other injurious substances act similarly and, *in vitro*, tumour cells occasionally arise. Usually, however, in experiments, as in the body, the effect of injuring respiration is to cause the death of the cell.

In many cases in plants it is well known that lack of oxygen for a time may result in cell-proliferation, and it may be significant in crown gall that *Bacterium tumefaciens* is an organism which is very strongly aerobic. Thus it is possible that in the intercellular spaces it modifies the oxygen relations of the cells so that the internal changes resulting in proliferation are set up. Whether the tissues in crown galls show a similar type of metabolism to that which Warburg has found in animal tumours will be seen from further work. It certainly appears possible that in their cellular physiology the plant galls may have at least some points in common with the animal tumours.

[*May* 4, 1927.

The Development of *Schistosoma mansoni*.

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(*Communicated from the Pathological-anatomical Laboratory of the Military Hospital of Paramaribo, Dutch Guiana.*)

DURING an investigation into the development of *Schistosoma mansoni* at the beginning of this year, undertaken more out of interest in the cause of a disease endemic here than with the intention of discovering anything new in connexion with it, a few details were observed, some not yet mentioned in the literature of the subject and some not entirely in keeping with facts therein stated.

Therefore, notwithstanding the excellent work of Leiper on the development in the intermediate host, and that of Yamagiwa, Lütz, Faust, and others, on the development in the final host, I venture to mention briefly certain observations of my own in Surinam on the development of this highly interesting trematode.

(I) THE DEVELOPMENT IN THE INTERMEDIATE HOST.

The full-grown parasites of this species of Schistosoma live in the hepatic portal vein and its branches. The parasites—paired—swimming against the blood-current, enter the narrower veins, where the thinner female, quitting the male, deposits its ova.

The ova, distinguished by a lateral spine, measure about 165 microns, and the spine about 26 microns. In order to continue the species, a number of the ova penetrate the intestinal wall and reach the intestinal canal.

The part played by the lateral spine is not quite understood. Possibly the ovum is forced back against the intestinal wall by the blood-current and remains sticking to it by means of this lateral spine. With the fæces the ova are ejected.

If the ovum be placed in a hypotonic medium (e.g., water, or very diluted eosin-solution, added to the fæces) the embryo, if alive, visible through the egg-shell, starts moving, while, after an interval—depending on light, temperature (optimum 35°) and unknown influences—the shell ruptures laterally somewhat behind one of the poles.

The miracidium slowly creeps out, and, escaping from the shell, swims away. I have never observed in the case of *Schistosoma mansoni* the dilatation of the egg-shell as described by Brumpt for *Schistosoma hæmatobium*—"L'œuf se gonfle."

The duration of the life of the free swimming miracidia—estimated at two to three days—is shown in Surinam (laboratory temperature 33° max.) to be forty hours at the longest: according to Faust, in China, three days (*Schistosoma japonicum*); according to Christopherson, in the Sudan, at the most nine hours

(*Schistosoma hæmatobium*). The morphological structure is clearly discerned by staining with eosin, especially in dying forms, as a mobile protoplasmic mass, ever changing in shape, clothed with cilia, the greatest measurements being between 110 and 240 microns. At one pole is found a headlobe or papilla surrounded by a ring of cilia. The germ-cells, salivary glands, cerebral ganglion commissures, the blind, pouch-like intestine and the excretory apparatus are clearly seen.

The ova being deposited with the fæces in water frequented by certain species of snail, further development takes place within these molluscs.

According to Leiper, who visited Surinam in 1921, *Planorbis olivaceus* is found in this colony exclusively. He found 5 per cent. of these snails infested with *Schistosoma mansoni*. After a systematic examination of some thousands of snails from infected areas around Paramaribo, I found that not only *Planorbis olivaceus*, but also *Planorbis guadeloupensis* is found naturally infected in the locality.

The highest percentage of infested *Planorbis olivaceus* was 18 per cent. for the month of September, that of infested *Planorbis guadeloupensis* 15 per cent. for the month of July (1925).

Observations published hitherto on the penetration of the snail by the miracidium are more or less in agreement. The miracidia are said to pierce the soft parts of head and foot (Miyairi and Suzuki, 1913, Franco and Mello, 1921, Faust, 1924), of the tentacles (Lütz, Brumpt and others, 1917) or of the mantle-cavity (Manson-Bahr and Fairley, 1920).

