نجاح انتاج الجيجانتو بلهارسيا فى الكتاكيت
بعدواهم بسركاريا شستوماتيد عزلت من قواقع الميلانيا توبر كلانا
فى محافظة أسيوط بمصر

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الملخص

لأول مرة يسجل وجود الجيجانتو بلهارسيا فى مصر وقد أمكن بنجاح انتاج الدودة الناضجة فى الكتاكيت بعد تعرضهم للعدوى من سركاريا قواقع الميلانيا توبركلانا . وكانت فترة الحضانة للعدوى من ٧ – ٨ أسابيع وكان شكل البويضة بيضاوى أو شبه مستدير ومزود بأشواك طرفية دقيقة .

وقد وصفت شكل الدودة الناضجة ، والسركاريا وحويصلاتها وقد صمم جهاز لتجميع الديدان من أوردة الكتاكيت . وقد أقترح أن الدودة المفلطحة المعزولة هى نوع جديد من أنواع الجيجانتو بلهارسيا .

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SUCCESSFUL PRODUCTION OF *GIGANTOBILHARZIA* IN CHICKENS BY INFECTING THEM WITH A SCHISTOSOMATID CERCARIA RECOVERED FROM *MELANIA TUBERCULATA* IN ASSIUT GOVERNORATE* (EGYPT)

(With 6 figures)

By

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SUMMARY

Gigantobilharzia has for the first time been reported in Egypt. The adult worms could be experimentally produced in chickens, after they were exposed to an apharyngeal brevifurcate ocellate cercaria recovered from *Melania tuberculata*. It is found that the prepatent period ranged from 7-8 weeks. The ova were oval or subglobular in shape and they were provided with a minute terminal spine. The morphology of the adult worms, the cercaria and sporocyst were described. A simple perfusion apparatus has been designed to collect the trematode from the veins of the chicken. It is suggested that the present trematode is a new species of *Gigantobilharzia*.

INTRODUCTION

In Egypt LEIPER (1915) found an ocellate brevifurcate cercaria from *Melania tuberculata* which he thought to be cercaria of *Bilharziella polonica Kowalewski*, 1895.

Later AZIM (1935) recovered another type of apharyngeal brevifurcate ocellate cercaria from *Melania tuberculata* in Egyptian Oasis. He tried without success to infect some mammals, birds and even himself with this type of cercaria. In the present work, an apharyngeal brevifurcate ocellate cercaria has commonly been found in *Melania tuberculata* collected from the branches of the River Nile of Assiut Governorate (Egypt). Many attempts were done to produce the adult worms in a variety of hosts. The ckicken was suggested to be the suitable experimental host for this trematode.

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* Part of a Ph.D. Thesis, Assiut University (1973) : "Studies on the relation of snails to parasitic infections" by LAILA A.M. OMRAN

MATERIAL AND METHODS

Collection of schistosomatid cercaria from *Melania tuberculata* : The snails were collected from different localites of Assiut Governorate. They were put into separate test tubes containing dechlorinated water and the tubes were exposed to a bright electric light for a few hours. Then the cercariae were discharged from the snails, they were collected in test tubes for further investigations. Some of these cercariae were fixed in 70% alcohol and they were stained with acetic acid alum carmine. They were identified as apharyngial brevifurcate ocellate type of cercaria (schistosomatid cercaria) (Fig. 1).

Attemps to infect a variety of experimental animals with this type of cercaria :

1. In chickens : Six chickens were given the infection through both the skin and mouth on different occasions.

2. In ducklings : Two ducklings were given the infection through both routes mouth and skin.

3. In white rats and white mice : Paddling method (MOORE, YOLLES and MELENEY 1949) was used in the same way of *Schistosoma mansoni* infections. Six rats and six mice were used in the present experiment.

The stools of the previously mentioned animals and birds were examined twice weekly for trematode ova.

When only the chickens passed terminal-spined ova, they were sacrificed, one on each occasion. Each chicken was submitted to autopsy for the purpose of collecting the proposed blood flukes.

