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towards the life cycle of *Distoma hepaticum*

Adolpho Lutz
Towards the life cycle of *Distoma hepaticum* *\n
In the preliminary note that follows, I summarize all the observations which I have been able to make until now on an epizootic disease prevalent in the Sandwich Islands. It is caused by a *Distoma*, which, so far as I can judge by the literature on the subject, does not differ either in build or size, nor in its mode of life cycle, from *Distoma hepaticum*. My description will also show that the cercariae, rediae, etc. display so few differences that I feel justified in designating it as *Distoma hepaticum*.

As to the distribution of this *Distoma*, it has certainly established itself on three of the Hawaiian islands, to wit, Oahu, Maui, and Kauai (and it most likely is not entirely absent from the island of Hawaii). On Oahu, it was found several years ago on the Koolau side, which faces the trade winds and is consequently rainier, and was finally recognized as *Distoma hepaticum*. However, official attention was only recently called to the parasite, on account of the poor quality of the beef cattle. It now appears that the other side is also extensively infected; in some places, almost all cattle have died from distomiasis, and in other places, the disease is just as common, though not so intense.

Of farm animals, beef cattle have been the most affected until now; horses kept in the same places seem to be infected but to a lesser degree. There is no information in regard to goats, sheep, and pigs, which are not bred to any extent around Honolulu, but I have been told that the flukes have also been found in wild pigs (that is, pigs that have turned wild) killed in the vicinity of infected pastures.

These circumstances first came to my notice through one of my patients, who told me about the high mortality rate on his dairy farm near the city. From his description, I suspected liver flukes and asked him to have one of the sick animals slaughtered in my presence. This was done a little later; the victim was a very emaciated, slightly jaundiced cow. We found that the liver was small, strongly adherent and filled with flukes, masses of which could be pressed out of the bile ducts. The distended gall bladder contained two handfuls of living flukes, which I

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1 Former name of the Archipelago of Hawaii. [E.N.]
took for culture. I would like to mention that in the lungs I also found three small foci of lobular pneumonia similar to those seen in Filaria pneumonia; in one of them there was a young Distoma, while the inhabitants of the other two seemed to have emigrated again.

In the eggs that I took and carefully washed, embryos developed in 2 to 3 weeks; however, since I was very occupied with other work at the time, most of the culture was lost, with the embryos hatching and dying off. Only a small part was used for infection experiments.

I later made another culture in which I used the contents of the gall bladders of six diseased and slaughtered animals. The earliest hatching of embryos occurred on the 12th day, with the atmospheric temperature quite high; but this only happened with eggs that were covered with a minimum layer of fluid. One part was also stored in the same manner as employed with Ascaris, as described earlier, i.e., in a bottle with a minimum of fluid in which the bottle was rolled to make the eggs stick evenly to the damp inner walls, so the eggs would stay just moist enough to prevent their drying out; this lot developed just as rapidly. I also found, as did Leuckart, that a deeper layer of liquid retards development, so that even when the water is only a little deep, the process is much slower and may take weeks or even months in the open. Processes of decomposition in the water are bad for cultures; mycelia (*Saprolegnia*) develops on the eggs and probably penetrates into them; in other cases, the eggs become filled with a mass of bacteria that distend them like balloons and eventually burst open their opercula. In nature, development occurs in water that is running for the most part, conditions are not rarely more favorable, but many eggs probably perish in similar fashion or die when the water level drops, because they cannot tolerate desiccation even for short periods.

Even after the embryo is formed and is already clearly contracting, one should not expect hatching to occur immediately; the best sign of maturity is the shape of the stigma, which is not easy to describe. Seen from the front, it should look clearly X-shaped, with longer lower limbs; at this stage, one can induce the embryo to hatch by using Leuckart’s method, illuminating it after keeping it in the dark or by applying cold water to it. I have clearly observed the cilia moving inside the egg during examination in an ammonia solution (the percentage of which I cannot specify with precision). The hatched embryos are such lively and agile swimmers that they can undoubtedly cover considerable stretches even without the aid of currents; their movements are, however, uncertain and irregular.