My observations do not entirely agree with these authors' views. Under the binocular microscope the attraction of the miracidia can be clearly observed. Only a few adhere to the external soft parts of the snails; the bulk disappear into the buccal and the mantle cavities. I have never observed the mutilation of the tentacles, described and illustrated by Lütz and Brumpt, which would be caused by a mass invasion of these organs. I seldom found miracidia in the tissue, in serial microscopic sections, although they were prepared immediately after the infection was definitely observed under the microscope.

Either the miracidia have certainly penetrated, and have not been found, even when many serial sections have been made, or they have been observed in the buccal or the intestinal canal. Therefore I think it justifiable to assume that infection of the snails through the mouth is not only possible, but may be considered most frequent.

The infection-method of other trematodes, the miracidia of which do not spontaneously escape from the egg-shell, but are liberated in the stomach of the intermediate host (*Clonorchis sinensis, Distomum macrostomum, Dicrocœlium lanceolatum, Opisthorchis felineus*) is analogous and fully justifies this statement.

Further development in the snail is easily traced. The unchanged miracidium that has penetrated, as a rule—even in sections made immediately after the infection—is difficult to find; the other stages may be clearly seen in the microscope sections. After a few days a sporocyst in its first stage of development can be detected as a thick-walled pouch in or close to the intestinal wall. As this primary cyst steadily grows it changes into a thin-walled tube constricted in places; daughter-sporocysts, which develop from local thickenings of the mother-sporocyst wall ("germ-cells"), arise; they are isolated from the wall, and eventually fill the mother-sporocyst. In these daughter-sporocysts, regenerating by transverse fission and furrowing, the so-called cercariæ develop again out of local thickenings of the wall (buds). The danger to man lies with these cercariæ.

The moment of the rupture of the primary cyst varies in different individuals. In the third, or at the beginning of the fourth week, the liberated daughter-sporocysts are found between the hepatic islands, in the form of thin-walled pouches, filled with tail-less cercariæ. In these wandering daughter-sporocysts reproduction is still going

on by transverse fission and furrowing. The hepatic tissue degenerates in consequence of pressure-atrophy. The thin wall of the daughter-sporocysts expanding approaches the mantle-cavity of the snail, and at the spot of least resistance when the cyst is entirely filled with cercariæ it ruptures. The cercariæ escape in bunches into the mantle-cavity. From here they swim away into the surrounding water.

The number of cercariæ produced by an infested snail during its life-time of two or three months is so fabulously large that it can hardly be estimated. For instance, from a snail infected in the laboratory 600 cercariæ per hour escaped on the thirty-seventh day after the infection (7 a.m. to 5 p.m.), 92 per hour on the fifty-sixth day, 190 per hour on the fifty-seventh day, and 44 per hour on the seventy-eighth day. During night-time cercariæ do not escape.

From many controlling observations on an artificially infected snail, from which cercariæ continued to escape during sixty-five days, I was able to estimate the number approximately at 172,000 (2,646 daily). Liberation is enhanced by light and heat, under the influence of which the snail moves about with activity.

The lifetime of these cercariæ in pure water at laboratory temperature is more than twenty-four hours. In a suspension of red-blood cells in physiological NaCl solution, at body temperature, it is not more than six hours.

Excepting sections in series the different stages of development of infected snails are most clearly demonstrated by a binocular microscope. The snails are macerated in a mixture of glycerine and chloral hydrate 3/7 (Vonk) in which they remain some days. The isolation of sporocysts and daughter-sporocysts, otherwise not possible, is easily effected by this method.

The duration of this parthenogenetic development[1] was found in our laboratory to be never more than thirty-four days and never less than thirty-three days.

Experiments were carried out to discover what influence light and temperature have on the period of development. To that end a large number of snails infected at the same time (*Planorbis olivaceus* and *Planorbis guadeloupensis*) were kept in different groups under different conditions. The result was that the period of the development proved practically independent of the influence of light and temperature. The mortality of the infected snails is greater at high temperature and less with snails kept in the dark, than under ordinary conditions.

