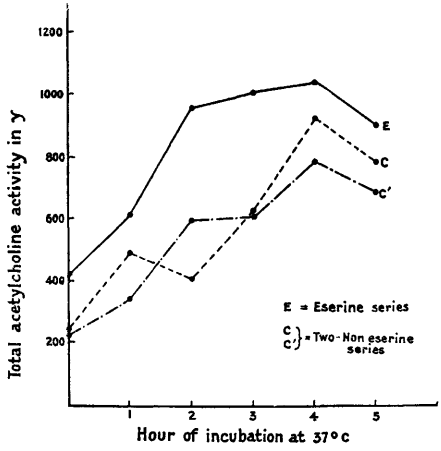

FIG. 2.

The liberation of reserve acetylcholine increases during incubation and reaches a maximum at the 4th hour, the average hourly increment during the first 4 hours being 169, 138 γ acetylcholine activity for the 2 non-eserine series respectively. Under the protection of eserine, the initial free acetylcholine activity was higher but the average increment during incubation was only 119 γ per hour.

Elucidation of the mechanism and significance of the present finding in relation to our previous claim[3, 4] constitutes some of the objectives of the experiments which are now in progress.

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In Vitro Experiments on the Viability and Excystment of Paragonimus Cyst.

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Cysts were obtained from infected crabs (*Potamon denticulatus* Milne-Edwards) collected from Lan Ting, where the first 2 human cases of paragonimiasis in China were reported by Ying.[1]

Experiments were carried out in hollow glass slides in which

[3] Chang, H. C., and Wong, A., *Chinese J. Physiol.*, 1933, **7**, 151.

[4] Wong, A., and Chang, H. C., *Chinese Med. J.*, 1933, **47**, 987.

[1] Ying, Y. Y., *Nat. Med. J. China*, 1930, **16**, 638.

actively motile encysted cercariae were placed in the medium to be tested. Freed cysts which had been kept in ice chest for 7 days could live in wine made from millet (*Sorghum vulgare* L.) in (a) 10% alcohol, for 43 hours at 22°C., and 20 hours at 37°C., (b) 25% alcohol, for 1 hour at 22°C., and (c) 50% alcohol for a few seconds at the same temperature. In yellow rice wine with 14% alcoholic content, death took place in 18 hours at 22°C., and in 15 hours at 37°C.* They could be kept alive in 10% formalin for 23 days and in 0.9% saline for a like period in the ice chest (10°C.).

The metacercariae caused to excyst by the methods described in the next series of experiments were actively motile in trypsin 1% plus sodium carbonate 0.2% for at least 3 hours at 37°C. if bacterial growth was not checked and could remain dormant for 43 hours in the ice chest in the same medium. When they were placed alternately at 22°C. and at 16°C. they could live 84 hours. In 12% bile and in artificial intestinal juice plus bile (5%), their viability was 42 hours in the ice chest.

These experiments demonstrate that in a diluted millet wine containing 10% alcohol and in rice wine (14% alcohol), the encysted metacercariae were viable up to 43 and 18 hours at room temperature (22°C.), respectively, and that they could be kept alive in the ice chest (10°C.) in 10% commercial formalin or in 0.9% saline for over 3 weeks. Therefore, the customary mode of preparing crabs, as practiced by the villagers in the endemic area where the infection rate for crabs varies from 25 to 100%, by soaking them, very often only over night at room temperature so as not to spoil the taste, in a weak solution of salt and yellow rice wine seasoned with spices, cannot kill all the cysts of Paragonimus. This would account for the high rate of infection, 87% in one village (Chen and Rose),[3] prevailing in the Lan Ting district.

The early Japanese investigators believed that, under natural conditions, the excystment took place spontaneously. However, Yoshida[4] showed that this was not the case. Kobayashi[5] thought that gastric or intestinal juice alone did not cause hatching, as the larvae were seen escaping from the cysts when transferred from the crabs

* If these experiments show discrepancies with those of Ameel[2] with alcohol, they may be due to the fact that the wines contain ethereal and other extracts which may modify its action.

[2] Ameel, D. J., *Am. J. Hyg.*, 1934, **19**, 299.

[3] Chen, W. L., and Rose, G., Ninth Congr. Far East. Assn. Trop. Med. Abst., 1934, 62.

[4] Yoshida, S., *J. Parasit.*, 1916, **2**, 175.

[5] Kobayashi, H., *Mitteil. Med. Hochsch. Keijo*, 1921, **4**, 5.

to tap water, normal saline, or a higher temperature. Ameel[6] did not obtain consistent results by the successive uses of artificial gastric and pancreatic juice at 37°C. In order to test this point, the following experiments were made *in vitro*.

Paragonimus cysts measuring from 0.336 to 0.506 mm. with a wall thickness of 12 to 20 microns, containing actively motile metacercariae were suspended at 37°C. in the following media with the results noted. (1) In artificial gastric juice there was no excystment after 5½ hours and larvae were dead in 15 hours; (2) in 0.2% HCl, they died in 18 hours and the cyst walls in some were broken; (3) in dog's or artificial gastric juice for 3 hours, the larvae remained quiescent but became restlessly active on being transferred to an artificial intestinal juice made up of trypsin 1% plus 0.2% sodium carbonate, and excysted in 3¾ through a triradiate opening in the intact wall; (4) in artificial intestinal juice plus 5% fresh cow's bile *without* previous contact with gastric juice, the excystment took place in 1¾ hours; (5) in the same medium but *without* the bile, the larvae escaped in *between* 45 to 90 minutes; (6) in bile alone, there was excystment in 75 minutes in 12% dilution, and in 2¾ hours in 1.5%, 6%, and 100%; (7) In Na_2CO_3 (0.2%) alone, the larvae remained dormant for 24 hours and died after 3 hours' immersion in artificial intestinal juice; (8) no change was observed in pure or diluted *boiled* bile, the larvae being still motile at the end of 13 hours; or in *boiled* artificial intestinal juice in which the larvae were found alive after 6 hours but dead in 16 hours.

To determine the rôle played by digestive active of the alimentary tract, experiments with dead cysts were made with the result that there was no visible solution or dissolution of the cyst wall when cysts were immersed in dog's gastric juice or artificial intestinal juice for 58 hours. Some cysts burst after 18 hours in 0.2% HCl; and in 0.4% Na_2CO_3, they turned black and appeared hard and brittle and the walls of some of them gave way. Observations on empty cyst walls kept in artificial intestinal juice, renewed every third day, showed that some of them did not totally disappear even after 4 weeks.

The process of excystment does not differ from that of *Clonorchis sinensis* (Faust and Khaw[7]) and *Opisthorchis felineus* (Vogel[8]) except that the larva makes its escape long before the true outer cyst wall has been demonstrably and visibly digested off.

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[6] Ameel, D. J., *Am. J. Hyg.*, 1934, **19**, 279.

[7] Faust, E. C., and Khaw, O. K., Studies on *Clonorchis sinensis*, *Am. J. Hyg.*, 1927, Monogr. Series B.

[8] Vogel, H., *Zoologica*, 1934, **33**, Heft 86.