I was very much interested in following the development of these embryos. As Leuckart’s observations have shown that *Limnaeus minutus* is the intermediate host of the liver fluke, I first tried to ascertain whether this species occurs here or not. I remembered having seen similar forms in a constantly flooded taro plantation and in a brook. The former location had since been drained, but I soon found that similar shells could be found on taro plantations. The forms I found were small, at the most the size of *Limnaeus minutus* and not unlike them in other ways. They generally contained 6 to 20 specimens of a *Distoma* enclosed in hyaline cysts,

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2 Another name for yam. [E.N.]
which resembled *D. echinatum*, although feeding experiments on a duckling produced no results. Stages in the life cycle of *D. hepaticum* were not to be expected from the nature of these locations, nor were they found.

With a view to more easily demonstrating the penetration of the embryos, I chose the smaller specimens for my experiments, because they could be pulled out of their shells and crushed between two slides and then easily sampled *in toto*. Infection occurred rapidly and without any difficulties; the embryos were easily recognized by the two round spots of pigment that are soon produced after cleavage of the X-shaped masses of pigment. After 12 days, I found the first emigrated rediae in moderate numbers. This stage, like the earlier ones, also agreed entirely with Leuckart’s careful description, except for the somewhat more rapid development of the rediae, probably because of the higher temperatures.

After finding the probable intermediate host for the Sandwich Islands, I tried to detect spontaneously infected specimens in appropriate places.

Two samples from suspect locations produced negative results. My third search was successful, however, and the material obtained much more favorable than any available to any of the earlier investigators.

I had heard from another of my patients, who had caught ancylostomiasis in Oahu, that in a pasture belonging to him and others, most of the cattle had died of distomes and the rest were sick. I gave him a sample and asked him to bring me as many such snails as possible from the cattle’s drinking place.

After a few days, he returned with about one hundred snails of different sizes. The first large specimen that I examined had over one hundred rediae, most displaying mature cercariae, several hundreds of which encysted while I watched. I found that all the large specimens were infected, and usually very much so. There might have been up to 200 cercariae, but mortality is very high in such snails, which soon perish because of the infection. The upper part of the shell is found to be filled by a thick plug of rediae; when the upper whorls are broken, they escape “as if the snail had been devoured by maggots,” as aptly remarked by a layman. All that may be left of the liver are small pieces, so that it is just as appropriate (or even more appropriate) to call the disease a liver plague in the case of snails as in the case of cattle. The sexual organs also seem to atrophy.

At first glance, all the snails seemed to belong to the same species, evidently a *Limnaeus* identical to the one I had found earlier. Notwithstanding, there were also some considerably larger specimens, which agreed wholly with Leuckart’s illustration of *Limnaeus pereger*. Moreover, my earlier specimens, which contained spermatozoa but not eggs and thus could not have been fully grown, resembled the figure of *L. pereger* more than that of *L. minutus*. Although I had no data other than these two figures from Leuckart’s book, it seemed probable that *L. pereger* was the real host, because it was precisely the quite large snails (which could not belong to *L. minutus*) that displayed the largest broods of cercariae; furthermore, I soon became convinced that only the youngest specimens of my species could be infected, which again agreed with Leuckart’s findings on *L. pereger*. This seemed even more likely after Leuckart kindly sent me the descriptions of both species, which spoke decidedly in favor of my view. However, as the snails found here might also be a different species, a little earlier I had sent Leuckart a number of shells to be
submitted to a conchologist. The decision stated “L.pereger,” and so we can take it as a fact that at least in a tropical climate, L.pereger may serve as a very prolific intermediate host of the liver fluke, whereas in Europe, according to Leuckart’s experiments, Distomum hepticum only develops in this host to the stage of rediae.3

Among the shells received last, there were some that spiraled to the left, although they were of the same size and very similar in shape.