Attempts to isolate the blood flukes from the infected chickens

Many attempts were done to isolate the blood flukes from infected chickens. The mesenteric veins were dissected as well as the liver parynchyma and the tributaries of the portal vein. These attempts were always accompanied with failure. No trematodes could be collected during the period of about six hours (per chick). For this reason it was found that this technique is time consuming and is not feasible to obtain such trematodes. It was then expected that the worms might be minute and fragile, that they might die as soon as the birds die, and it would be difficult to be collected (DONALD, Mc MULLEN and BEAVER 1945). For this reason the chick was perfused under general

anaesthesia according to the modified technique by YOLLES, MOORE, De GUISTE,, RIPSOM and MELENEY (1947) originally used for isolation of human schistosomes from experimentally infected hamsters. A simple apparatus has been designed by one of us* and it was found that it could be easily made and easy to use.

The apparatus (Fig. 2) consists of a bottle (500 ml. capacity) with a nozzle near its bottom, to which is attached a rubber tube. At the other end of the tube is fitted a fine needle (No. 18) A clamp is put at any point of the tube to be opened and closed according to the amount of fluid needed during perfusion. A blood pressure manometer is fitted to the inlet of the bottle, in such a way that air can be pumped under special pressure inside the bottle (usually 100-120 mm. kg. pressure is enough for perfusion). The bottle is filled to its half with 1% citrated saline (1% sodium citrate in normal saline).

Perfusion technique

The bird was defeathered under open ether anaesthesia. The skin was washed under tap water to remove any bristles or feathers from the skin.

The abdomen was opend, and all precautions were done to avoid loss of blood. Bleeding points were closed by an artery forceps. The pectoral muscles and the underlying keel were removed to expose the internal viscera including the heart, liver, gizzard and duodenum. The aorta was noticed to be hidden beneath the heart. A single ligature was applied to the thoracic aorta below the aortic arch to prevent back flow of the perfusing fluid. The fine needle attached to the apparatus was inserted gently into the thoracic aorta below its ligature. The bird was put in a slanting position, that the legs were directed downwards. The citrated saline was injected into the aorta. The portal vein was cut to allow the blood and the proposed worms in the superior and inferior mesenteric veins to flow out through the portal vein. The vessels became glistening and colourless. Coils of intestine which did not bleach out, were first manipulated gently to relieve kinking of the vessels. After perfusion, the viscera were washed with a strong stream of citrated saline to remove any worms which may have remained on them after being washed from the vessels. Bleached vessels were inspected under the stereomicroscope for any worms remaining inside them. The collected fluid was filtered through a special sieve of 16 mesh per cm. The sieve was then invereted onto a wide petri-dish, and saline solution was poured on the sieve to dislodge any possible worms. The fluid was then examined microscopically for blood flukes.

The collected worms were transferred to another petridish, containing clean saline solution. They were stained with acetic acid alum carmine after being fixed in 70% alcohol. Drawings were made by the aid of a Camera lucida.

RESULTS

The eggs (Fig. 3) were detected in the chicken's droppings 7-8 weeks after the infection. The majority of ova contained viable miracidia, while these detected in compression smears prepared from the liver or intestinal mucosa, showed immature or rarely mature miracidia. Usually the villi of the duodonum were heavily packed with ova (Fig. 4). The ova were oval or subglobular in shape, measuring 90-100 μ in length and 70-80 μ in breadth. A minute terminal spine measuring 5-6 μ in length could be seen originating from a shallow depression of the egg shell (Fig. 5). The miracidia appears to be surrounded by a thin membrane, which might be the cause of non-hatchability of the ova when left in dechlorinated water for about 24 hours. During that time, the miracidium was actively moving within the ovum in attempts to escape from its prison, but without success.

