancylostome, Ascaris, and Trichuris, and the average degree of infection, in each of three groups of West African natives classified according to their physique and general fitness.

Groups based on physique and general fitness	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostomes per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—32 For Ancylostomes, Ascaris and Trichuris—50	84	22	44	2,619	2,418	102	15,600	42,630	933	109
B (Moderate)	For Ancylostomes only—5 For Ancylostomes, Ascaris and Trichuris—31	83	16	39	4,381	1,651	335	36,830	17,733	7,800	183
C (Bad)	For Ancylostomes only—11 For Ancylostomes, Ascaris and Trichuris—8	84	0	62	9,420	0	340	124,000	0	2,200	392

It is obvious that when one is dealing with a comparatively small number of cases a single pre-eminently heavy infection may be sufficient to raise to a considerable extent the average degree of infection of the whole group; and on enquiring into the question it was found that the maximum infections in Group B and Group C, shown in Table IV, were in fact outstanding, as the next highest infection in Group B was 23,100, and in Group C 17,760. Turning to a consideration of the Ascaris and Trichuris infections it was

similarly discovered that in each case there was a pre-eminently high infection, viz., 42,630 Ascaris ova per gm. in Group A, and 7,800 Trichuris ova in Group B. A truer picture is, therefore, obtained by omitting these predominantly high infections, and this is done in Table IVA.

TABLE IVA.

Table IV modified by the omission of the four predominantly high infections.

Groups based on physique and general fitness	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostomes per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—32 For Ancylostomes, Ascaris and Trichuris—49	84	20	44	2,619	1,597	102	15,600	19,500	933	109
B (Moderate)	For Ancylostomes only—5 For Ancylostomes, Ascaris and Trichuris—30	83	16	37	3,453	1,651	86	23,100	17,733	1,000	144
C (Bad)	For Ancylostomes only—10 For Ancylostomes, Ascaris and Trichuris—8	83	0	62	3,054	0	340	17,760	0	2,200	128

It is seen from Table IVA that no definite correlation exists between the physical standard of a group and the percentage of infected cases, or the degree of infection occurring in that group.

Turning to the subject of the effect of intense infections, Table IV shows that the total number of cases examined for ancylostomes was 137; of these eighty-two (60 per cent.) fell into Group A, thirty-six (26 per cent.) fell into Group B, and nineteen (14 per cent.) into Group C. Eighty-nine natives were examined for Ascaris and Trichuris infections and were found to be distributed amongst the three groups in the following proportions; Group A, fifty (56 per cent.), Group B, thirty-one (35 per cent.), and Group C, eight (9 per cent.). It is now necessary to consider the distribution of the ten heaviest infections with each species of worm amongst the three groups, and to compare this distribution with that of the uninfected cases.

TABLE V.

Showing the ten heaviest Ancylostome, Ascaris, and Trichuris infections observed amongst West African natives, and the standard of physique and general fitness for each case.

ANCYLOSTOME				ASCARIS			TRICHURIS		
Case	Standard of physique	Number of ova per gm. of faeces	Computed number of Ancylostomes	Case	Standard of physique	Number of ova per gm. of faeces	Case	Standard of physique	Number of ova per gm. of faeces
113	C	124,000	5,167	38	A	42,630	43	B	7,800
43	B	36,830	1,537	74	A	19,500	20	C	2,200
44	B	23,100	962	66	B	17,733	15	B	1,000
71	B	21,100	879	30	A	17,400	73	A	933
114	C	17,760	740	43	B	16,630	74	A	900
33	A	15,600	650	115	B	10,300	60	A	460
35	B	13,700	570	117	A	9,430	119	A	400
60	A	13,600	567	57	A	9,130	14	A	360
76	A	13,400	558	72	B	8,700	49	B	330
38	A	13,200	550	65	B	4,260	22	A	300

From Table V it can be seen that the ten heaviest infections with Ancylostome, Ascaris, or Trichuris, are in each case distributed according to the size of the group. Amongst the ten most intense Ancylostome infections 40 per cent. occur in Group A, 40 per cent. in Group B, and 20 per cent. in Group C ; an examination of twenty-three natives who were not infected with Ancylostomes showed that 61 per cent. fell into Group A, 26 per cent. into Group B, and 13 per cent. into Group C. Of the ten heaviest Ascaris infections 50 per cent. occurred in Group A, and 50 per cent. in Group B ; and of the seventy-three cases negative for Ascaris, 53 per cent. fell in Group A, 36 per cent. in Group B, and 11 per cent. in Group C. Amongst the ten heaviest Trichuris infections 60 per cent. occur in Group A, 30 per cent. in Group B, and 10 per cent. in Group C ; fifty cases free from Trichuris infection occurred in the different groups in the following percentages, Group A, 56 per cent., Group B, 38 per cent., and Group C, 16 per cent. These figures, therefore, show no noticeable association between an intense infection with Ancylostome, Ascaris, or Trichuris, and a lowered standard of physique and general fitness.

Conclusions regarding the influence of Ancylostome infection on the physique and general fitness of 137 *West African natives, and that of Ascaris, and Trichuris infections on eighty-nine of the same cases.*

1. A group of individuals with a lower standard of physique and general fitness does not necessarily show a noticeably greater percentage of infected cases than a group with a higher standard of physique and general fitness.

2. A group with a lower standard of physique and general fitness does not necessarily show a noticeably greater average degree of infection than a group with a higher standard of physique and general fitness.

3. Individuals with a high degree of infection do not necessarily show a low standard of physique and general fitness.

(C) *Mentality*. It has already been stated in the Introduction that the mental examination was not directed to ascertaining how much the individual knew, but rather to discovering his mental alertness and ability to learn. In Table V are set out the percentage of infected cases, and the degree of infection, occurring amongst West African natives classified on this basis.

Table VI.

Showing the percentage of individuals infected with Ancylostome, Ascaris, and Trichuris, and the average degree of infection, amongst West African natives arranged in three groups according to their mentality.

Groups based on mentality	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostomes per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—8 For Ancylostomes, Ascaris and Trichuris—17	76	12	47	1,218	1,578	65	8,260	17,400	300	51
B (Moderate)	For Ancylostomes only—10 For Ancylostomes, Ascaris and Trichuris—43	85	19	42	4,050	2,350	132	23,100	42,630	1,000	169
C (Bad)	For Ancylostomes only—30 For Ancylostomes, Ascaris and Trichuris—29	85	21	45	5,193	1,523	392	124,000	17,733	7,800	216

The four predominant infections shown to be present in Table IV are, of course, also present in Table VI; the two Ancylostome infections and the one Trichuris infection occurring in Group C, and the predominant Ascaris infection in Group B. Omitting these four cases we have the results shown in Table VIA.

TABLE VIA.

Same as Table VI except that four predominantly high infections have been omitted.

Groups based on mentality	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostomes per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—8 For Ancylostomes, Ascaris and Trichuris—17	76	12	47	1,218	1,578	65	8,260	17,400	300	51
B (Moderate)	For Ancylostomes only—10 For Ancylostomes, Ascaris and Trichuris—42	85	17	42	4,050	1,391	132	23,100	19,500	1,000	169
C (Bad)	For Ancylostomes only—29 For Ancylostomes, Ascaris and Trichuris—28	84	21	43	2,553	1,523	128	21,100	17,733	2,230	106

These figures of Ancylostome, Ascaris, and Trichuris infections in relation to mentality require careful consideration; in the first place it is clear that the percentages of Ancylostome and Trichuris infections are about equal in all three groups; the percentage of positive Ascaris cases is higher in Groups B and C, than in Group A, but the difference is not marked and the number of positive Ascaris cases dealt with is small. If we now consider the average degree of infection in the different groups, it is seen that in the case of Ascaris infection it is equal in all three groups, but that the Ancylostome and Trichuris infections are noticeably more intense in Groups B and C, than in Group A. In the Ancylostome infections Group B shows nearly four times, and Group C twice, as heavy an average degree of infection as Group A; this can hardly be explained on the assumption that Ancylostome infection has exerted a deleterious effect on the mentality, for if this were so we would expect to find that Group C was more intensely infected than Group B, whereas the

reverse is the case ; a similar state of affairs is also shown by the figures dealing with Trichuris infection. Table VIA, therefore, tends to disprove the theory that any relationship exists between the mentality of a group and the percentage of Ancylostome, Ascaris or Trichuris infected cases, or the degree of infection, occurring in that group.