After noticing this peculiarity, I found a slight difference in the opening of the shell, a very different radula, a thin, blackish foot, and longer, thread-like feelers; by these characters, I determined them as belonging to the genus Physa, based on Claus’s Handbuch der Zoologie. Some of these shells were also in the sample sent to Europe, which were likewise identified as Physa.4 This Physa could not be infected with Distoma hepaticum at any age, although it contained the hyaline Distoma cysts. The embryo of D.hepaticum is thus not only more selective than other distomes, but also contrasts sharply with the adaptability of the adult liver fluke.

No other species of snail that might serve as an intermediate host has so far been found in the infected places.

Since then I have found infected snails in two other localities and feel sure that in highly infected places, one ought as a rule be able to find the host, provided that the surroundings have not been disturbed and that one knows what is being sought. It may be interesting to describe the infected places in brief, since, as far as I know, nothing similar has so far been published.

Honolulu is basically surrounded by a chain of volcanic mountains, two to three thousand feet high, with a narrow plain stretching in front of them. On the side of Honolulu, the slopes are gentle but furrowed by a dense system of erosion valleys, which vary in breadth and depth but are always very steep. Each has one or two swift mountain brooks that completely (or almost completely) dry out at the height of summer but that on occasion can also swell rapidly and violently. These waters have eroded the valleys and their sediments have formed the plain, supported by reef-building corals. In the upper part of the valleys (from 3 to 10 kilometers long), rainfall is extremely abundant, whereas precipitation drops off sharply towards the lower end and may be insufficient in the plain. The latter depends in part on the brooks just mentioned, in part on the artesian wells used to feed the rice and taro fields, which are kept constantly flooded and are the main lowland crop but also stretch to the foothills. Cattle raising is relegated to the unused portions of the valleys and adjacent slopes.

Our Limnaeus occurs both in the brooks, up to the foot of the range, and also in the fields watered by them. It is found there either on stones and rocks, partly in and partly out of the water, or climbing the stems of taro and most certainly as well in floating masses of Conferva5 or on decaying leaves. Its food consists of algae

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3 Despite the remarkable similarity between the shells that were sent (especially the largest) and L. pereger (var. curta), according to Dr. O. Böttcher of Frankfurt, who was kind enough to examine them painstakingly, it was determined that the species in question belongs to L. oahuensis, described by Souleyet (Leuckart’s note).

4 According to O. Böttcher: Ph. sandwichensis Gould (ibid.).

5 Conferva – a genus of algae. [E.N.]
and decomposing plants; in captivity, they prefer macerated cabbage leaves to any other leaf, which means these leaves can be used as bait for catching specimens in the environment. In infection experiments, I often found their intestines crammed with *Distoma* eggs, which had neither hatched nor undergone any perceptible change.

The last two places where I collected specimens were the streams that rushed through two of these valleys, used for raising and watering cattle. They contained masses of distomes; these were, however, mostly small, none having attained full size. In examining the larger specimens, about every 5th or 6th individual displayed free rediae and cercariae. When one takes into account the large number of snails and how hard it is for eggs to develop easily in the swift currents and occasional floods, these results seemed remarkably positive and proof of infection was easy enough. Proof could easily be obtained even in mild infections by crushing a large number of the collected snails and keeping them a while in a little water, since the cercariae and their cysts would soon clump together; in good lighting, it would be impossible not to see them, even if only one snail were infected. In both cases, infection could only have occurred in the brooks because no snails were found outside them, e.g., in pools or puddles. Even then, infection occurred more often at the drinking place rather than in the pasture. Of the eliminated eggs, the only ones to develop were those that were freed along with feces directly into the streams or that had been swept into them by violent downpours before becoming desiccated.