Gigantobilharzia sp. :

Fragments of the worms (Fig. 5) could be isolated from one chichen, only when its mesenteric veins were perfused. The worms when fresh, are whitish, slender, thread-like and coiled. In acetic acid alum carmine, the stained fragments could be differentiated into males and females on the basis of the following characters : The oral sucker is large in the male, measuring 0.035 mm by 0.013 mm. The sucker is kidney-shaped which is concave ventrally. In the female, the oral sucker is either rudementary or absent. The gynaecophoric canal could be detected in the male, being 0.810 mm. in length. The testis were not observed in the present stained material. Other structers of the male genital organs are found lying at one side of the gynaecophoric canal and extending anteriorly. The female possesses a subterminal opening, possibly the metraterm. This opening is leading to the uterus which contains about 13 ova arranged in one line. The uterus measures 0.973 mm in length, extending from the ovary to the metraterm. The ovary is spiral or wavy lying at 1.04 mm far from the anterior end of the body. The ovary measures about 0.405 mm in length by 0.054 mm in breadth. The posterior

end of the worms are rounded and broader than the anterior end, measuring about 0.675 mm in both sexes. What appears to be a ventral sucker measuring 0.054 mm in diameter, was detected posterior to the ovary.

DISCUSSION

OISO (1927) was the first report on the life history of an avian schistosome, Bilharziella yokogawi. However, Mc. LEOD (1937) transferred this species to the genus Pseudobilharziella.

Since that time many discoveries of the life cycles of other avain schistosomes were recorded. SZIDAT (1929) reported on the life cysle of *Bilharziella* polonica KOWALEWSKI, 1895. Later BRUMPT (1931) discoverd that *Cercaria ocellate* LA VALETTE (1855) developed into *Trichobilharzia kosserewi Skrjabin* and *Zaharow,* 1920. BRACKETT (1942) recovered the adult of *Cercaria gyrauli* BRACKETT, 1940 from black birds and placed it in the genus *Gigantobilharzia.* NAJIM (1952) worked out the life history of *Gigantobilharzia huronensis* NAJIM, 1950 from gold finches.

Due to the difficulty in obtaining the very long, fragile worms from the minute intestinal veins of the definitive host, satisfactory specimens were frequently not obtainable and this lack of adequate material has resulted in much confusion regarding the taxonomy and life cycle of this group of trematodes DONALD *et al.* (1945). Many workers have reported on the difficulty of obtaining entire worms, thus usually fragments of males or females or both are usually described. This was the main cause of much controversy about the generic and specific diagnosis of the avian schistosomes. Some workers such as YAMAGUTI (1958) relied upon the morphological characters of males and females, while others such as LEIGH (1953) found that the morphological characters of the ova are necessary for indentification. More recently KHALIFA (1972) suggested that the morphological characters of the cercaria should be included to identify the genus of the avain schistosomes.

According to YAMAGUTI (1958), the present material is related to the subfamily Gigantobilharziinae, Family Schistosomatidae LOOSS, 1899. According to LEIGH (1953) and KHALIFA (1972), the generic name of the present trematode is Gigantobilharzia (ODHNER, 1910). Many species are reported, so far, from different localities of the world. However, *Gigantobilharzia* has never been reported in Egypt, and accordingly this

is the first time to report on the presence of *Gigantobilharzia* in experimentally infected chickens in Egypt (Assiut Locality). The natural host of this trematode in this locality awaits further investigations. It is worthwhile mentioning here that FARLEY (1971) stated that *Gigantobilharzia* from Africa are all with incomplete description and unknown life cycles. Accordingly the present material is not comparable with the five African species of *Gigantebilharzia* described by FAIN (1955 and 1960). According to the available literature, chicken were used for the first time in this experiment to produce *Gigantobilharzia*. It is as well, the first time to confirm that *Melania tuberculata* is a new intermediate host for *Gigantobilharzia*.

The prepatent period of the infection with this type of bird schistosome has been identified. The fact that the ova did not hatch within a long period when submerged in dechlorinated water may throw the light on the mode of infection of the snail and it could be suggested that the snail might swallow the ova. In the gut of the snail, hatching might take place, resulting in free miracidia which could grow further in the snail's liver.

The apparatus and the technique devised by the authors have helped much to collect the blood flukes from the infected chicken within a short time. However, it is suggested that more training is needed to get the intact worms for accurate description and identification of this species of *Gigantobilharzia*. Fragmentation of the worms might be attributed to the attempts done to stretch out the coiled worms after their exit from the host.

The present trematode might be a new species of *Gigantoblharzia* on the basis of the fact that it is recorded in a new locality, a new intermediate host, and a new definitive host.