It will be seen from Table VI that the proportionate sizes of the three groups in the mental classification bear no resemblance to those of the physical classification shown in Table IV ; in the mental classification the 137 natives examined are distributed amongst the three groups in the following proportions : Group A, twenty-five (18 per cent.), Group B, fifty-three (thirty-nine per cent.), Group C, fifty-nine (43 per cent.). Whereas all cases were examined for Ancylostomes, only eighty-nine cases were examined for Ascaris and Trichuris infections ; of these seventeen (19 per cent.) occurred in Group A, forty-three (48 per cent.) in Group B, and twenty-nine (33 per cent.) in Group C. Group A, therefore, forms much the smallest group in the mental classifications, whereas it formed much the largest group in the physical classifications. It is necessary to bear this fact in mind when considering the group distribution of the ten heaviest infections shown in Table VII.

TABLE VII.

Showing the ten heaviest Ancylostome, Ascaris, and Trichuris infections observed amongst West African natives, and the standard of mentality for each case.

ANCYLOSTOME				ASCARIS			TRICHURIS		
Case	Standard of mentality	Number of ova per gm. of faeces	Computed number of Ancylostomes	Case	Standard of mentality	Number of ova per gm. of faeces	Case	Standard of mentality	Number of ova per gm. of faeces
113	C	124,000	5,167	38	B	42,630	43	C	7,800
43	C	36,830	1,537	74	B	19,500	20	C	2,230
44	B	23,100	962	66	C	17,733	15	B	1,000
71	C	21,100	879	30	A	17,400	73	B	933
114	C	17,760	740	43	B	16,630	74	B	900
33	B	15,600	650	115	A	10,300	60	B	460
35	B	13,700	570	117	B	9,430	119	B	400
60	B	13,600	567	57	C	9,130	14	B	360
76	B	13,400	558	72	B	8,700	49	B	330
38	B	13,200	550	65	C	4,260	22	A	300

Table VII shows that the distribution of the ten heaviest Ancylostome infections is, Group B, 60 per cent., Group C, 40 per cent. ; of the twenty-three natives not infected with Ancylostomes, 26 per cent. fell into Group A, 35 per cent. into Group B, and 39 per cent. into Group C. The distribution of the ten heaviest Ascaris infections was 20 per cent. in Group A, 50 per cent. in Group B, and 30 per cent. in Group C ; seventy-three cases negative for Ascaris occurred in the three groups in the following proportions :—Group A, 21.5 per cent., Group B, 48.5 per cent., Group C, 30 per cent. Amongst the ten heaviest Trichuris infections, 10 per cent. occurred in Group A, 70 per cent. in Group B, and 20 per cent. in Group C ; of the fifty natives not infected with Trichuris, 18 per cent. fell into Group A, 50 per cent. into Group B, and 32 per cent. into Group C. Analysis of these figures shows that the ten heaviest infections are distributed according to the size of the groups ; they also show that the proportion of cases amongst the ten heaviest infections occurring in the C, or mentally bad group, corresponds very closely with the proportion of cases occurring amongst uninfected natives in the same group. Intense infection, therefore, with Ancylostome, Ascaris, or Trichuris, does not necessarily result in a lowered standard of mentality.

Conclusions regarding the influence of Ancylostome infection on the mentality of 137 *West African natives and that of Ascaris and Trichuris infections on eighty-nine of the same cases.*

The conclusions reached are the same as those for physique and general fitness.

Reference has already been made to Strong's (1916) interesting monograph on the effects of hookworm disease on the mental and physical development of school children. It is not clear where the experiments described were carried out, but presumably they were in one of the American States, and possibly dealt with a less resistant race than the West African native.

Butler (1915), working at the Bo School for the sons of Chiefs in Sierra Leone, wrote as follows : ' *Examination for Ankylostomiasis.*— The same seventy-five boys were examined also for this condition, and in only one case was there a negative result ; that is, 98.6 per cent. showed the presence of ancylostome ova. I think these cases may be regarded as fairly heavy infections, because in fifty-nine of the cases (that is, roughly, 80 per cent.) the ova were found in a single

examination of crude faeces. None of the individuals showed any symptoms or signs suggestive of ankylostomiasis. Duffers and individuals of acute intelligence appeared equally infected, and the standard of the school sports is quite as high as the average English Public School, so that I could not detect any evidence suggesting that these individuals suffered any disability from harbouring the parasite at that particular time, though symptoms of ankylostomiasis might quite likely appear if the individual was placed under some untoward condition, such as semi-starvation, when the ancylostome toxins might get the upper hand.' Easmon (1923), writing of the same school, repeats Butler's observations. These two papers are not based on any exact data, and are not quoted here as supporting the theory regarding the non-pathogenicity of ankylostomiasis; they are referred to merely because the present writer believes that they represent the views of the majority of medical men in Sierra Leone.

(D) *Energy*. The definition of energy has already been given as the keenness with which an individual attempts any mental or physical task allotted to him. The data collected regarding the percentage and degree of infection amongst West African natives, classified in three groups on this basis, are set forth in Table VIII.

TABLE VIII.

Showing the percentage of individuals infected with Ancylostome, Ascaris, and Trichuris, and the average degree of infection, amongst West African natives arranged in three groups according to their energy.

Groups based on energy	Number of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostomes per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—20 For Ancylostomes, Ascaris and Trichuris—27	72	19	37	1,990	1,677	94	12,660	17,400	933	83
(B) (Moderate)	For Ancylostomes only—0 For Ancylostomes, Ascaris and Trichuris—33	85	18	45	4,064	2,586	99	15,600	42,630	900	169
C (Bad)	For Ancylostomes only—28 For Ancylostomes, Ascaris and Trichuris—29	91	17	45	5,681	1,429	423	124,000	17,733	7,800	237

In Table VIII the predominant Trichuris, and the two predominant Ancylostome infections, both occur in Group C ; while the predominant Ascaris infection occurs in Group B. If, as before, we omit these four infections, the results are as shown in Table VIIIA.

TABLE VIIIA.

Same as Table VIII except that four predominantly high infections have been omitted.

Groups based on energy	Numbers of cases examined	Percentage infected with			Average number of ova per gm. of faeces			Maximum number of ova per gm. of faeces			Computed average number of Ancylostomes per individual
		Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	Ancylostome	Ascaris	Trichuris	
A (Good)	For Ancylostomes only—20 For Ancylostomes, Ascaris and Trichuris—27	72	19	37	1,990	1,677	94	12,660	17,400	933	83
B (Moderate)	For Ancylostomes only—0 For Ancylostomes, Ascaris and Trichuris—32	85	16	45	4,064	1,334	99	15,600	19,500	900	169
C (Bad)	For Ancylostomes only—27 For Ancylostomes, Ascaris and Trichuris—28	91	17	43	2,963	1,429	160	23,100	17,733	2,230	123

Consideration of Table VIIIA shows that Ascaris and Trichuris infections are in no way associated with a lowered standard of energy. As regards Ancylostome infection, it is seen that the percentage of infected cases in each group increases with each reduction in the standard of energy ; but this increase in the percentage of infected cases is only as 72-85-91 and is, therefore, obviously too small to allow of any conclusions. The average degree of infection is higher in both Group B and Group C than it is in Group A, but, as was also found in the mental classification, the degree of infection in Group B is higher than in Group C, which represents a lower standard of energy, the ratio of A-B-C being as 2-4-3. Before studying the results of intense infection on the energy of the individual, as shown in Table IX, it is necessary to consider the proportionate sizes of the different energy groups shown in Table VIII ; from this table it can be seen that, of 137 natives

examined for ancylostomes, forty-seven (34 per cent.) occurred in Group A, thirty-three (24 per cent.) occurred in Group B, and fifty-seven (42 per cent.) in Group C. Eighty-nine natives were examined for Ascaris and Trichuris infection ; of these twenty-seven (30 per cent.) fell into Group A, thirty-three (37 per cent.) into Group B, and twenty-nine (33 per cent.) into Group C.