Conditions were much simpler and clearer in the first collection place, which I visited repeatedly. Here, a spring came out of the ground near the pasture; it flowed in a rather inaccessible bed between rocks, but at a certain point it formed a quite shallow basin about the size of a bedroom, with walls of lava rock and level muddy ground, in which watercress and duckweed grew abundantly. Hoof tracks and excrements showed that the cattle waded into the water. The eggs that were not carried off by the weak current could easily develop in the shallow water; the embryos found the snails right there, for I myself or others, at my request, gathered hundreds of them, without killing them. I even found some cysts on the duckweed, which were evidently only waiting to be gathered. The excrements in the water, the snails filled with rediae and cercariae (which upon the slightest damage released their brood of parasites), and, lastly, the bones of an ox which had died of distomiasis quite close to the pool served to illustrate the life history of the fluke, and pointed in the most inarguable way possible to the cause and effect relation.

I will now proceed to my observations of the naturally infected snails gathered at the places just described. I have hardly anything to add to the anatomical details presented by Leuckart on rediae and cercariae; I re-examined most of these characters and can only confirm his findings. Thanks to the remarkable transparency of the tissues, the strong enlargements sometimes necessary can even be successfully used with living animals; microscopic preparations were only necessary for a few particularities.

For fixing, solutions of purified pyruvic acid or picric acid can be used; as a coloring, I used borax (hydrated sodium borate) or picrocarmine, but hematoxylin glycerin might yield better results.

The size of the rediae (in releasing the brood of cercariae) is given by Leuckart as 1mm; I feel this is not constant. Although this measurement is often the case, in
some cases, it is much larger, even twice as large. However, such giant cases are very scarce. I saw up to 20 fully developed cercariae, but generally there were fewer; not rarely, there were only 2 or 3 full-grown cercariae in a redia. I feel that the estimate of an average of 60 cercariae (according to my own observations of the conditions found here) at least three times too high. On the other hand, the assumption that 6 rediae constitute the offspring of one sporocyst seems too low, since I never observed such small a number of rediae in the larger snails.

In immature cercariae, I found the contents of the granule-cells composed of large, regular granules, of the same size and similar to grinding-stone crystals. As they mature, the granules become smaller and must therefore be partly diluted.

During formation of the cyst, a sheath of fine fiber is differentiated, inside of which the granules form a more compact shell. The former is evidently an adhesive substance that is secreted in a fluid state and attaches the cysts to the substratum; it probably derives from the cells themselves. Inside these cysts there soon develops a new, rather rough, hyaline wall, which can be expressed in toto by pressure. Inside these, and filling them completely, lies the larva, rolled up into a ball, like a hedge-hog, moving very little. Its tegument is extremely delicate and its tissues have lost all firmness with formation of the cyst, so that only the suckers, pharynx, and concrements display any consistency; it is therefore very difficult to extract the larva from the cyst alive and in a fairly well-preserved condition. In older cysts and under great magnification, one can clearly see the very fine covering of spines and the serpentine intestine. Contrary to Leuckart, I find that the little rods diminish rapidly and finally disappear in encysted worms. Small fragments are sometimes found later in the cells and others between the skin of the larva and the wall of the cyst. As no equivalent to them is visible, I believe that in their dissolved state they are used to reinforce the cyst wall, while the transformations of the larvae are hardly sufficient to consider them as larval nourishment.

To judge by how they are much less stainable, the material of which the rods are formed is different from that of the granules of the lobar organ. The latter are remarkably open to different dyes, and in immature cercariae they can be deeply stained inside the rediae while the latter hardly accept any stain. This property also holds true for the granules of cyst wall; the latter is originally pure white but soon acquires a yellowish-brown hue. If there are even traces of dye in the water, it soon appears distinctly stained in the same color. It thus suffices to put a piece of orange paper in the vessel, so that the water hardly shows a noticeable color, and the result will be orangish-red cysts; a bit of borax tints them deep red. At present, I use this simple stain to make the cysts quite distinct.

Shapes of liver flukes at different ages.

1 – 8-9 days old.
2 – 27-31 days old.
3 – 32 days old.
4 – 44 days old.
5 – Full grown.

1-4) From the liver and peritoneal cavity of a guinea pig.
5) From the gall bladder of a cow.
While working on *Distoma hepaticum*, I found a different kind of redia that most likely has not been described yet. It occurs by the hundreds and even by the thousands in the viscera, especially the kidneys, of a large *Melania*; the slender cercariae also encapsulate in the open, in flagon-shaped cysts, out of which the larvae can emerge at any time. This noteworthy arrangement shall be described at another opportunity. I have only mentioned it here because in some respects (discussed shortly) it coincides with *Distoma hepaticum*.