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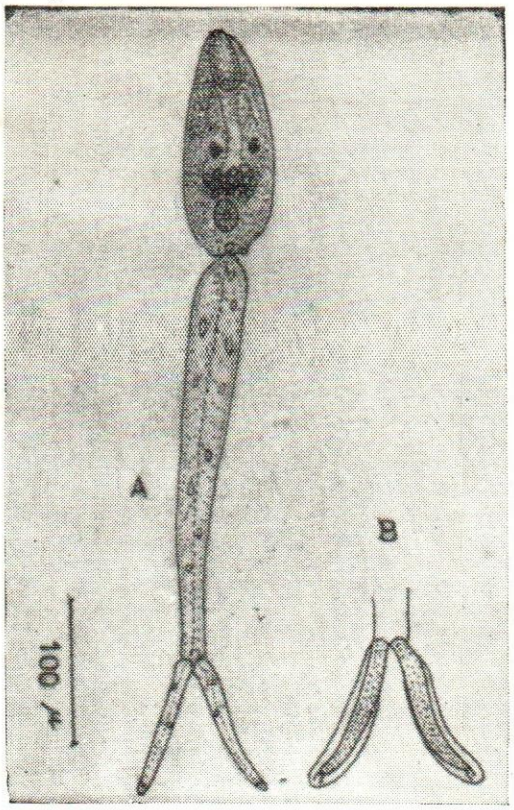

Fig. 1.—Cercaria of *Gigantobilharzia*.

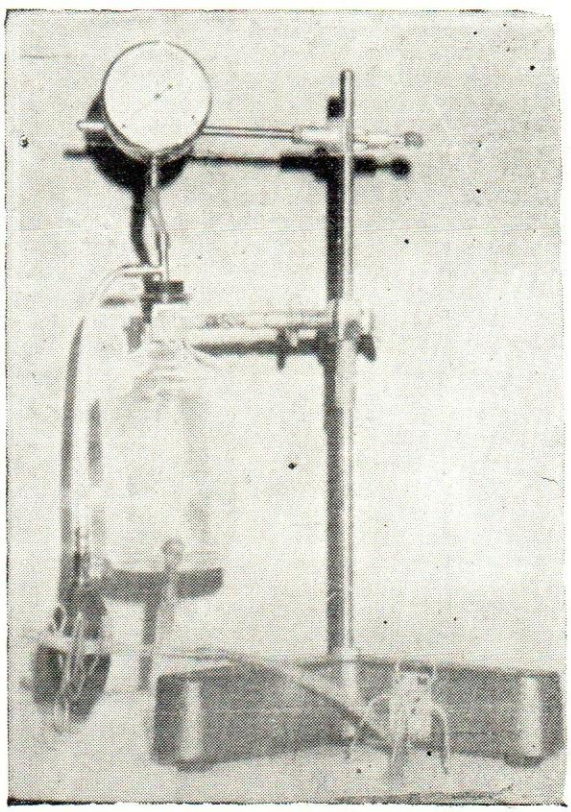

Fig. 2.—Apparatus used for perfusion of the mesenteric veins of the chicken.