Table IX.

Showing the ten heaviest Ancylostome, Ascaris, and Trichuris infections observed amongst West African natives, and the standard of energy for each case.

Ancylostome				Ascaris			Trichuris		
Case	Standard of energy	Number of ova per gm. of faeces	Computed number of Ancylostomes	Case	Standard of energy	Number of ova per gm. of faeces	Case	Standard of energy	Number of ova per gm. of faeces
113	C	124,000	5,167	38	B	42,630	43	C	7,800
43	C	36,830	1,537	74	B	19,500	20	C	2,230
44	C	23,100	962	66	C	17,730	15	C	1,000
71	C	21,100	879	30	A	17,400	73	A	933
114	C	17,760	740	43	C	16,630	74	B	900
33	B	15,600	650	115	A	10,300	60	B	460
35	B	13,700	570	117	A	9,430	119	B	400
60	B	13,600	567	57	B	9,130	14	A	360
78	B	13,400	558	72	B	8,700	49	C	330
38	B	13,200	550	65	A	4,260	22	A	300

The figures in Table IX show that amongst the ten most intensely infected ancylostome cases, 50 per cent. fall into Group B and 50 per cent. into Group C ; twenty-three natives were negative for ancylostomes and these were distributed in the proportions of 56 per cent. in Group A, and 22 per cent. in both Group B and Group C. The distribution of the ten heaviest Ascaris infections was, 40 per cent. in Group A, 40 per cent. in Group B, and 20 per cent. in Group C ; seventy-three cases were negative for Ascaris and were distributed as follows : Group A, 30 per cent., Group B, 37 per cent., Group C, 33 per cent. Amongst the ten heaviest Trichuris infections 30 per cent. occurred in Group A, 30 per cent. in Group B, and 40 per cent. in Group C ; fifty cases were negative for Trichuris ; of these 34 per

cent. occurred in Group A, 34 per cent. in Group B, and 32 per cent. in Group C.

An examination of the figures in Table VIII has already shown that the numbers of natives examined for Ascaris and Trichuris infections were about equally distributed in the three groups. It will be seen from the figures above recorded that the intensely infected Ascaris and Trichuris cases, and also the cases free from these infections, likewise distribute themselves in more or less equal groups ; that is to say, they occur in the proportions that would be expected if these two infections had no influence on energy.

The Ancylostome figures are of interest as they appear to indicate some connection between intense infection with ancylostomes and a lowered standard of energy ; thus none of the ten heaviest infections occur in Energy Group A, whereas of the twenty-three uninfected cases, 56 per cent. fall into this category ; Group C contains not only 50 per cent. of the ten heaviest infections, but the five most intense of these ten infections all fall into this class, whereas it contains only 22 per cent. of the uninfected cases. The figures seem to suggest, therefore, that very intense Ancylostome infections, represented by more than 15,000 ova per gm. of faeces, may possibly have a deleterious effect on the energy of the individual so infected.

Conclusions regarding the influence of Ancylostome infection on the energy of 137 *West African natives, and that of Ascaris and Trichuris on eighty-nine of the same cases.*

1. A group of individuals with a lower standard of energy does not necessarily show a noticeably greater percentage of infected cases than a group with a higher standard of energy.

2. A group with a lower standard of energy does not necessarily show a noticeably greater average degree of infection than a group with a higher standard of energy.

3. (*a*) Individuals with a high degree of infection with Ascaris or Trichuris do not necessarily show a low standard of energy.

(*b*) The figures, such as they are, suggest that there may be some correlation between Ancylostome infections of more than 15,000 ova per gm. of faeces, and the low standard of energy observed in such cases ; but it is obvious that to justify any definite conclusion of this kind the work must be repeated with very much larger groups of cases.

(E) *Urine.* Eighty-two natives—in whom the degree of infection with Ancylostome, Ascaris, and Trichuris, was already known—were examined for the presence of albumin and (or) casts in the urine; twenty-seven (33 per cent.) of the cases were positive for albumin; none of the cases showed the presence of casts. No association between the presence of Ancylostome, Ascaris, or Trichuris ova in the faeces and albumin in the urine could be demonstrated; nor was a high degree of infection with any of these worms necessarily associated with the presence of albumin in the urine. The high percentage of albuminurias is probably due to the frequent occurrence of chronic gonorrhoea amongst certain classes of the native population.

VI. EFFECT OF MIXED INFECTIONS

Mixed infections were common amongst the natives examined and it is obviously impossible to set forth briefly the results of different worm combinations. Tables showing the effects of mixed infections with any two species of worms under consideration have been prepared by the writer, with the result that no association has been demonstrated between any such double infection and a lowered standard of haemoglobin percentage, physique, mentality, or energy. Only five of the eighty-nine natives examined for Ancylostome, Ascaris, and Trichuris revealed the presence of all three infections in the one individual, and the full data regarding these five cases is set forth in Table X.

Table X.

Showing the degree of infection and the corresponding classification according to haemoglobin percentage, physique, mentality, and energy, of five cases of mixed infection with Ancylostome, Ascaris, and Trichuris, occurring amongst West African natives; also the presence or absence of albumin in the urine of such cases.

Case	Number of Ancylostome ova per gm. of faeces	Number of Ascaris ova per gm. of faeces	Number of Trichuris ova per gm. of faeces	Haemoglobin group	Physique group	Mentality group	Energy group	Albuminuria
38	13,200	42,630	33	A (80%)	A	B	B	Negative.
43	36,830	16,330	7,800	—	B	C	C	Positive.
64	363	366	163	A (85%)	A	C	C	Negative.
74	333	19,500	900	B (75%)	A	B	B	Negative.
75	3,300	2,630	100	A (80%)	A	C	B	Positive.

Table X shows that mixed infections with Ancylostome, Ascaris, and Trichuris, are not necessarily associated with albuminuria or a lowered standard of haemoglobin percentage, physique, mentality, or energy.

VII. SUMMARY OF CONCLUSIONS

A study of the effects of ankylostomiasis on the health of 137 West African natives, and those of Ascaris and Trichuris on eighty-nine of the same cases, has shown that these infections, or a combination of these infections, produce no noticeable effects on the haemoglobin percentage, the physique and general fitness, or the mentality of the cases examined, nor is their presence in any way associated with albumin or casts in the urine. Ascaris, and Trichuris infections do not appear to be associated with a low standard of energy, nor are the percentage of Ancylostome infected cases, or the average degree of infection, necessarily noticeably greater in a group of individuals with a lower standard of energy than in one with a higher standard. On the other hand, the figures suggest the possibility of some association between Ancylostome infections of more than 15,000 ova per gm. of faeces, and the low standard of energy observed in such cases ; but only a few such intense infections were observed, and it is obvious that in order to justify any such definite conclusion the work must be repeated with very much larger groups of cases.

It therefore follows from these conclusions that before treatment, and especially before the so-called ' Mass treatment,' of ankylostomiasis, is applied to any native race, careful investigation should be made whether ankylostomiasis has any definite pathogenic effect on that race, and if pathogenic effects are noted, with what degree of infection they are associated.

ACKNOWLEDGMENTS

The writer is greatly indebted to W. N. Martin, Esq., M.A., Principal of the Albert Academy, Freetown, Dr. J. Y. Wood, of the West African Medical Service, and Inspector Warren, of the West African Police Force. These gentlemen made the present work possible by preparing lists of the physical and mental condition of the natives who were in their charge.

REFERENCES

Adler, S. (1925). The Hookworms of Man in Sierra Leone. *Ann. Trop. Med. & Parasitol*, Vol. XIX, No. 1, p. 138.

