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STUDIES ON THE COMPLEMENT FIXATION TEST FOR PARAGONIMIASIS AS THE METHOD OF CRITERION OF CURE

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Introduction

It is well recognized that paragonimiasis is one of the most important tropical disease in Asia, especially in Japan, Korea, China and Formosa. But the accurate treatments for this disease were not found until quite recently.

Recently, Yokogawa *et al.* (1960) found that Bithionol is quite effective for this disease and several other investigators have been confirmed the efficacy of this drug. However, there are many questions to decide the recovery of paragonimiasis after treatment by sputum and stool examinations for ova. For example, the parasites sometimes may show an interruption of the ovulation only during the period of treatment and may begin the ovulation soon after treatment, so it is often very difficult to decide the cure of this disease during short period after treatment. Yokogawa (1953) reported that the examination for ova in sputum and stool should be followed up at least for more than 3 months after the treatment to decide the cure of paragonimiasis and also Yokogawa (1961) reported that positive reaction in complement fixation test for paragonimiasis would turn negative soon after the complete recovery of this disease. In this study, the authors conducted to use the complement fixation test for evaluation of the effect of treatment for paragonimiasis. The complement fixation tests were followed up before, during and after treatment on 48 paragonimiasis patients treated with the combined method with emetine hydrochloride and sulfonamide or Bithionol.

Object and Method

The sera for the complement fixation test were prepared periodically from the paragonimiasis patients before, during and after the treatment with Bithionol or the combined method of emetine hydrochloride and sulfonamid, and the changes of antibody titers in complement fixation test were compared with the technique of the 50 percent end point titration of complement (Yokogawa *et al.*, 1956).

Namely, 1 : 5,000 of V. B. S. antigen prepared from adults of *Pargonimus westermani* as the same method of V. B. S. antigen for skin test was employed in this test and 2.5 50 percent hemolysis units of complement were used. More than 1 : 10 in dilution of the serum at the 50 percent hemolysis end point titration was considered as the positive reaction in this test. The details of this test was described in the previous report (Yokogawa *et al.*, 1956, 1961), so ommited to described in this paper.

The sera of the patients for complement fixa-

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tion test were inactivated as soon as possible after sero-separations and kept in −20°C. These sera were tested at the same time under the same conditions. From the results of our detailed studies, it was proved that no significant differences were found in the antibody titers of complement fixation test carried out with those sera which were stored in −20°C and those which were used immediately after sero-separated.

Results

The results of complement fixation tests carried out periodically on 48 human cases of paragonimiasis summarized in Table 1, 2 and 3. 23 cases out of 48 cases were treated with emetine combined with sulfonamid, and the rests of them were treated with Bithionol.

These 48 cases were divided into 3 groups as follows.

Table 1. Changes of dilution titer of antiserum in complement fixation tests on the individuals before and after treatment with emetine hydrochloride combined with sulfonamid (effective cases — Group No. 1)

Case No.	Before treatment	After treatment							
		1 M.	2 M.	3 M.	4 M.	5 M.	6 M.	7 M.	8 M.
1	>×160	>×160	× 54		× 39			× 16	—
2	>×160	× 89	× 14			—	—		
3	>×160	× 80	× 10			—			
4	>×160	× 54	× 23	× 17		× 11		—	
5	>×160	× 40	× 32			× 31			—
6	>×160	× 40			× 10	—			
7	×149	>×160		× 28		× 12			—
8	×145	×123		× 33		× 25	× 25		—
9	×120	× 62		× 24		—			
10	× 20	× 25		× 10		—			
11	× 16	× 33		× 15		× 16	—		

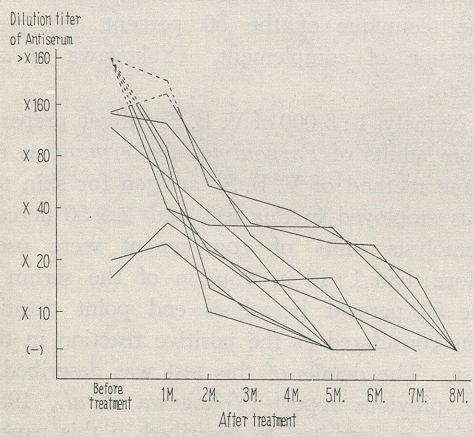

Fig. 1. Changes of dilution titer of antiserum in complement-fixation tests on the individuals before and after treatment with emetine hydrochloride combined with sulfonamid (effective cases ··· Group No. 1)