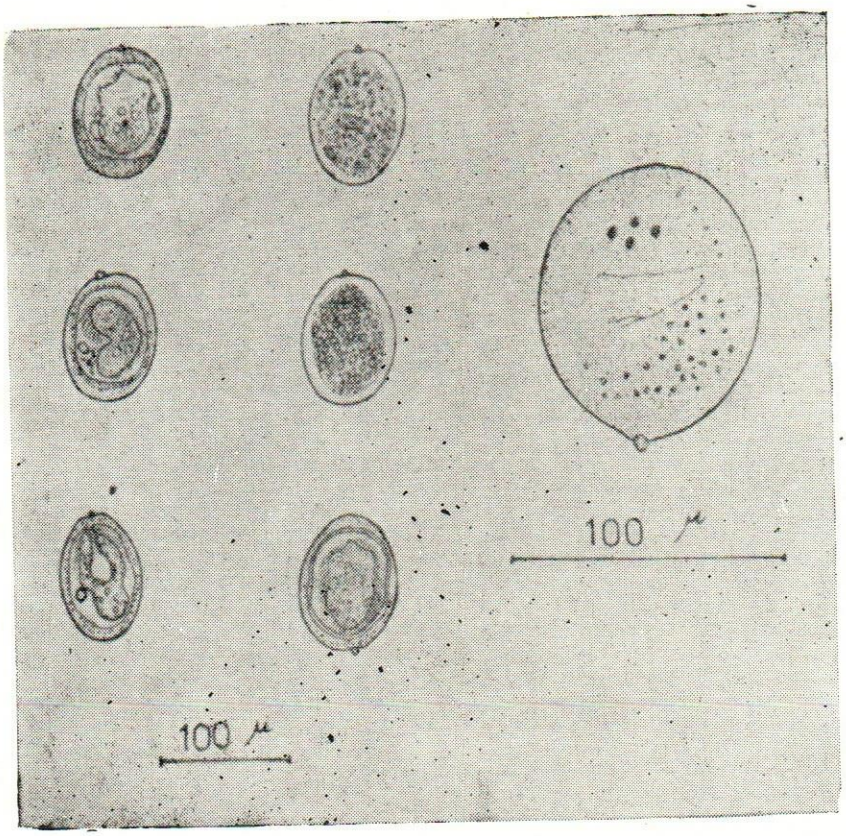

Fig. 3· —Ova of *Gigantobilharzia*, notice the mature miracidia in some of the ova.

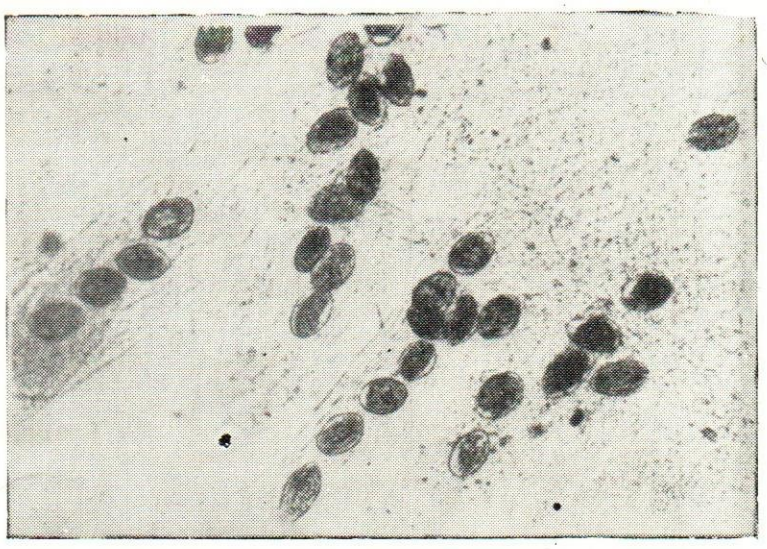

Fig. 4.—Ova of *Gigantobilharzia* within the villi of the small intestine. (× 80).

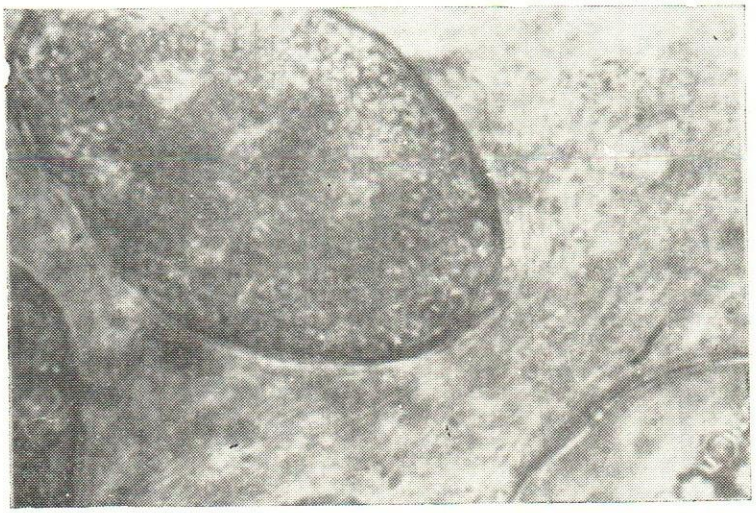

Fig. 5.—*Gigantobilharzia* ova, notice the minute terminal spine. (× 800).