Butler, G. C. (1915). Some Observations made on apparently healthy boys at the Bo School for the sons of Chiefs. *Sierra Leone Annual Report of the Medical Department for the year ending 31st December*, 1915, p. 26.

Chandler, A. C. (1925). The Measure of Hookworm Infection in Communities. *Ann. Trop. Med. & Parasitol.*, Vol. XIX, No. 2, pp. 191-210.

——— (1925b). Notes on some Methods for Diagnosing Hookworm Infection and for Estimating the Egg-output. *Ind. Med. Gaz.*, Vol. LX, No. 9, pp. 403-406.

Cort, W. W. (1924). Investigations on the Control of Hookworm Disease. XXXII: Methods of Measuring Human Infestation. *Amer. Journ. Hyg.*, Vol. IV, No. 3, pp. 213-221.

Cort, W. W., Payne, G. C., and Riley, W. A. (1923). A Study of a Heavily Infected Group of People on a Sugar and Coffee Estate in Porto Rico, before and after Treatment. *Amer. Journ. Hyg.*, Vol. III, July Supplement, pp. 85-110.

Darling, S. T. (1922). The Hookworm Index and Mass Treatment. *Amer. Journ. Trop. Med.*, Vol. II, No. 5, pp. 397-447.

Darling, S. T., Barber, M. A., and Hacker, H. P. (1920). Hookworm and Malaria Research in Malaya, Java, and the Fiji Islands. *Report of the Uncinariasis Commission to the Orient*, 1915-1917, p. 106 *et seq.*

Darling, S. T., and Smillie, W. G. (1921). Studies on Hookworm Infection in Brazil. First Paper. *Monograph of the Rockefeller Institute for Medical Research*, No. 14, pp. 1-42.

Davis, N. G. (1924). Experience with the Stoll Egg-Counting Method in an Area Lightly Infested with Hookworm. *Amer. Journ. Hyg.*, Vol. IV, No. 3, pp. 226-236.

Easmon, M. C. F. (1923). Report on Ankylostomiasis at Bo and Pujehun. *Sierra Leone Annual Report of the Medical Department for the year ending 31st December*, 1923, Appendix IX, p. 69.

Hill, R. B. (1923). The Use of the Egg-Counting Method in an Intensive Campaign. *Amer. Journ. Hyg.*, Vol. III, July Supplement, pp. 37-60.

Lane, Clayton (1917). Final Report on the Ankylostome Inquiry in the Darjeeling District of India. *Ind. Journ. Med. Res.*, Vol. V. No. 2, pp. 350-385.

——— (1919). Diagnosis on a Large Scale in Hookworm Infection. *Ind. Journ. Med. Res.* Special Indian Science Congress Number, 1919.

——— (1923b). On Ankylostome Infestation: The Changing Problem. *Brit. Med. Journ.*, March 31st, 1923, pp. 551-553.

——— (1923-1925). The Mass Diagnosis of Ankylostome Infection. Part I. *Trans. Roy. Soc. Trop. Med. & Hyg.*, Vol. XVI, No. 6, pp. 274-313. Parts II-VII, *Ibid.*, Vol. XVII, Nos. 6 and 7, pp. 407-436. Parts VIII-XIII, *ibid.*, Vol. XVIII, Nos. 5 and 6, pp. 278-310. Part XIV, *ibid.*, Vol. XIX, No. 3, pp. 156-176.

Löbker and Bruns (1906). In Fantham, H. B., Stephens, J. W. W., and Theobald, F. V. (1916), The Animal Parasites of Man. John Bale, Sons, and Danielsson, London, p. 682.

Maplestone, P. A. (1924). A Critical Examination of Stoll's Method of Counting Hookworm Eggs in Faeces. *Ann. Trop. Med. & Parasitol.*, Vol. XVIII, No. 2, pp. 189-194.

Mhaskar, K. S. (1924). Report of the Ankylostomiasis Inquiry in Madras. *Ind. Med. Res. Memoirs*, Vol. I, No. 1, pp. 1-95.

Mhaskar, K. S., and Kendrick, J. F. (1923). Anti-Hookworm Campaign in the Tea Estates of Madras. *Ind. Journ. Med. Res.*, Vol. XI, No. 1, pp. 52-64.

Moosbruger. In Brumpt, E. (1922). Précis de Parasitologie, 3rd ed. Masson et Cie, Paris, p. 615.

Rockefeller Foundation International Health Board (1922). Bibliography of Hookworm Disease.

Smillie, W. G. (1922). Studies in Hookworm Infection in Brazil 1918-1920. Second Paper. *Rockefeller Inst. Med. Res.*, No. 17, pp. 1-73.

Stoll, N. R. (1923). An Effective Method of Counting Hookworm Eggs in Faeces. *Amer. Journ. Hyg.*, Vol. III, No. 1, pp. 59-70.

——— (1923b). On the Relation between the number of Eggs found in Human Faeces and the number of Hookworms in the Host. *Amer. Journ. Hyg.*, Vol. III, No. 2, pp. 156-179.

——— (1924). The Significance of Egg Count Data in *Necator americanus* Infections. *Amer. Journ. Hyg.*, Vol. IV, No. 5, pp. 466-500.

Stoll, N. R., and Tseng Hsien Wu (1925). The Severity of Hookworm Disease in a Chinese Group, as Tested by Haemoglobin Readings for the Anaemia, and Egg Counts for the Degree of Infestation. *Amer. Journ. Hyg.*, Vol. V, No. 4, pp. 536-552.

Strong, E. K. (1916). Effects of Hookworm Disease on the Mental and Physical Developments of Children. *International Health Commission.* Publication No. 3, pp. 1-123.

Sweet, W. C. (1925). Notes on Methods of Diagnosing Hookworm Infection and on Egg-Counting Methods. *Amer. Journ. Hyg.*, Vol. V, No. 4, pp. 497-507.

EXPLANATION OF PLATE VII

FIG. 1. Showing the physical condition of six boys selected at random from amongst the negative or lightly infected Ancylostome cases.

FIG. 2. Showing the physical condition of six boys selected at random from amongst the heaviest Ancylostome infections.

Number of Ova per gm. of faeces	*Ancylostome*	433	0	200	900	0	0
	Ascaris ...	8,200	9,400	17,730	0	2,733	0
	Trichuris ...	0	100	0	0	400	166

FIG. 1

Number of Ova per gm. of faeces	*Ancylostome*	36,830	23,100	21,000	15,600	13,200	13,700
	Ascaris ...	16,330	0	0	0	42,630	0
	Trichuris ...	7,800	0	0	0	33	66

FIG. 2

C. Tinling & Co., Ltd., Imp.

A NEW VARIETY OF *ANOPHELES MARSHALLI*, FROM SIERRA LEONE

BY
A. M. EVANS

(*Received for publication* 14 *November*, 1925)

PLATE VIII

During a recent survey of the *Anophelini* in and around Freetown, Professor Blacklock and the author collected several larvae which gave rise to an *Anopheles* apparently related to *A. marshalli* Theo., but differing from it and its allies in certain characters. Further, the larvae were markedly distinct from those of *A. marshalli*, possessing palmate hairs on the thorax as well as on the first two segments of the abdomen, and it seems probable that larval characters will be found to be a good means of separating the closely allied species of the *marshalli* group.

Anopheles marshalli var. *freetownensis* n.var. (Plate VIII).

A variety of *A. marshalli* having the mesonotum chiefly clothed with hairs and the tarsi entirely dark.

FEMALE.