From the literature, I gained the impression that it is assumed that the mature cercariae break out of their host voluntarily. I must gainsay this completely for the two species under discussion. The cercariae may emerge from the birth-opening of the redia but it remains inside the host’s tissues, relatively quiescent and without taking solid nourishment until it is released either by the death of the host or by the breaking of the shell. Every undamaged snail thus contains its full complement of cercariae produced until then. If a strongly infected *Limnaeus* is drawn out of its shell, which is relatively easy, the upper end of the parasite-filled visceral sac can be sampled in toto and the rather slow movements of the mature cercariae can be observed, while the mature rediae remain rather mobile, especially the neck. If a little water is added, the cercariae become somewhat more lively but they only acquire full mobility if the body-wall of the host is torn and water touches them directly. The tail then begins to move so energetically that its contours are only clearly perceptible at both ends of the trajectory, in the form of an 8.

I interpret this as an expression of discomfort in the new medium, to which an end is put as soon as possible, by forming a cyst. The cyst material can, however, only be expelled when the body has gained a point of support, often after swimming about for a long while; once this has been accomplished, no time is lost in forming the cyst, and soon after the larvae again falls into a listless state, in strong contrast to the convulsive movements of the free cercariae.

In the same way that cercariae rarely emigrate from a living, undamaged *Limnaeus* (or *Melania*), they do not encapsulate inside them. This can only happen if the upper end of the shell has been injured. Such injuries occur more easily when the first coils of the shell have been eroded (probably by microorganisms that dissolve calcium). They are also sometimes occupied by a bunch of *Conferva*. In contact with water, even immature, hardly mobile cercariae form cysts. Their covers are easily recognized by their much coarser material; they are often unfinished or, at any rate, useless since such premature specimens soon perish. Under these circumstances, rediae also become immobile and then die off gradually.

When kept in a large vessel, the cercariae encapsulate on the surface as well as on the bottom. Plant parts are accepted willingly but there is no indication that they are preferred. In aquaria, *Limnaeus* with encysted cercariae on the shell are not rare; in nature, this of course must occur much less often. I have found enclosed distomes still alive after two months, but some of them perish, probably for the same reason as the eggs. This is especially true when the water is not renewed regularly. In running water they probably enjoy a considerable lifespan.

When the cysts are attached to plant parts that gradually decay, they loosen and gradually sink to the bottom. However, in one way or another, their attachment to the substratum grows looser over time, especially when the latter is smooth, so
that water currents are enough to detach the cysts. Most cysts therefore eventually end up gathering in the bottom sediment; given their low specific weight, they most likely can be dislocated by disturbances. This means that they can easily reach the stomachs of animals that come to drink, especially if they wade into the shallow water first. Anyone who has observed larger animals drinking knows that large amounts of bottom deposits are sucked up with the water. I consider this mode of infection as by far the most common, and in the places I studied, the only important one. Snails are certainly swallowed but probably not very frequently; it is very doubtful whether infection can be produced in this manner. In any case, the non-encapsulated cercariae are more easily swallowed during drinking.

The ingestion of plants on which there are cysts may certainly occur at the watering place; after all, the cabbage leaves that I threw in to catch snails were consumed by the cattle. Nevertheless, it is well known that cattle are not fond of the grasses growing in marshy places, and when pastures are flooded, the cysts must perish soon after the water level drops. In isolated instances such conditions may play a role, for instance, in epidemics among rabbits. Nevertheless, it has not been proven that rabbits do not drink water. The food available to them in nature is certainly not as juicy as what I provide for my rabbits, but all the same, the latter do drink water.

The influence of wet years on the liver plague is explained by the easier development of *Distoma* eggs; the spreading of *Limnaeus* is also favored. Conversely, the place and mode of infection were most likely not influenced by the weather.