Lütz, in Brazil, saw the first cercariæ escaping after 30 days (*Planorbis guadeloupensis* and *Schistosoma mansoni*). Cawston, in South Africa, mentioned that the first cercariæ never escape before the thirty-fifth day after artificial infection (*Physopsis* and *Schistosoma hæmatobium*). Faust and Meleney, in China, found the duration of the development in the snail to be about seven weeks (*Onchomelania hupensis*, *Schistosoma japonicum*).

(II) The Development in the Final Host.

These experiments were carried out with two different species of cercariæ of the order of the Schistosomidæ, viz., with the larvæ of *Schistosoma mansoni* and with a furcocercous cercaria of different size, without pharynx and eye-spots, derived from *Planorbis guadeloupensis*.

The measurements of the living cercariæ were as follows:—

	Cercaria of *Schistosoma mansoni*	Cercaria of unknown species
Body	75 to 225 microns	... 75 to 150 microns
Unforked tail ...	150 to 225 ,,	... 125 to 150 ,,
Tail fork	90 ,,	... 180 ,,

Fixed in 10 per cent. formalin, these measurements became:—

Of cercaria *Schistosoma mansoni* 115 microns, 190 microns, 38 microns
Of cercaria spp. ... ... 120 ,, 125 ,, 150 ,,

[1] The germ cells being considered as parthenogenetically developing ova.

These measurements of *Schistosoma mansoni* do not agree with the numerous and variable statements published.

Guinea-pigs, rabbits, cats, mice, ducks, chickens, etc., were used for experiments.

Method of Infection.

(1) Submersion of the partly shaved or plucked animal in highly infected water. (2) Pouring of water, containing cercariæ, into the mouth. (3) The same in the conjunctiva. (4) The same through the anus into the rectum. (5) Subcutaneous injection with water containing cercariæ.

Precautionary measures were taken :—

(1) To avoid the skin being punctured during shaving or plucking, which might facilitate penetration. (2) To avoid cercariæ coming into contact with the skin during the artificial infection by way of the mucous membranes.

At intervals after the infection the mammals were killed, and the organs (*porte d'entrée*, heart, lungs, diaphragm, liver, spleen, kidneys, brains, intestine and mesentery) microscopically examined.

(1) *Infection through the Skin.*

Infection through the skin proved successful. At first it was difficult to find the cercariæ which had penetrated in the skin. Even by violently exaggerating the conditions appertaining in nature, e.g., submerging the ear previously macerated, in strongly infected water for many hours, cercariæ which had penetrated were looked for in vain in a large number of serial sections. The water used for the infection showed, however, that infection had taken place, as many immobile tails minus bodies were found in it.

These experiments were frequently carried out and the results were always uniform. I therefore assumed that the relatively few cercariæ that do penetrate remain in the skin and the subcutaneous tissue only for a short time. The results of repeated investigations confirm this assumption. If the duration of the infection was shortened to fifteen to thirty minutes it proved easier to find the cercariæ in the skin. The results were somewhat different when the skin was incised ; the number of cercariæ left behind was smaller, the number of the tails abstracted larger, and the cercariæ were easier to find in the skin.

The best results were obtained when experimenting with new-born mice, which could be bodily submerged in the infected water and microscopically examined. In these experiments nearly all cercariæ entered the body of the subjects of experiment, the great quantitative difference being undoubtedly due to the tenderness of the skin of the new-born animals.

These latter experiments also showed that the cercariæ do not make use of the hair-follicles or pores of the skin. Sections show that the skin—perhaps after previous lysis (by the action of the salivary glands of the cercariæ)—is severed and lifted up.

In view of these experiments I think that, under natural conditions, infection through the intact skin of the body or extremities of adult men or animals does not easily take place. This is not to be considered as contrary to the generally accepted opinion that the skin is the most frequent *porte d'entrée*. The tender skin of the præputium, the soft skin between the toes, and also the frequently present lesions or wounds on the extremities, offer sufficient chances for the infection of peasants and labourers working in swamps and morasses, and also for infection of careless bathers.

Moreover the question of the difficulty of the penetration of the experimental animals by the cercariæ should be duly considered, viz., as to whether this difficulty might not arise from the unsuitability of the animals submitted to infection.