Head: occiput with the usual anterior-median white upright-forked scales and black or dark brown ones behind. *Palpi* with three white bands, the proximal one narrow and the two distal ones very broad and equal in length, apex white. Distal bands separated by about half the length of one of them, and extending so as to occupy rather more than the distal third of the palp. *Thorax:* integument dark greyish-brown, a thick group of long and narrow, curved white scales in the middle in front, rest of mesonotum clothed with pale brown hairs, interspersed with a few rather long, very narrow, curved, pale scales, the scales rather more numerous at the sides. *Abdomen:* blackish-brown with fine dark brown hairs. *Legs.* Entirely dark except apices of femora and tibiae which are

obscurely pale. *Wings* (Pl. VIII): costa with six dark areas, the fifth rather long. First vein with light and dark areas coinciding with those of costa on distal half, but the white areas more extensive basally; second vein with two white spots on the stem, one at the bifurcation and two on the upper branch; third vein with two dark areas, a large one sub-basally and a small one sub-apically; fourth vein with a large and a small white area on the stem, and one at the bifurcation, both branches white at the apex, the upper with an additional small white spot; fifth vein with the stem mostly white-scaled, one dark area towards the base and a large one at the bifurcation involving the base of the upper fork, which has two more dark areas, lower branch white on basal two-fifths and at apex; sixth vein with two large white areas on basal half, distal half dark. Fringe at the apex of the wing largely white, but a small dark spot present just above the apex of the lower branch of the second vein. White spots at the apices of the branches of the fourth and fifth vein, but not at the apex of the sixth. Scales relatively rather short and narrow, the longer lateral squames near the apex of the third vein measuring 0·05 mm. in length, and having the greatest width about one-fifth to one-fourth of the length, and five or six striae. Wing length *c.* 3·5 mm.

Male.

Palpi. Long segment with sub-median ring and apex white; last two segments white-scaled with narrow, basal black rings. Other scale characters as in female except that the wing scales are shorter and less dense.

Type: ♂ and ♀, bred from larvae taken in a stream, Kissy Bridge, near Freetown, Sierra Leone, 2.VII.25, Professor D. B. Blacklock and A. M. Evans. Other specimens, thirteen ♂♂ and fourteen ♀♀ from this and other localities near Freetown. Types in the collection of the Liverpool School of Tropical Medicine.

Variation. The most marked variation was exhibited by a female specimen in which the wings were considerably darker than in the majority of specimens. In the first vein the basal white area was interrupted by a dark spot opposite that on the costa, and the upper branch of the second vein was entirely dark.

The larva will be described in a joint paper by Professor Blacklock and the author, which is shortly to be published in these Annals.

EXPLANATION OF PLATE VIII

Anopheles marshalli var. *freetownensis* n. var

Wing of female.

(The width of the white scales is slightly exaggerated.)

A.M.E., del.

C. Tinling & Co., Ltd., Imp.

MISCELLANEA

ADDENDUM

With reference to my paper on ' A New Cestode from Nigeria,' in *Annals of Tropical Medicine & Parasitology*, Vol. XIX, No. 2, pp. 2 and 3, Dr. Joyeux has called attention to a species (*L. mahdiaensis*) described by him in *Archives de l'Institut Pasteur de Tunis*, Vol. XII, No. 2, p. 146.

Joyeux's species is distinct from those listed in my paper.

T. Southwell.

FILARIA MEDINENSIS

' The National Diseases here (Gold Coast of Guinea) are the *Small Pox* and *Worms ; . . .* with the latter they are miserably afflicted in all parts of their Bodies, but chiefly in their Legs ; which occasions a grievous Pain, which they are forced to bear till they can get the *Worm* quite out, that being sometimes a Month : The manner which the Artists take to get it out is this ; as soon as the Worm is broken thro' the Tumour, his Head commonly first making its way, after they have drawn it out a little way, they make it fast to a stick, about which they every day wind a small part of it, till continuing this tedious Method, they have entirely wound out the whole, and the Patient is freed from his Pain. But if the Worm happens to break, they are put to a double Torture, the remainder part of the worm either rotting in the Body, or breaking out at some other place. The Negroes are most afflicted with these Worms : But though the Europeans are but seldom troubled with them, yet they do not escape them entirely. I have seen some Negroes who had nine or ten of them at once, with which

they were inexpressibly tormented. This *Worm-Disease* is frequent all the Coast over; but our Men are most tormented with it at *Cormantyn* and *Apam*; which perhaps may be occasioned by the foul Water which they are obliged to drink there. If you would know the length of these Worms, Monsieur *Focquenbrog* obligeth you with a pathetical Description; by which you are informed that they are some of them an Ell-long, and some as long as Pikes, and have not the patience to stay till the Man is dead, but seize him alive.'

(*A New and Accurate Description of the Coast of Guinea, divided into the Gold, the Slave, and the Ivory Coasts.* p. 108. Written originally in Dutch, by William Bosman, 1705. Reprinted for Sir Alfred Jones, K.C.M.G., at the Ballantyne Press, London, 1907.)

J. W. W. Stephens.

THE PREDACEOUS HABIT OF THE LARVAE *MUCIDUS SCATOPHAGOIDES*

The following interesting note on the predaceous habit of the larvae of this mosquito has just been received from Dr. Innes, of Bathurst.

'I am glad to be able to send you two ♂♂ and one ♀ *Mucidus scatophagoides*. I got them as large creamy-coloured larvae, in shallow grassy pools (rainwater) with some other culicine and anopheline larvae. Two pupated on the way to my office. Others pupated later. The pupa stage lasts about two days and the pupae also are creamy coloured. The larvae are larvivorous: I fed larvae to them which were all quickly devoured. I think this accounts for the very few larvae of other species of mosquitos which I found in the pools in association with these ogres.—Frank A. Innes, Bathurst, Gambia, *October 21st*, 1925.'

INDEX

INDEX

INDEX OF AUTHORS

GENERAL INDEX

INDEX OF GENERA, SPECIES AND VARIETIES NEW TO SCIENCE

UNIVERSITY OF LIVERPOOL

December 16, 1925 Vol. XIX. No. 4

Annals of Tropical Medicine and Parasitology

ISSUED BY

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

LIVERPOOL:

THE UNIVERSITY PRESS OF LIVERPOOL LIMITED

LONDON:

HODDER AND STOUGHTON LIMITED

Price Seven Shillings and Sixpence Nett

THE MODERN TREATMENT OF LEPROSY

The value of injections of esters of acids of the Chaulmoogric Series—either alone, or in association with iodine—in the treatment of this disease has been clearly indicated by reports from leper settlements.

Jnl. Chem. Soc. 1904 .. .. *pp.* 838, 851, 884
,, ,, ,, 1907 .. ..*p.* 557
,, *Trop. Med. & Hygiene, Oct.* 15, 1920 .. ..*p.* 249
,, *A.M.A., Nov.* 27, 1920 .. .. *p.* 1484
,, ,, *Jan.* 22, 1921 .. ..*p.* 248
,, ,, *April* 16, 1921 .. .. *p.* 1121
,, ,, *May* 28, 1921 .. .. *p.* 1470
Practitioner, April, 1921, *etc.*
Indian Medical Gazette, May, 1922.. .. .. *p.* 190

TRADE MARK 'MOOGROL' BRAND

Mixture of Esters of Acids of the Chaulmoogric Series

has been prepared by Burroughs Wellcome & Co. as the result of researches extending from 1904 to the present time, and is now available for the use of the Medical Profession.

'MOOGROL' is supplied in bottles of 100 *c.c. at* 12/- *each*

Full information on request

BURROUGHS WELLCOME & CO., LONDON

D 206

Volume XIX December 16, 1925 No. 4

ANNALS

OF

TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

THE INCORPORATED
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

FOUNDED BY SIR ALFRED LEWIS JONES, K.C.M.G.