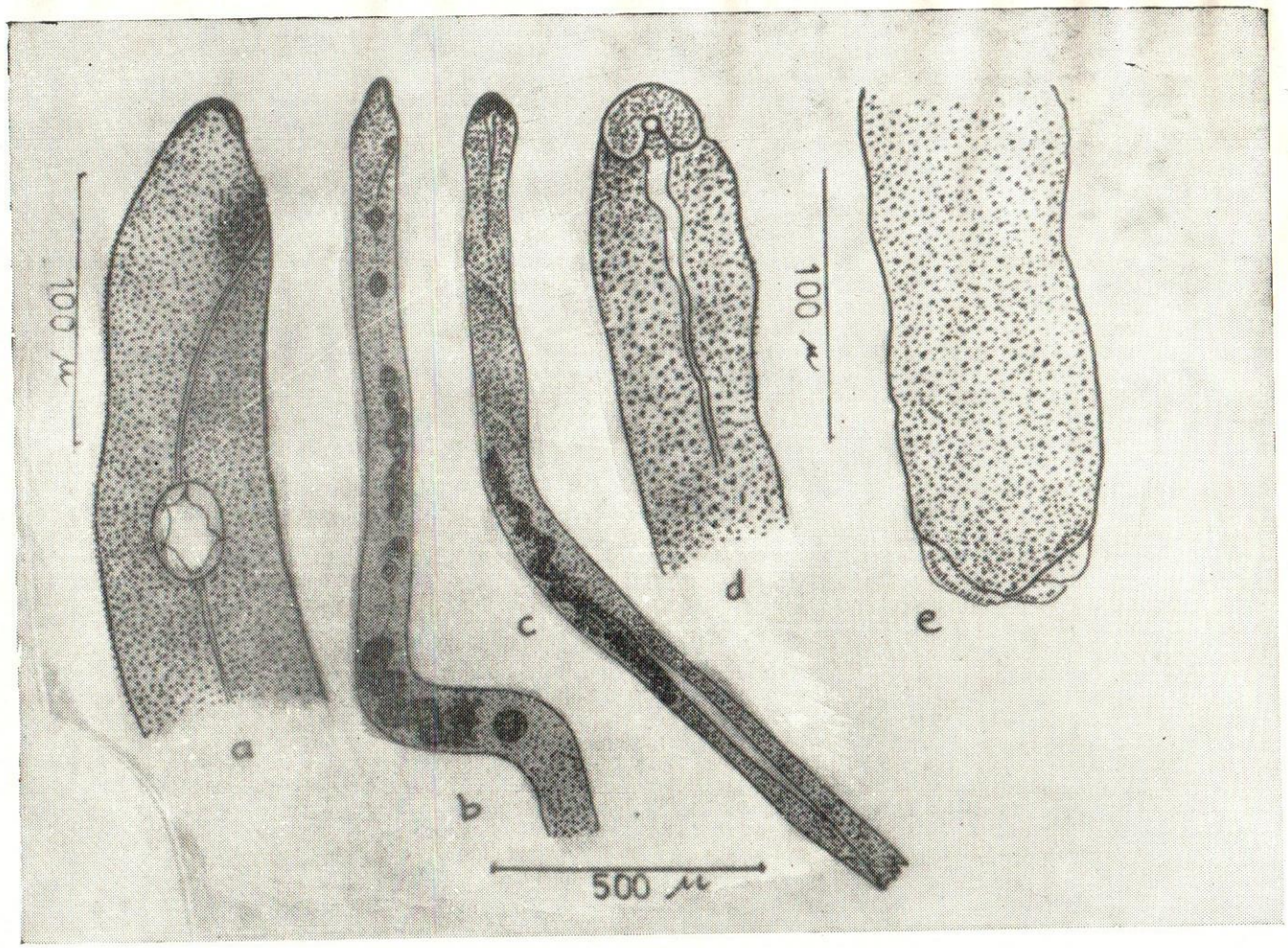

Fig. 6.—*Gigantobilharzia* **sp. recovered from the mesenteric veins of experimeatally infected chickens : (a, b) female ; (c,d) male ; (e) posterior end of the worms.**