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With regard to these experiments with mammalia, it may be stated that new-born mice were easily infected, the mucous membrane of large animals succeeded well, and cercariæ injected subcutaneously always reached complete development.

(2) *Infection through the Mucous Membranes.*

This mode of infection always produced good results. Considering the fact that the cercariæ need not enter through hair-follicles or pores, this was to be expected.

(3) *Subcutaneous Infection.*

This required great care. When too many cercariæ are injected, the animal dies, generally after a few days, with diffuse infiltration of both lungs (see later on). If this stage be passed, death ensues after some weeks in consequence of a blocking up of the small ramifications of the hepatic portal vein by young parasites.

(4) *Experiments with Birds.*

As might be expected, thesec ercariæ of mammalian trematodes never attained a full development when birds were used for experiment. Birds were infected in order to discover whether these cercariæ had any tendency to penetrate extraneous animals. With birds the infection through the intact skin proved successful, and infection through the wounded skin and mucous membranes (*per anum*) readily produced definite results.

It is not impossible that this infection of an extraneous animal plays an important part in the epidemiology of this disease (*diversion of parasites*), the more so because the cercariæ that penetrate never arrive at sexual maturity or undergo further reproduction.

(5) *The Route by which the Penetrating Cercariæ reach the Vena Porta.*

This was always the same in the case of both species of Schistosomidæ and was used with all experimental animals.

The cercariæ, in whatever way they may be brought into contact with the experimental animal, always find their way into the veins, after having wandered for some time within the connective tissue of the skin or mucous membrane. They then immediately feed on the blood; a few remaining behind in the tissue die. The majority reach the veins and so the right heart. Six hours after infection cercariæ may be found in coagulated blood in the heart. Serial sections of the heart show, however, that the cardiac muscle or the septum is never pierced.

Cercariæ, once in the blood-circulation, find their way into the lungs. The lung is therefore a good place in which to find cercariæ. In the lungs, they leave the thinner branches of the pulmonary artery, and then—filled with blood—penetrate the stroma between the alveoli. At first they were found diffusely spread; later on they accumulated in the bases of the lung.

Microscopic sections of apices and bases of lung show a marked difference two or three days after the infection. Most of the cercariæ are then congregated in the bases of the lungs. This, it seems, is the critical period of the development of this parasite. Except for a steady growth and further aggregation in the pulmonary bases, further migration into other organs does not occur in the majority of cases. They wander about within the lungs and die. Small hæmorrhages, induration-areas and giant-cells are proof of the death of many. Only a few parasites are able to find a way out of the lungs. The visceral pleura is pierced and the diaphragm entered. This may occur adjacent to the pleural cavity, adjacent to the mediastinum, or adjacent to the pericardial cavity.

Very often an adherent pleuritis diaphragmatica is set up by which means a safe path is established for the cercariæ. In a high percentage of the observed cases this pleuritis was found to be present.

Three days after the infection, cercariæ can be found in the diaphragm. After piercing the diaphragm some parasites reach the body-cavity. Presumably these die. In no section of the free margin of the liver have I seen any penetrating cercariæ. The remainder then reach the liver at the place of adhesive connexion with the diaphragm.

In the stroma of the liver, development proceeds with great speed. After a few days the cercariæ begin to resemble adult parasites. The time of reaching the portal veins appears to vary considerably. The reserve of food (blood) seems to suffice for many days. After eight days parasites can be found in the stroma of the liver, filled with remains of previously sucked blood, but apparently this is the limit. After this the young parasites are found only in the ramifications of the portal veins, where further development takes place.

Only very exceptionally are a few cercariæ found in the arterial circulation. Twice a *Cercaria mansoni* was found in an artery of the hilus of the kidney. Once a number of dead bodies of the unknown species were found in the mesenteric artery. Presumably in these cases a few cercariæ reached branches of the vena pulmonalis, and thence the great circulation.

The observation of Turner, who found in a pulmonary vessel full-grown bilharzia parasites, has to my knowledge never been corroborated by another observer. [Ruffer recorded this.—R. T. L.] My opinion, based upon pathological-anatomical alterations in the lungs of bilharzia patients, is that full-grown parasites in the lungs need not be rare. The important alterations, many times observed by myself in Surinam, cannot easily be explained by emboli alone. Moreover in one case I succeeded in squeezing out of the pulmonary-vessels a few full-grown bilharzia parasites. In cases of severe infections it appears most probable that a few cercariæ, remaining behind in the pulmonary vessels, may completely develop under favourable conditions.