(Affiliated with the University of Liverpool)

Hon. President: H.R.H. THE DUKE OF YORK, K.G., G.C.V.O.
Chairman: SIR FRANCIS C. DANSON
Vice-Chairman: Professor R. CATON, C.B.E., M.D., F.R.C.P., LL.D.
Hon. Vice-Presidents: THE EARL OF DERBY, K.G., G.C.V.O., C.B., LL.D.
BARON KYLSANT, G.C.M.G.
SIR EDWARD MEREWETHER, K.C.V.O.
SIR H. J. READ, K.C.M.G.
MR. O. HARRISON WILLIAMS

COMMITTEE

Vice-Chancellor J. G. ADAMI, C.B.E., M.D., F.R.S. — *University of Liverpool*
Professor R. CATON, C.B.E., M.D., F.R.C.P., LL.D. — *Council of University of Liverpool*
Mr. H. WADE DEACON, C.B.E. — *Council of University of Liverpool*
Professor J. M. BEATTIE, M.A., M.D., C.M., M.R.C.S., L.R.C.P. — *Senate of University of Liverpool*
Professor W. J. DAKIN, D.Sc., F.L.S., F.Z.S. — *Senate of University of Liverpool*
Mr. E. G. BUCKLEY — *Royal Southern Hospital*
Mr. T. WOODSEND — *Royal Southern Hospital*
Mr. ENFIELD E. FLETCHER — *Steamship Owners' Association*
Mr. J. N. SELLERS — *Shipowners' Association*
Mr. F. C. BOWRING — *Shipowners' Association*
Mr. G. BROCKLEHURST
Mr. H. D. DICKIE
Professor E. E. GLYNN, M.A., M.D., C.M., F.R.C.P., M.R.C.S.
Professor E. W. HOPE, O.B.E., D.Sc., M.D.
Mr. DAVID JONES
Mr. J. PICKERING JONES
Mr. R. RANKIN
Mr. O. HARRISON WILLIAMS
Professor J. W. W. STEPHENS, B.A., M.D., F.R.S.
Professor R. NEWSTEAD, M.Sc., F.R.S., A.L.S., F.E.S., Hon. F.R.H.S.
Professor W. YORKE, M.D.
Professor B. BLACKLOCK, M.D., D.P.H., D.T.M.

Mr. J. A. TINNE, M.P., *Hon. Treasurer*

Mr. J. L. MCCARTHY, *Secretary*
C 17—18, Exchange Buildings, Liverpool

J. MIDDLEMASS HUNT,
Hon. Dean

Staff, 1925

Alfred Jones Professor of Tropical Medicine	JOHN WILLIAM WATSON STEPHENS, B.A., M.D., Cantab., F.R.S.
Dutton Memorial Professor of Entomology	ROBERT NEWSTEAD, J.P., F.R.S., M.Sc., A.L.S., F.E.S., Hon. F.R.H.S.
Walter Myers Professor of Parasitology	WARRINGTON YORKE, M.D.
Professor of Tropical Diseases of Africa	BREADALBANE BLACKLOCK, M.D., D.P.H., D.T.M.
Lecturer on Entomology	ALWEN M. EVANS, M.Sc.
Assistant Lecturer on Entomology	Vacant.
Lecturer on Protozoology	Vacant.
Lecturer on Helminthology	T. SOUTHWELL, Ph.D., M.Sc., A.R.C.Sc., F.Z.S.
Clinical Pathologist	D. U. OWEN, M.B., Ch.B., D.T.M.
Hon. Lecturer on Clinical Veterinary Parasitology	A. W. NOEL PILLERS, F.R.C.V.S.
Assistant Lecturer and Demonstrator on Veterinary Parasitology	Vacant.
Lecturer on Tropical Surgery	ROBERT ERNEST KELLY, C.B., M.D., B.Sc., F.R.C.S.
Lecturer on Tropical Sanitation	R. H. KENNAN, B.A., M.D., F.R.C.S., D.P.H., D.T.M.
Hon. Lecturer on Tropical Sanitation	SIR WILLIAM THOMAS PROUT, M.B., C.M.G., C.M.
Hon. Statistician	WALTER STOTT.

Royal Infirmary, Liverpool

Physician	JOHN WILLIAM WATSON STEPHENS, B.A., M.D., Cantab., F.R.S.
Assistant Physician	WARRINGTON YORKE, M.D.
Consulting Surgeon	W. THELWALL THOMAS, Ch.M., F.R.C.S.

The Manáos Research Laboratory

Director	HAROLD WOLFERSTAN THOMAS, M.D., C.M.

Sierra Leone Research Laboratory

Director	BREADALBANE BLACKLOCK, M.D., D.P.H., D.T.M.
Assistant Director	RUPERT MONTGOMERY GORDON, M.D., Ch.B.
Research Assistant	G. MACDONALD, M.B., Ch.B., D.T.M.

THE MARY KINGSLEY MEDAL

This medal was struck in commemoration of the work of the late Miss Mary Kingsley in West Africa, and is conferred in recognition of distinguished scientific achievement.

HONORARY RECIPIENTS

Her Royal Highness Princess Christian
Lord Lister
The Right Hon. Joseph Chamberlain
Prince Auguste d'Arenberg
Mrs. Pinnock
Mr. William Adamson
Professor William Carter

RECIPIENTS

1905—

Colonel Sir David Bruce, K.C.B.
Geheimrath Professor Robert Koch
Dr. A. Laveran
Sir Patrick Manson, K C.M.G.

1907—

Professor Danielewsky
Dr. Charles Finlay
Mr. W. M. Haffkine
Professor Golgi
Colonel Gorgas
Professor Theobald Smith

1910—

Sir William Macgregor, G.C.M.G.
Professor R. Blanchard
Dr. Anton Breinl
Professor Angelo Celli
Dr. C. W. Daniels
Surgeon-General Sir Alfred Keogh
Colonel W. G. King
Professor Nocht
Professor G. H. F. Nuttall
Major Leonard Rogers
Professor J. L. Todd
Surgeon-General Walter Wyman

1913—

Professor Fred V. Theobald

1917—

Dr. Griffith Evans

1919—

Dr. J. W. Scott Macfie
The Oswaldo Cruz Institute, Rio de Janeiro

1920—

Major E. E. Austen, D.S.O.
Dr. A. G. Bagshawe, C.M.G.
Dr. Andrew Balfour, C.B.
Dr. A. L. G. Broden
Mrs. Chalmers, in recognition of the work of the late Dr. A. J. Chalmers
Professor B. Grassi
Professor R. T. Leiper
Professor F. Mesnil
Dr. Edmond Sergent
Dr. C. W. Stiles
Dr. T. Zammit

THE ALAN H. MILNE MEDAL

This medal was struck to commemorate the late Alan H. Milne, C.M.G., the first Honorary Secretary of the School (1899-1917), and is awarded twice yearly on the recommendation of the examiners for the Diploma in Tropical Medicine.

1921—

George Phillip Farmer Allen

1922—

Quinton Stewart

1923—

John Cecil Cruickshank

1924—

George Maclean
Frederick John Carlyle Johnstone
Bernard Langridge Davis

1925—

Khwaja Samad Shah

NOTICE

The following courses of instruction are given by the Liverpool School of Tropical Medicine each year:—

(1) Two courses for the Diploma in Tropical Medicine, commencing on the 1st October and the 7th January. The D.T.M. examinations are held in December and March.

(2) Two courses for the Diploma in Tropical Hygiene, commencing on the 7th January and the 24th April. The D.T.H. examinations are held in March and July.

(3) A short advanced course, of one month's duration, in June.

(4) Two courses in Veterinary Parasitology, commencing on 1st October and the 7th January.

DIPLOMA IN TROPICAL MEDICINE

This Diploma shall be awarded only to candidates who possess a qualification to practise Medicine recognised for this purpose by the University, and who present satisfactory certificates of having attended approved courses of study, and pass the prescribed examination.

DIPLOMA IN TROPICAL HYGIENE

This Diploma can only be taken by those who have already obtained the D.T.M.

'The course for this Diploma will not be conducted unless at least five applications are received, and no application for admission can be considered later than December 1st and March 1st respectively.'

