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Survival of Adult *Clonorchis Sinensis* in the Biliary Passages of Dead Human Bodies

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Routine medico-legal autopsies in the Department of Pathology, Kyungpook National University School of Medicine, have revealed live adult *C. sinensis* in the biliary passages of some corpses.

To the best of the author's knowedge, there has been no previous report available on the survival rate of worms from the biliary passages of dead human bodies. This paper purports to determine the survival rate of worms obtained from nine autopsy cases and to clarify its usefulness as an antigen for intradermal tests.

MATERIALS AND METHODS

Nine cases, harboring an abundance of worms, were selected from autopsy cases in the Department of Pathology, Kyungpook National University, Taegu, Korea. The test cases were comprised of 2 females and 7 males ranging from 24 to 51 years of age.

The adult worms were obtained partially from the biliary passages by pressing on the outer side of the liver cut. They were washed repeatedly with a large amount of tap water and rinsed with physiological saline. After removing the residual saline with a pipette, the worms were transferred to a sterile beaker containing Tyrod's solution supplemented with 100 units of penicillin and 100 ug of streptomycin per ml. The worms were then incubated at 37 degrees C in a waterbath. Immediately after the incubation, the movement of the surviving worms was detected microscopically. These worms were then transferred to another beaker and counted. The total elapsed time from the autopsy of a dead human body to the incubation of the worms was approximately one hour.

OBSERVATIONS

The ratio of surviving to dead worms obtained from the biliary passages of the corpses is shown in Table 1. The survival rate of the worms decrease with the aging of the corpse. Although cases 2 and 7 were killed by a traffic accident, the survival rate for the worms in these cases varied from 13.9 to 22.0 percent(Table 1). The variation in the survival rate of the worms from the dead autopsied immediately after death appears to be due to differences in temperature and

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Table 1. Survival of adult *Clonorchis sinensis* in the biliary passages of dead human bodies

Case No.	Sex	Age	Days after death	No. of worms collected	Worms survived	
					No.	Percent
1	M	36	4	284	41	14.4
2	M	24	3	309	43	13.9
3	F	35	5	251	23	9.2
4	M	41	4	612	112	18.3
5	M	38	5	126	8	6.3
6	M	45	4	875	120	13.7
7	F	26	3	127	28	22.0
8	M	32	2	228	65	28.5
9	M	51	2	785	166	21.1

Table 2. Survival rates for adult *Clonorchis sinesis* in the Tyrod's medium

Days after death	No. of worms collected	No. of worms survived	Mean of worms survived	Range of survival percent
2	1013	231	22.8	21.1-28.5
3	436	71	16.3	13.9-22.0
4	1771	273	15.4	13.7-18.3
5	377	31	8.2	6.3-9.2

viability changes.

The appoximate numbers of the worms that survived in the Tyrod's medium at 37 degree C are presented in Table 2. The number of the surviving worm population proved to be unexpectedly high (22.8%) when the worms were collected within 2 days after death. This data was not shown by statistical analysis because there were few cases and it seemed likely that the temperature must have been a limiting factor for the survival of worms in the corpses' biliary passages. Generally, the rates for the surviving worms for 3 to 4 days after death were more or less constant, with ranges of after death 16.3 to 15.4 percent. Five days after death, the surviving worm burden tended to decrease and its rates were found to be 6.3 to 9.2 percent in the Tyrod's medium.

DISCUSSION

The study was begun in 1958. Because of the avoidance of postmortem examination, the author's have been making persistant effort to collect the case, 9 bodies could be obtained during the past 14 years.

One of the most striking aspects of this survey is the relatively high resistance of the adult *C. sinensis* toward the putrid bile of dead human bodies.

During microscopic observations of the surviving worms, ovipositions was seen in the Tyrod's medium. The mobility of the worms, in most instances, was so sluggish that the surviving worms were difficult to distinguish from the dead ones. However, the live worms were yellowish bile colored, while the dead were whitish bile in color.

Table 1 reflects the survival rate of adult *C. sinensis* in the biliary passages of nine case studies. A survival rate of 13.9 percent was observed in case 2, while a rate of 22.0

percent is shown in case 7. The variation in survival rates appears to be due to the difference in temperature, host parasite ralationship, and the degree of putrid bile changes.

To approach a valid conclusion concerning the effects of temperature on worms, it will be necessary to study different temperature groups using rabbits experimentally infected with *C. sinensis*. Lee et al. (1958) made a survey on the effects of temperature on the worms and reported that adult worms obtained from the rabbits have survived for more than 100 hours at 37 degrees C. However, they lost their viability within a few seconds when incubated at 38 to 40 degrees C in the saline medium. In view of the results described above, it is conceivable that the pattern of resistance in rabbits is similar to that which has been observed in man.

The percentage of worms surviving in the biliary passages of the corpses was unexpectedly high. Furthermore, there were several different levels of resistance among the worms obtained from experimental animals. This has been recognized in most cases of parasitic diseases and resistance to various anthelmintic drugs.

The biological significance of the living *C. sinensis* in the biliary passages of dead human bodies is not clear, but raises questions of relations to such experimental animals as rabbits, rats, and dogs. It is noteworthy that treatment of clonorchiasis with various anthelmintic drugs in man has been unsatisfactory. In contrast, it has proven considerably effective to cure the laboratory animals infected with *C. sinensis*. This may be due in part to the difference in therapeutic dosage and host parasite relationships. Experiments dealing with the life expectancy of adult worms among chronic clonorchiasis patients have been conducted by various workers (Kobayashi, 1922; Katsurada, 1922; Markell, 1966; Carlos Calero M, 1967; Choi et al., 1970). However, the results vary so remarkably that the life expectancy is still unknown. A period of more than 2 decades of *C. sinensis* infections have been observed, ranging from 9 to over 40 years old. Further clinical observation will be required to estimate their life expectancy.

Because of the limited number of cases studied, no valid conclusion regarding survival of worms in the corpses can be presented. The results, however, suggest that a small number of live worms can be obtained from corpses even 5 days after death. Additionally, they can be used as an antigen for intradermal tests of clonorchiasis as reported by the authors (1963).

SUMMARY

Adult *Clonorchis sinensis* were collected from the biliary passages of nine cases of medico-legal autopsies. The worms were washed repeatedly with tap water and rinsed twice with physiological saline. Incubation took place in Tyrod's medium in order to recover live worms. The number of worms surviving in the medium was determined.

The rate of surviving worms showed an unexpectedly high percentage (22.8%) in those cases autopsied 2 days after death. The surving worm burden dropped sharply to 6.3 and 9.2 percent for those samples taken 5 days after death.

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=국문초록=

시체 담도에서의 간디스토마성충의 생존

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지금까지 법의해부시체의 담도에서 간디스토마성충을 보아왔으나 본성충의 생존율에 관한 보고는 아직 찾아 볼 수 없었다.

저자들은 법의해부시체(9예)의 간을 압박하여 유출되는 간디스토마성충을 얻어 비커에 담아 여러번 물로 씻은 다음 생리적 식염수로 다시 2회 씻어 살아있는 성충을 찾아내기 위해 37°C tyrode 용액에 넣어 생존 충체의 수를 체아렸다.

본성충의 생존율은 이외로 높아 사후 2일에 부검한 시체에서는 22.8%였고 사후 5일에서는 급격히 떨어져 6.3~9.2%였다.