Leuckart’s feeding experiment involving grass snails from a suspect sheep pasture, cited in Friedberger and Froehner (*Lehrbuch der spez. Pathologie und Therapie der Hausthiere*, Stuttgart, 1886, p. 332), is quite unconvincing without explanatory details, and the expression “grass snails” hardly applies to *Limnaeus*. I hope to conduct experiments with infected *Limnaeus* soon.

For purposes of examination, one can allow the cercariae to encyst themselves on glass plates or in dishes in which thin leaves of insoluble gelatin have been placed. In conducting experiments, I take a piece of paper, write the date on it, and place it folded as a filter in a porcelain dish, or with its edges bent upward in a photographic dish. Water is then poured in and a snail placed inside, after its shell is cut open with a scissors, from the upper edge of the mouth to the apex. All the cysts are thus on the paper (colored, if one prefers) and after rinsing, they are free from all mixtures. They can be kept in water as long as desired; it is possible to scrape them off the softened paper without damaging them, even after some time; alternatively, they can be cut off the paper using a curved scissors and then added to food or drink. When placed on tissue paper, they can even be used for microscopic examination. Parts of plants are only recommended for immediate consumption.

Let us now go to the results of my transmission experiments. So far I have conducted them on only three guinea pigs, one young kid, and a suckling pig. My first successes, which are probably the first in this domain, were obtained on the

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6 Treatise on the special pathology and treatment of domestic animals. [T.N.]
guinea pig, which had previously not been known to be a host of the liver fluke, but proved to be a perfectly suitable experimental object, at least for the early stages. The small kid suffered an accidental death some 20 hours after it had been fed several hundred cysts, probably because it had been weaned much too soon. The stomach and bile ducts failed to show any traces of the cysts or their inhabitants, and these probably passed through the digestive tract, or its largest part, without hatching (probably because of insufficient digestive capacity). Over the course of a week, the suckling was repeatedly fed numerous cysts of different ages, yet no young distomes were found. Unfortunately, the stomach was full in spite of long fasting, and it was impossible to make an absolutely accurate examination of the already quite large liver, with a view to finding the little parasites, at most one millimeter long.

After mentioning these failures, I will now present the positive results. The animals experimented on were two full-grown guinea pigs and a very young one, hardly matured. The cysts were given them in part on green fodder, in part on wet bread. The overall result was such that one may presume that the majority of the cyst larvae of at least one week of age and well preserved reached their right destination. The two grown animals died of the infection and the younger one was killed.

The first guinea pig had received about twenty older cercariae on both the 23rd and 24th of December and on the 27th, twenty more, one week old. In the first lots some loss must be allowed for as the cysts were scraped off the walls of a glass container; the last lot were encysted on grass that had been kept with its roots in water. Some of both kinds may have been lost during eating.

The first animal was found dead on the 23rd of January (1892) and the postmortem revealed the following:

A great deal of fluid blood in the abdominal cavity, which coagulated soon after opening. In addition, a considerable serous emission, which mingled only slightly with the blood. The surface of the liver was normal only in some parts of the right lobe; elsewhere, it was hyperemic, finely granular, and covered with numerous coagula of fibrin. Beneath the serosa were many small cavities filled with blood and in the parenchyma, numerous small vermiform clots of blood. In one of the former, a Distoma of about 8 mm in length was easily found; it was engaged in lively contractions, which changed its shape considerably. The highly branched intestine was filled with a fluid brown mass, which flowed this way and that during the contractions but soon was for the most part expelled. The excretory vessels and thorny scales were very distinct, the latter especially so at the cephalic end.