FEES

Each Diploma Course	... Fifteen Guineas
Short Advanced Course	... Six Guineas
Course in Veterinary Parasitology	... Fifteen Guineas
Each Diploma Examination ...	... Five Guineas

Fee for use of a School microscope during one term ... One Guinea

For prospectus and further information, application should be made to the Hon. Dean, School of Tropical Medicine, University of Liverpool.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool :—

Diploma in Tropical Medicine

Date of Diploma	
1904	Augustine, Henry Joshua
1904	Bennett, Arthur King
1904	Bruce, William James
1904	Byrne, John Scott
1904	Clayton, Thomas Morrison
1904	Dalziel, John McEwen
1904	Dee, Peter
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1904	Maclurkin, Alfred Robert
1904	McConnell, Robert Ernest
1904	Nicholson, James Edward
1904	Philipson, Nicholas
1904	Sharman, Eric Harding
1904	Thomson, Frank Wyville
1904	Walker, George Francis Clegg
1905	Anderson, Catherine Elmslie
1905	Brown, Alexander
1905	Caldwell, Thomas Cathcart
1905	Critien, Attilio
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz
1905	Macfarlane, Robert Maxwell
1905	Maddock, Edward Cecil Gordon
1905	Moore, James Jackson
1905	Nightingale, Samuel Shore
1905	Radcliffe, Percy Alexander Hurst
1905	Young, John Cameron
1906	Adie, Joseph Rosamond
1906	Arnold, Frank Arthur
1906	Bate, John Brabant
1906	Bennetts, Harold Graves
1906	Carter, Robert Markham
1906	Chisholm, James Alexander
1906	Clements, Robert William
1906	Dundas, James
1906	Faichnie, Norman
1906	Jeffreys, Herbert Castelman
1906	Mackenzie, Donald Francis
1906	Pailthorpe, Mary Elizabeth
1906	Palmer, Harold Thornbury
1906	Pearse, Albert
1906	Sampey, Alexander William
1906	Smithson, Arthur Ernest
1906	Taylor, Joseph van Someron
1906	Taylor, William Irwin
1906	Tynan, Edward Joseph
1906	Watson, Cecil Francis
1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1907	Vallance, Hugh
1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
1908	Schoorel, Alexander Frederik
1908	Smith, John Macgregor
1908	Stewart, George Edward
1908	Tate, Gerald William
1908	Whyte, Robert
1909	Abercrombie, Rudolph George
1909	Allin, John Richard Percy
1909	Armstrong, Edward Randolph
1909	Barrow, Harold Percy Waller
1909	Beatty, Guy
1909	Carr-White, Percy
1909	Chevallier, Claude Lionel
1909	Clark, William Scott
1909	Cope, Ricardo
1909	Fleming, William
1909	Hanschell, Hother McCormick
1909	Hayward, William Davey
1909	Henry, Sydney Alexander
1909	Innes, Francis Alexander
1909	Jackson, Arthur Frame
1909	Kaka, Sorabji Manekji
1909	McCabe-Dallas, Alfred Alexander Donald

Date of Diploma	
1909	Meldrum, William Percy
1909	Murphy, John Cullinan
1909	Samuel, Mysore Gnananandaraju
1909	Shroff, Kawasjee Byramjee
1909	Thornely, Michael Harris
1909	Turkhud, Violet Ackroyd
1909	Webb, William Spinks
1909	Yen, Fu-Chun
1910	Brabazon, Edward
1910	Castellino, Louis
1910	Caulcrick, James Akilade
1910	Dowden, Richard
1910	Haigh, William Edwin
1910	Hamilton, Henry Fleming
1910	Hefferman, William St. Michael
1910	Hipwell, Abraham
1910	Homer, Jonathan
1910	Houston, William Mitchell
1910	James, William Robert Wallace
1910	Johnstone, David Patrick
1910	Korke, Vishnu Tatyaji
1910	Macdonald, Angus Graham
1910	Macfie, John Wm. Scott
1910	Manuk, Mack Walter
1910	Murison, Cecil Charles
1910	Nanavati, Kishavlal Balabha
1910	Nauss, Ralph Welty
1910	Oakley, Philip Douglas
1910	Pratt, Ishmael Charles
1910	Sabastian, Thiruchelvam
1910	Shaw, Hugh Thomas
1910	Sieger, Edward Louis
1910	Sousa, Pascal John de
1910	Souza, Antonio Bernardo de
1910	Waterhouse, John Howard
1910	White, Maurice Forbes
1911	Blacklock, Breadalbane
1911	Brown, Frederick Forrest
1911	Chand, Diwan Jai
1911	Holmes, John Morgan
1911	Ievers, Charles Langley
1911	Iles, Charles Cochrane
1911	Ingram, Alexander
1911	Kirkwood, Thomas
1911	Knowles, Benjamin
1911	Liddle, George Marcus Berkeley
1911	Lomas, Emanuel Kenworthy
1911	Mackarell, William Wright
1911	MacKnight, Dundas Simpson
1911	Mascarenhas, Joseph Victor
1911	Murray, Ronald Roderick
1911	Oluwole, Akidiya Ladapo
1911	Rao, Koka Ahobala
1911	Sinton, John Alexander
1911	Tarapurvalla, Byramji Shavakshah
1911	Taylor, John Archibald
1911	Woods, William Medlicott
1912	Aeria, Joseph Reginald
1912	Anderson, Edmund Litchfield
1912	Borle, James
1912	Bowie, John Tait
1912	Brassey, Laurence Percival

Date of Diploma	
1912	Christie, David
1912	Dillon, Henry de Courcy
1912	Dunn, Lillie Eleanor
1912	Hardwicke, Charles
1912	Jagose, Jamshed Rustomji
1912	Kochhar, Mela Ram
1912	McGusty, Victor William Tighe
1912	Milne, Arthur James
1912	Mitra, Manmatha Nath
1912	Myles, Charles Duncan
1912	Pelly, Huntly Nevins
1912	Prasad, Bindeshwari
1912	Prentice, George
1912	Ross, Frank
1912	Russell, Alexander James Hutchison
1912	Ruthven, Morton Wood
1912	Sandilands, John
1912	Seddon, Harold
1912	Smalley, James
1912	Strickland, Percy Charles Hutchison
1912	Watson, William Russel
1913	Austin, Charles Miller
1913	Banker, Shiavux Sorabji
1913	Becker, Johann Gerhardus
1913	Carrasco, Milton
1913	Clark, James McKillican
1913	Forsyth, Charles
1913	Grahame, Malcolm Claude Russell
1913	Grieve, Kelburne King
1913	Hargreaves, Alfred Ridley
1913	Hepper, Evelyn Charles
1913	Hiranand, Pandit
1913	Jackson, Oswald Egbert
1913	Khaw, Ignatius Oo Kek
1913	MacKelvie, Maxwell
1913	MacKinnon, John MacPhail
1913	Macmillan, Robert James Alan
1913	Mouat-Biggs, Charles Edward Forbes
1913	Noronha, John Carmel
1913	O'Connor, Edward
1913	Olubomi-Beckley, Emanuel
1913	Pestonji, Ardeshir Behramshah
1913	Puttanna, Dodballapur Sivappa
1913	Reford, John Hope
1913	Smith, Edward Arthur
1913	Stewart, Samuel Dudley
1913	Walker, Frederick Dearden
1913	Wilbe, Ernest Edward
1913	Wilson, Hubert Francis
1913	Yin, Ulg Ba
1913	Young, William Alexander
1914	Arculli, Hassan el
1914	Chohan, Noormahomed Kasembha
1914	Connell, Harry Bertram
1914	Gerrard, Herbert Shaw
1914	Gimi, Hirji Dorabji
1914	Gwynne, Joseph Robert
1914	Hodkinson, Samuel Paterson
1914	Jackson, Arthur Ivan
1914	Kaushash, Ram Chander
1914	Kelsall, Charles
1914	Luanco y Cuenca, Maximino
1914	Misbah, Abdul-Ghani Naguib