According to the described experiments, the cycle of development of *Schistosoma mansoni* in the final host takes place as follows through :—

(1) *Skin or mucous membrane.*
(2) *Connective tissue* (a few, remaining there, die).
(3) *Veins.*
(4) *Right heart.*
(5) *Pulmonary artery :—*
    (*a*) can seldom completely develop there (?).
    (*b*) can seldom obtain access to the pulmonary veins and so into the general circulation (?).
    (*c*) reach the stroma of the lungs and become aggregated in the base of the lung.
(6) *Base of the lungs :—*
    (*a*) the majority remain there and die.
    (*b*) the remainder pierce the pleura and reach the diaphragm :—
        (i) via pleural cavity.
        (ii) via mediastinum.
        (iii) via pericardial cavity.
        (iv) directly, after an adherent pleuritis diaphragmatica is set up.
(7) *Diaphragm :—*
    (*a*) from there arrive in the abdominal cavity, presumably to die.
    (*b*) reach the stroma of the liver at the point of connexion with the diaphragm.
(8) *Stroma of the liver.*
(9) *Hepatic portal vein.*

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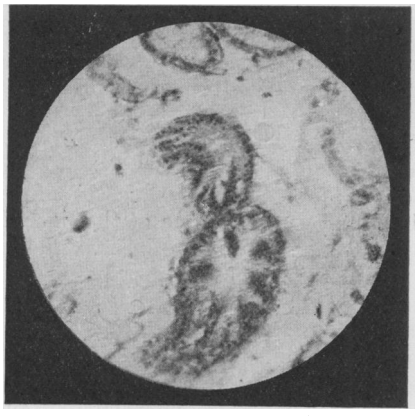

FIG. 1.

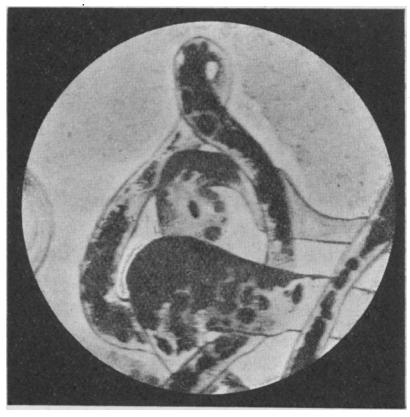

FIG. 2.

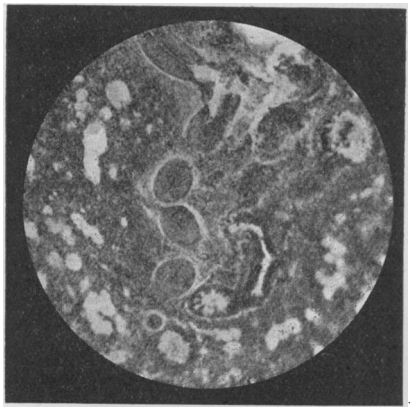

FIG. 3.

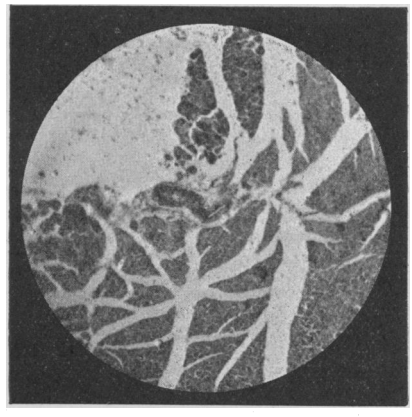

FIG. 4.

FIG. 1.—Primary sporocyst with local thickenings of the wall (buds). *Planorbis guadeloupensis.*

FIG. 2.—Snail macerated with glycerine chloral hydrate. Daughter sporocysts. (Somewhat diagrammatic.)

FIG. 3.—Cercariæ of *Schistosoma mansoni* heaped up in the stroma of the lung tissue. In a guinea-pig two days after infection.

FIG. 4.—Cercaria of *Schistosoma mansoni* penetrating the diaphragm. In a rabbit six days after infection through the skin.