As the large gall ducts (including the bladder) proved to be empty and the smaller ones could not be followed because of the small size of the object, examination was carried out in the following way:

The liver was kept in warm water while one lobe after the other was examined; the last one, accounting for about one fourth of the whole mass, was placed in alcohol for later examination. Each of the lobes was cut into pieces and then squashed between glass plates. In this manner, the whole of the liver (excepting the lobe preserved in alcohol) was viewed under incidental light, and all the distomes collected; a number of specimens that had come out of the tissues when the surface
of the liver was broken were fished out of the water. The count indicated 29 distomes, of which the smallest was roughly 4.75 mm in length with a maximum width of 1.5 mm; the largest measured 9.5 mm by 2.5 mm. The measurements were made using specimens that were either dead or made rigid by the cold, and differences in the degree of contraction must be allowed for. Even so, there were considerable differences in size, which can hardly be explained by differences in age alone; other factors must contribute to this. There was a small nodule in the lung, which may have housed a stray parasite.

Examination of the other organs was briefer and showed nothing abnormal; the liver itself was so carefully examined that hardly one specimen could have escaped.

Next day, the other guinea pig died of serous peritonitis and hemorrhage of the liver. The liver displayed the precise same conditions; its left lobes were also quite engorged, while the left ones were less so; fibrous deposits were very marked but limited to the surface of the liver.

In this case, 32 days before death, about 20 older cysts had been administered. Seventeen flukes were found, of which only one in the liver, whereas the other 18 were distributed throughout the peritoneal cavity. They were more uniform in size but they also displayed noticeable differences.

The third, very young guinea pig was killed 44 days after it had been fed a few older cysts and 9 and 8 days after it had been given many two-week-old cysts. In a deposit of fibrin on the surface there were about 20 1-mm-long flukes, in addition to a larger specimen. It was very difficult to find them and they were so delicate that only a very small part could be isolated without injuring them. The liver was crisscrossed by a mass of winding passages, the walls of which seemed to be filled with pus; these infiltrations were much thicker than the diameter of their inhabitants, and they evidently were not normal ducts.

From these experiments, I have deduced the following:

In guinea pigs (and probably also in other small rodents), the emigrated flukes soon seek the edge of the liver, and when the normal bile ducts become too narrow for them, they bore into the soft tissues. When they reach the surface, they perforate the peritoneum and thus reach the peritoneal cavity, where they probably live some time longer. However, even if the host survives this emigration, they do not reach full development. (This may explain Leuckart’s negative results in his experiments on rabbits).

No doubts can be entertained that the kind of flukes that I observed came from the cysts that were fed to the hosts, as they correspond wholly with Thomas’s and Leuckart’s descriptions; furthermore, my guinea pigs never had liver flukes. To illustrate the relationship of the shapes, I give the exact outlines of some of them, which were preserved in diluted glycerin. Be it noted that these specimens are more elongated than in the quiescent state. I shall have to await a future opportunity for the exact description of the history of development and give only a summary of results here.

The covering of the spines and excretory system soon appears in its fully developed state. In my smallest specimens (observed when full), the intestine seemed to present only curves but not any clear branching yet; however, the latter
soon appear and gain their complete shape. In young flukes, the ventral sucker is located on a very prominent cone, which must certainly be important for locomotion. Of the sexual organs, the bursa of the cirrus and the shell-gland seem to be the first; next come the uterus, ovaries, testicular ducts, and yolk-organ. It is, however, quite difficult to recognize the true proportions, as the first structures have no distinctive characters, and the parenchyma displays (instead of the future hollow formations) only small, round, densely packed cells. The young forms also appear capable of great liveliness and marked dynamism.

In conclusion, I would like to mention in brief the following experiment:

A number of three-day-old cysts, in part at rest on tissue paper, were tied up inside a small bag of parchment paper, along with a little water, and were pushed down a rabbit’s esophagus into its stomach. Four hours later, the rabbit was killed and the little bag recovered and opened. It could be seen that the outer wall of the cyst, colored by carmine, had rather broken open all over, apparently because of the swelling of the inner, uncolored part of the cysts. The latter were often stuffed but still intact. At the host’s body temperature, the larvae inside engaged in very lively movements; by applying pressure, part of them were induced to emerge in a more or less uninjured state and it was quite definitively ascertained that the little rods were missing in all of them. Spontaneous emergence was not observed with any certainty.

I intend to continue these observations.