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Excystment phenomena in clonorchis sinensis.

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Clonorchis sinensis is a digenetic trematode living in the bile passages of man and other mammalian hosts, particularly dogs and cats. The infection is found endemically only in the Far East. Although infection in reservoir hosts is common throughout the Sino-Japanese areas, that in man is confined almost entirely to one restricted area in Japan, to the southern half of Korea, to Kwangtung Province, China, and to Tonkin Province, French Indo-China. Cases found outside these endemic areas have without exception been traced back to them.

The Process of Encystment. The life cycle of Clonorchis involves a snail, *Bythinia striatula,* as first intermediate host and fresh-water fishes as second intermediate hosts. The second larval stage, the cercaria, attacks the fish, attempting to burrow under the scales and into the flesh. During this process it drops its tail and, after penetrating as far as it can into the new host, secretes a viscous fluid which gradually hardens to form a spherical cyst wall, *the inner (true) cyst capsule.* The presence of the encapsulated larva in the tissues of the fish, and the excretion of its waste products into the host's tissues causes the latter to lay down an outer false wall which is fibrous in nature and adheres firmly to the inner capsule. The process is comparable to the encystment of trichina larvæ that have migrated into the striped muscles of mammals.

Japanese investigators have found that various cyprinoid fishes are involved in this phase of the Clonorchis life cycle, but our studies have shown that practically any fresh-water fish which is exposed to the infection may serve as the host of the encapsulated larvæ. While the encysted larvæ are usually found some distance beneath the epidermis, a certain proportion fails to penetrate the superficial layers of the fish and becomes encapsulated either on the epidermis or attached to the under side of the scales. Here the larva obtains nourishment from the surrounding itssues of the host and increases in size, the elastic capsule enlarging to accommodate the growing larva. Our experiments have shown

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that cysts which have just been formed are viable when introduced into an experimental mammal and that they remain viable in the tissues of the fish for several months following encystment.

The Phenomena of Encystment. The experiments reported in this communication have to do with the processes of encystment and the arrival of the larvæ in the bile passages of the mammalian hosts. The data were derived from two types of experiments, (1) *in vitro* and (2) *invivo.* The former were undertaken to determine under what circumstances excystment took place. The latter were employed in order to confirm the former and to ascertain the exact route of migration of the larvæ into the bile tracts. We employed viable cysts from the under side of the scales of the knife-fish, *Hemiculter kneri,* obtained from the Peking Market.

(1) IN VITRO EXPERIMENTS.

(a) *Effect of gastric juice on the cyst.* Dog's gastric juice (preserved in toluol) was allowed to work on the encysted larvæ at 26° C. and at 37° C. for various lengths of time. In this medium digestion of the outer wall and of the fish scales took place but the inner true capsule remained intact. The action was much more rapid at 37° C. than at 26° C. The larva within at first became activated but later became quiescent and died after four or five hours. In case the cyst capsule was ruptured through artificial pressure the larva died on immediate direct contact with the medium. Boiled gastric juice and the HCl fraction had no effect either on the outer wall or on the true capsule, although the larva died as in the previous experiments. Fresh (unpreserved) gastric juice had the same digestive effect on the outer wall and the fish scales as the preserved juice but was less lethal to the larvæ within, which survived until about the twelfth hour.

(b) *Effect of intestinal juice on the cyst.* When the cyst was first placed in intestinal juice, either fresh or preserved (reaction neutral) without the previous action of gastric juice, no digestion of the inner or of the outer cyst wall took place up to 22 hours. If the capsule was ruptured by mechanical pressure, the larva escaped and was able to live in the fresh juice from 1 to 3 hours, but was digested after that time. In preserved intestinal juice it died almost immediately after rupture of the capsule.

(c) *Fresh (unpreserved) gastric juice followed by fresh (unpreserved) intestinal juice.* When the cyst was first submitted to the action of fresh gastric juice at 37° C. for 4 hours and was then transferred to fresh intestinal juice, the outer wall was

digested in the former medium and on introduction to the latter medium the larva became greatly activated, so that in 20 minutes *it caused the rupture of the cyst wall,* wriggled out into the free intestinal juice, and was active up to three hours, when observation was discontinued.[1] The cyst capsule had been somewhat weakened by the action of the intestinal juice but remained intact. The process as we observed it differed, therefore, from that which Kobayashi (1917) described for Clonorchis, to the effect that the digestive juices of the stomach and of the intestine had no effect whatever on the cyst capsule. It also differed from Ciura's statement (1917) regarding the related form. *Opisthorchis felineus,* in which he believed the larva was entirely passive, and excystment depended entirely on the digestion of the hyaline cyst capsule by the intestinal juice, which left the larva lying free in the medium.

From this series of experiments we have concluded that fresh gastric juice dissolves and digests the outer cyst wall and the fish scales but does not digest the true cyst wall. The larva within remains viable up to 12 hours *in vitro.* Transfer to fresh intestinal juice weakens the true cysts capsule *pari passu* with increased activity of the larva, which causes the bursting of the cyst wall, through the opening of which the larva wriggles out. The freed larvæ appear to live comfortably for three hours or more in fresh intestinal juice..

(2) *In Vivo Experiments. In vivo* experiments were carried out on guinea-pigs and small puppies that had been fed on a meal of raw infected fish[2] from 5 to 72 hours previous to autopsy. In both series five hours after feeding the food mass with the cysts still remained in the stomach. The cysts had been freed from the scales and flesh, which had been partly digested. The cysts capsule in each case was intact and the larvæ viable. In both series, eight hours after feeding, most of the larvæ were still found in the stomach, although about 20 per cent had reached the duodenum, and of these latter a few had become excysted and were freely crawling about in the lumen. Likewise, in both series, after fourteen hours cysts remaining in the stomach were non-

[1] When bile in distilled water or in 0.5 per cent Na_2CO_3 was substituted for the intestinal juice there was no spontaneous excystment.

[2] In the case of the guinea pigs the flesh was made into a paste and smeared onto cabbage or spinach leaves; for the puppies the fish was fed in small pieces.

viable. Those in the duodenum were excysted; most of those found were attached to the mucosa of the duodenum near the opening of the common duct. After 22 hours the larvæ found in the duodenum were all attached to the mucosa and were massed in the vicinity of the opening of the common duct.[3] After 48 hours larvæ had migrated into the common duct; none were found in the duodenum. From the 72nd hour they were found passing into the bile passages. Larvæ were never found in the jejunum, the gall bladder or the pancreatic duct.[4] Only a portion of the viable larvæ which entered the duodenum actually excysted, and from our experiments we conclude that neither encysted nor excysted larvæ which passed into the jejunum or ileum were able to survive but were digested along with the food mass. Even in the duodenum it was necessary for the excysted larvæ to secure attachment to the surface of the mucosa in order to migrate into the common duct. It seems highly probable that only about 20 per cent of the viable encysted larvæ entering the body with the food mass excysted and that only about 5 per cent actually reached the common duct and the bile passages. These data favor the view that the migration into the bile passages is direct and does not involve previous passage through the portal vein which Lutz (1892-93) claimed was involved in the case of *Fasciola hepatica*.

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The influence of chaulmoogra on sulphur metabolism.

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Further metabolism studies with Chaulmoogra oil have been undertaken with special reference to sulphur excretion. Estimations were made to find out whether the cyclic pentene nucleus of Chaulmoogric acid is excreted like phenol as the ethereal sulphate,

[3] It seems probable that this is a chemotactic reaction.

[4] As determined both by examination of the contents of these tracts and by scraping their epithelial linings.