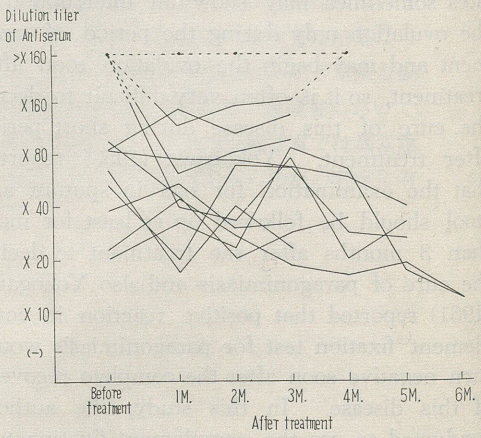

Fig. 2. Changes of dilution titer of antiserum in complement-fixation tests on the individuals before and after treatment with emetine hydrochloride combined with sulfonamid (ineffective cases ··· Group No. 2)

Table 2. Changes of dilution titer of antiserum in complement fixation tests on the individuals before and after treatment with emetine hydrochloride combined with sulfonamid. (ineffective cases — Group No. 2)

Case No.	Before treatment	After treatment 1 M.	2 M.	3 M.	4 M.	5 M.	6 M.	Notes
1	>×160	>×160	>×160	>×160	>×160			ineffectiveness
2	>×160	×117	×150	×160	>×160			relapse
3	>×160	× 62	× 81	× 96				ineffectiveness
4	>×160	× 40	× 33	× 76	× 20	× 32		relapse
5	× 93	× 46	× 20	× 28	× 34			relapse
6	× 85	×144	×104	×133				ineffectiveness
7	× 84	× 75	× 74	× 67	× 58	× 40		relapse
8	× 65	× 17	× 40	× 23				ineffectiveness
9	× 53	× 20	× 70	× 67	× 28	× 26		relapse
10	× 38	× 54	× 30	× 32				ineffectiveness
11	× 23	× 45	× 27	× 18	× 16	× 19	× 12	relapse
12	× 20	× 32	× 23	× 85	× 65	× 17	× 12	relapse

Group 1 : 11 cases, which were treated with emetine combined with sulfonamid for 12 days and were seemed to be completely cured.

Group 2 : 12 cases, which were treated with emetine combined with sulfonamid as same as group 1, but were ineffective.

Group 3 : 25 cases which were given 5–15 doses of Bithionol every other day and cured completely.

On group 1, the *Paragonimus* eggs in stools and sputa disappeared completely during the period from 10th day to 40th day and no ova were found during the follow-up studies for 8 months after treatment. The antibody titers in complement fixation test of all these patients decreased gradually with lapse of time after the treatment and became negative as shown in Table 1 and Fig. 1.

On group 2, the therapeutic effects were not recognized at all in spite of the same treatments were given same as group 1. As shown in Table 2, in 5 cases *Paragonimus* eggs in stools and sputa were not cleared during the period of the treatment and in 7 cases the eggs were cleared for a while, but the eggs were found again during the period from 2 months to 4 months after the treatment. The antibody titers of them in complement fixation test showed only strong fluctuation and no tendency of decrease.

On group 3, the *Paragonimus* eggs in stools and sputa were cleared during the period from the 4th day to 6th day after the beginning of the treatment and no relapses were found during the period from 6 months to 12 months after the treatment. The antibody titers in complement fixation tests showed the tendency of decrease as group 1 and became negative during the period from 1 month to 12 months after the treatment.

From the above mentioned results, the variations of antibody titers in complement fixation test were summarized as follows. The antibody titers of all the cases in group 1 and 3 which had cured completely showed a tendency of decrease soon after the treatment and finally became negative. On the contrary, on the cases in group 2 which were ineffective, the antibody titers showed only a strong fluctuation but a negative reaction. Accordingly, it may be possible to guess the therapeutic effects in early stage after the treatment through the periodical observations of complement fixation tests. No relations were found among the antibody titers, age, sex and lapse time of infection of the patients.

Table 3. Changes of dilution titer of antiserum in complement fixation tests on the individuals before and after treatment with Bithionol (effective cases — Group No. 3)

Case No.	Before treatment	After 10 times medication	After treatment						
			1 M.	2 M.	3 M.	4 M.	5 M.	6 M.	12 M.
1	>×160	>×160	>×160	>×160	× 57	× 12	—		
2	>×160	>×160	>×160	>×160	× 29	—			
3	>×160	>×160	>×160	× 40	× 42		—	—	
4	>×160	>×160	×150		× 33	× 10	—		
5	>×160	>×160	×148	× 94	× 93	× 11	—	—	
6	>×160	>×160			× 45				—
7	>×160	>×160		× 64	× 41	× 11	—	—	
8	>×160	>×160	×108	× 43	× 24	—	—	—	
9	>×160	>×160	×108	× 40	× 22	—	—	—	
10	>×160	>×160	× 50	× 54	× 40	× 11	—		
11	>×160	>×160	× 51	× 25	× 14	—	—	—	
12	>×160	×106	× 40	× 26	× 11	—		—	
13	>×160	× 93	× 29	× 13	—	—	—	—	
14	>×160	× 87			× 55			× 18	—
15	>×160	× 53	× 32					× 18	—
16	>×160	× 53						× 24	—
17	× 68	× 41						× 28	—
18	× 63	× 49	× 20	—	—	—	—		
19	× 47		× 22					—	—
20	× 40	—	—					—	
21	× 31	× 12	—					—	
22	× 29	× 12	—					—	
23	× 27	× 11	—	—	—	—			
24	× 25	× 23			× 20			—	—
25	× 22	× 20	× 15		× 19			—	—