Date of Diploma	
1914	Naidu, Bangalore Pasupulati Balakrishna
1914	Rowe, John Joseph Stephen
1914	Roy, Raghu Nath
1914	Shiveshwarkar ,Ramchandra Vishnu
1914	Sur, Sachindra Nath
1914	Talati, Dadabhai Cursedji
1914	Wilkinson, Arthur Geden
1914	Wright, Ernest Jenner
1915	Lobo, John Francis
1915	Madhok, Gopal Dass
1915	Pearson, George Howorth
1915	Swami, Karumuri Virabhadra
1915	Wood, John
1916	Barseghian, Mesroob
1916	Chaliha, Lakshmi Prasad
1916	Lim, Albert Liat Juay
1916	Lim, Harold Liat Hin
1916	Metzger, George Nathaniel
1916	Söderström, Erik Daniel
1916	Wheeler, Louis
1917	Chapman, Herbert Owen
1917	Krishnamoorthy, Yedatore Venkoba
1917	Lipkin, Isaac Jacob
1918	Watts, Rattan Claud
1919	Bowle-Evans, Charles Harford
1919	Burnie, Robert McColl
1919	Celestin, Louis Abel
1919	Cummings, Eustace Henry Taylor
1919	Darling, Georgina Renington
1919	Drake, Joan Margaret Fraser
1919	Fraser, William James
1919	Gordon, Rupert Montgomery
1919	Krige, Christian Frederick
1919	Maplestone, Philip Alan
1919	Oluwole, Isaac Ladipo
1919	Rustomjee, Khusshuyee Jamesidjee
1919	Sawers, William Campbell
1919	Thompson, Mary Georgina
1919	Turner, Gladys Maude
1919	Young, Charles James
1920	Adler, Saul
1920	Anderson, William Jenkins Webb
1920	Campbell, George
1920	Cobb, Charles Eric
1920	Cobb, Enid Margaret Mary
1920	Connolly, Evelyn Mary
1920	Fernandez, Daniel David
1920	Lim, Chong Eang
1920	McHutcheson, George Browne
1920	van der Merwe, Frederick
1920	O'Farrell, Patrick Theodore Joseph
1920	Renner, Edowo Awunor
1920	Vaughan, James Churchwill
1920	Waller, Harold William Leslie
1921	Allen, George Phillip Farmer
1921	Corfield, Charles Russell
1921	Hamid, Abdul
1921	Longhurst, Bell Wilmott
1921	Macvae, George Anthony
1921	Madan, Hans Raj
1921	Mulligan, William Percival
1921	Nixon, Robert
1921	Richmond, Arthur Stanley
1921	Shri Kent, Shamsher Singh
1921	Skinner, James Macgregor
1921	Stewart, Robert Bell
1921	Thomson, Marion
1922	Bhatia, Jagat Ram
1922	Cohen, Morris Joshua
1922	Crawford, Andrew Clemmey
1922	Gilmore, Edward Raymond
1922	Gracias, Cajetan Manuel
1922	Jennings, Arthur Richard
1922	Lethem, William Ashley
1922	Paul, Sachchidananda Hoshen
1922	Pinder, John
1922	Rieley, Stanley Desmond
1922	Rutherford, Gladys
1922	Stewart, Quinton
1923	Abelman, B.
1923	Basu, Dhirendranath
1923	Cruickshank, John Cecil
1923	Doherty, Winifred Irene
1923	Edghill, Winifred M.
1923	Elsohn, John
1923	Fraser, N. D.
1923	Lee, R.
1923	Pierce, E. R.
1923	Raja, Rojaporum
1923	Reid, C. B. B.
1923	Richmond, A. E.
1923	Steven, J. B.
1923	White, Charles Francis
1924	Bilimoria, H. S.
1924	Carson, J. C.
1924	Chopra, B. L.
1924	Davis, B. L.
1924	Hardy, M. J.
1924	Jennings, C. B.
1924	Johnstone, F. J. C.
1924	Keirans, J. J.
1924	Lee, S. W. T.
1924	Macdonald, G.
1924	Maclean, G.
1924	Mathur, W. C.
1924	Mitchell, J. M.
1924	Owen, D. U.
1924	Palmer-Jones, Beryl
1924	Sankeralli E. J.
1924	Singh, H.
1924	Theron, Elizabeth M.
1925	Black, John
1925	Crawford, E. J.
1925	Ellam, Mary Muriel
1925	Green, Frederick Norman
1925	Mackay, E. K.
1925	Shah, Khwaja Samad
1925	de Waal, Jacobus Johannes

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

EDITORIAL NOTICE

Articles for publication should not exceed twenty-five pages of the Annals, and will be understood to be offered alone to this Journal. They should be typewritten and addressed to:—The Editors, School of Tropical Medicine, The University, Liverpool.

Illustrations for text figures or charts should be drawn clearly and firmly in Indian ink, if possible on Bristol board. N.B.—*Blue or other coloured* ruling in squares or lines cannot be reproduced.

All lettering, names or legends on text-figures, charts or maps should be printed *sufficiently large* to allow of *clear legibility* on reduction if necessary.

Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way:—'According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.' The references should be collected in alphabetical order of authors' surnames at the end of the paper, and arranged in the following way:—

> Robinson, S. (1914). 'The spleen in malaria.' *Annals of Nosology*, Vol. XX, pp. 20-25.
>
> Smith, J. (1900). 'Enlargement of the spleen in malaria.' *Journal of Pathometry*, Vol. I, pp. 1-20.

Twenty-five reprints are supplied of each paper, free of charge. Additional copies (up to 100) can be supplied at cost price.

Subscription: £1 2s. 6d. per volume, post free, payable in advance to the Secretary, The University Press of Liverpool Limited, 177 Brownlow Hill, Liverpool, to whom correspondence concerning advertisements should also be addressed.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY
Vol. XIX. No. 1. March 31, 1925.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY
Vol. XIX. No. 2. July 16, 1925

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY
Vol. XIX. No. 3. September 30, 1925.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY
Vol. XIX. No. 4. December 16, 1925

THE NEMATODE PARASITES OF VERTEBRATES

BY

WARRINGTON YORKE, M.D.

Professor of Parasitology

University of Liverpool and Liverpool School of Tropical Medicine

AND

P. A. MAPLESTONE, M.D., D.S.O.

WITH A FOREWORD BY

C. W. STILES, *Professor of Zoology*

United States Public Health Service

This Volume contains a description of all the known genera of Nematode Parasites of the Vertebrate Animals with keys for their identification. The essential features of each genus are illustrated by camera lucida drawings of the type species or, when this is not available, of one of the other species; after the description of each genus is a list of the species it contains and of the hosts in which they are found. In preparing the work the authors had two objects in view, firstly to assist workers in the field in the identification of the Nematode parasites, and secondly to provide a reliable book of reference for the expert. The book is well illustrated and contains carefully prepared generic and specific indexes and bibliography.

xii + 536 pp. **Price 36s. net.**

Publishers - - J. & A. CHURCHILL

7 Great Marlborough Street, LONDON, W.1.

CONTENTS

EDITORIAL NOTICE

Articles for publication should not exceed 25 pages of the Annals, and will be understood to be offered alone to this Journal. They should be typewritten and addressed to :—

The Editors
School of Tropical Medicine
Pembroke Place
Liverpool

Illustrations for text figures or charts should be drawn clearly and firmly in Indian ink, if possible, on Bristol board. N.B.—*Blue or other coloured* ruling in squares or lines cannot be reproduced.

All lettering, names or legends on text-figures, charts or maps should be printed ***sufficiently large*** to allow of ***clear legibility*** on reduction if necessary.

Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way :— 'According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.' The references should be collected in alphabetical order of authors' surnames at the end of the paper, and arranged in the following way : —

ROBINSON, S. (1914). 'The spleen in malaria.' *Annals of Nosology* Vol. XX, pp. 20-25.

SMITH, J. (1900). 'Enlargement of the spleen in malaria.' *Journal of Pathometry*, Vol. I, pp. 1-20.

Twenty-five reprints are supplied of each paper, free of charge. Additional copies (up to 100) can be supplied at cost price.

Subscription : £1 2s. 6d. per volume, post free, payable in advance to The University Press of Liverpool Limited, 177 Brownlow Hill, Liverpool, to whom correspondence concerning advertisements should also be addressed.

C. TINLING & CO., LTD., PRINTERS, 53 VICTORIA STREET, LIVERPOOL.