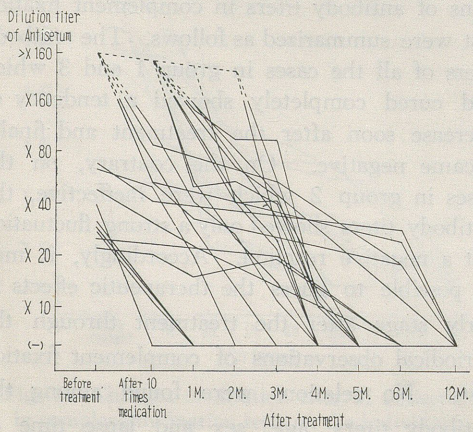

Fig. 3. Changes of dilution titer of antiserum in complement-fixation tests on the individuals before and after treatment with Bithionol (effective cases ··· Group No. 3)

Discussion

The complement fixation test for paragonimiasis has been introducted by Ando (1910), and he pointed out from the results of animal experiments that it was helpful to evaluate the efficacies of treatment for paragonimiasis. Recently, Yokogawa *et al.* (1956) reported the method and the diagnostic value of complement fixation test in human paragonimiasis. Yokogawa (1956, 1961) has also often described that the complement fixation test for paragonimiasis is closely connected with the survival of the worm, *Paragonimus westermani* in the final hosts. That is, the intradermal test using V. B. S. antigen keeps positive reactions during

the long period from 10 years to 20 years after the recovery of paragonimiasis. On the contrary, the antibody titers in complement fixation test became negative after the death of the worm in the final hosts. Accordingly, the complement fixation test would be helpful to evaluate the effects of treatment. Yokogawa (1956) reported that the antibody titers of the patients who received lobectomy to remove the worm cyst in the lung decreased gradually and became negative within the period of 6 months after operations.

Kushi *et al.* (1960) and Takano (1960) recognized the same results as Yokogawa from the results of follow up studies on paragonimiasis patients who had cured completely with treatment of emetine combined sulfonamid, and reported that the complement fixation test is one of the suitable methods to decide the cure of paragonimiasis after treatment.

Takano (1960) carried out the rapid flocculation tests with bentonite antigen and cholesterol lecithin antigen on the patients treated with various drugs, and reported that the tendency of decrease of antibody titers in the tests were recognized when the drugs were effective.

However, the results of rapid flocculation test seemed to be instable and its method is quite complicated compared with that of complement fixation test.

Conclusion

The changes of the antibody titers in complement fixation tests were studied periodicaly on 48 paragonimiasis patients before, during and after treatment. The antibody titers of the patients who had cured with the treatments of emetine-sulfonamid or Bithionol showed a tendency of decrease immediately after treatment and finally became negative during the period from 5 months to 12 months after the treatment. On the contrary, the antibody titers of the patients who were not cured showed only a strong floctuations.

It was confirmed that it may be possible to evaluate the efficacies of the treatments from the results of complement fixation tests in the early stage after treatment.

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肺吸虫症治癒判定法としての補体結合反応の検討

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肺吸虫症の治癒判定に補体結合反応の応用を試み，本症治療患者48名に就いてその継時観察を行つた．その結果，治療により虫卵が陰転しその後長期に亘つて再発をみなかつた塩酸エメチン・サルファ剤併用による完全治癒11症例及びビチオノール治療による完全治癒25症例では，抗体価は治療直後より漸減傾向を示し間もなく陰転したが，塩酸エメチン・サルファ剤併用治療で虫卵が消失せず，又たとえ一時的に消失しても再発をみた無効の12例では，抗体価は全然変化をしないか或は著しい上下変動を示して陰転をみなかつた．

肺吸虫症の治癒判定には虫卵検査を必要とすることは云うまでもないが，同時に補体結合反応の継時的観察を行えば比較的早期にその治癒傾向を推察することが出来る．特に虫卵排出をみない異所寄生例に於ては，本法が最も適切な手段として応用し得るものと考えられる．