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INFLUENCE OF TEMPERATURE ON THE ANTIGENICITY OF *SCHISTOSOMA JAPONICUM* LYOPHILIZED EGGS FOR CIRCUMOVAL PRECIPITIN TEST (COPT)

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The influence of temperature on the antigenicity of lyophilized COPT eggs for the diagnosis of schistosomiasis japonica was investigated.

Although alterations in temperature caused a gradual decline of antigenicity, stable temperatures, which ranged from 4°C to 60°C for 8 month preservation of the eggs, did not so much affect the antigenicity. A little decline of antigenicity was observed by incubation at temperature as high as 70°C to 120°C for 1 hour. The eggs incubated at 120°C for 24 hours and at 150°C and 200°C for 1 hour showed a marked decrease in antigenicity. No precipitin with eggs incubated at 200°C for 6 hours was observed. These results demonstrated that the egg antigens involved in COPT have a heat stable nature.

The COPT using lyophilized eggs for the diagnosis of schistosomiasis japonica is strongly recommended in field surveys of the endemic tropics since the egg antigen for COPT is sensitive, specific, and stable in nature and the technique is simple.

INTRODUCTION

It is well known that COPT is useful and specific for the diagnosis of schistosomiasis japonica.^{3,4,9,10,13)} The procedure for COPT has been simplified, and the reaction has been found to be stable^{8,9)}; therefore, COPT is of great value for the diagnosis of schistosomiasis japonica in field work, particularly in the tropics, where laboratory facilities for carrying out the reaction are insufficient.

OLIVER-GONZÁLEZ (1954) first applied the COP reaction by using fresh eggs as antigens for the schistosomiasis mansoni. The lyophilized eggs were then well applied to the diagnosis of schistosomiasis japonica.^{4,9,11,12)} Recently, we studied the standardization of antigenicity in lyophilized eggs used in COPT of schistosomiasis japonica²⁾. As little attention has been paid to the preservation of these eggs. The author has reported in this paper the influence of temperature on antigenicity in order to determine the most suitable conditions for the preservation of lyophilized eggs used in schistosomiasis japonica diagnosis.

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MATERIALS AND METHODS

The lyophilized eggs used in this study were purified in the Schistosomiasis Control and Research Project, Leyte, the Philippines. The ddY strain mice reared in the Project were 7 to 10 weeks old. All mice were subcutaneously injected with 30 cercariae of the Philippine strain of *Schistosoma japonicum*, which was established on Leyte Island, the Philippines. The eggs were collected from the intestines and the livers of infected mice on 44 days and 51 days after the infection, respectively, then, lyophilized according to the procedure of KAMIYA et al. (1980). The lyophilized eggs were preserved for 10 months in a refrigerator at 4°C with silica gel prior to the start of the present experiment. The lyophilized eggs were then divided into small vials, which were preserved with silica gel, and exposed to various temperatures. The COP reaction was conducted by using the lyophilized serum (standard serum) of a rabbit at 12 weeks after exposure to 600 *S. japonicum* cercariae (Philippine, Leyte strain) through skin penetration. The standard serum were resuspended in the same volume of 0.85% sodium chloride as the original volume. The preparation of specimens and the reading criteria for COP followed the procedures of YOKOGAWA et al. (1967) and MATSUDA et al. (1977). The reaction was carried out in a moisture chamber at 37°C for 48 hours.

After the 3~5 examinations of 100 eggs, the COP index and the percentage of eggs positive were recorded according to the formula cited in the "Technical Guide for Schistosomiasis Control in the Philippines", published by the Schistosomiasis Control and Research Project with the cooperation of the Japan International Cooperation Agency in 1976. The eggs used in the present study were also checked for any nonspecific precipitin reaction in the fresh normal rabbit serum. The Olympus differential interference microscope, model BH-NIC, was used for the observation of COP reaction.

RESULTS

Influence of preservation period under different temperatures to lyophilized egg antigens: The eggs were kept in vials under various temperature conditions for 8 months (tab. 1). The eggs kept at 4°C exhibited the highest antigenicity during the experiments; however, the antigenicity of eggs derived from the intestines of infected mice was more markedly decreased than that of the eggs removed from the liver at 8 month incubation. The eggs preserved at room temperature in a transparent bottle and in a brown bottle also showed reduced antigenicity. The influence of ultraviolet rays against the egg antigens was not obvious, although the antigenicity declined gradually during the experiment. The antigenicity of eggs from the liver decreased gradually at 60°C, but not so markedly at 30°C or 37°C.

Influence of high temperature on lyophilized egg antigens (tab. 2; figs. 1-8): The eggs used in this experiment were purified from the liver of the infected mice.

TABLE 1 Long time observation of the influence of temperature on COPT lyophilized eggs for diagnosis of schistosomiasis japonica

EGGS COLLECTED FROM	TEMPERATURE OF PRESERVATION	DURATION OF TREATMENT					
		1 week		4 weeks		8 months	
		%*1	COP Index*2	%	COP Index	%	COP Index
Intestine	4°C*3	32.7	31.7	29.3	27.5	24.5	22.8
	Room-temperature-I*4 (0-30°C)	26.3	24.7	20.7	17.6	16.5	14.2
	Room-temperature-II*5 (0-30°C)	28.7	27.8	28.7	25.9	16.3	14.4
	30°C	29.7	27.9	25.7	25.1	21.8	19.2
	37°C	not tested	not tested	30.0	28.2	16.3	14.8
	60°C	31.7	30.8	19.3	16.1	11.3	9.0
Liver	4°C	30.3	29.2	31.2	31.0	29.0	23.5
	Room-temperature-I*4 (0-30°C)	24.3	22.4	26.3	24.1	23.0	19.8
	Room-temperature-II*5 (0-30°C)	30.3	28.3	29.7	25.9	23.0	19.2
	30°C	28.7	27.0	32.0	30.6	28.0	22.6
	37°C	not tested	not tested	29.3	28.4	24.0	22.0
	60°C	27.3	25.6	23.3	22.8	23.5	18.1

The lyophilized eggs were divided into small vials and kept in the dark with silica gel, except for the eggs of "Room-temperature I and II".

*1 Percentage of eggs with precipitin.

*2 Index was calculated by using the formula "Index = $\frac{\sum ni}{N} \times \frac{100}{3}$ ", cited in the "Technical Guide for Schistosomiasis Control in the Philippines", published in 1976 by the Schistosomiasis Control and Research Project with the cooperation of the Japan International Cooperation Agency.

*3 The lyophilized eggs were prepared about 10 months before starting the experiment and kept at 4°C with silica gel after the preparation.

*4 The eggs were placed in a transparent bottle with silica gel and kept in the laboratory.

*5 The eggs were placed in a brown bottle with silica gel to prevent the effect of ultraviolet rays and kept in the laboratory.

TABLE 2 *Influence of high temperature on lyophilized egg antigens of COPT for schistosomiasis japonica*

TEMPERATURE OF PRESERVATION	DURATION OF TREATMENT (hour)	PERCENTAGE OF EGGS WITH COP	COP INDEX
4°C (control)* ¹	10 months	33.3	28.8
70°C	1	27.3	25.2
80°C	1	30.7	28.6
90°C	1	30.0	26.3
100°C* ²	1	37.3	36.4
120°C	1	29.5	25.2
	12	27.5	22.7
	24	17.0	12.2
150°C	1	17.7	13.7
	6	14.8	9.8
	12	5.4	2.5
200°C	1	8.0	4.2
	6	0	0

*1: Original antigenicity of eggs used in the experiment.

*2: Many eggs were ruptured.

The higher temperature treatment from 70°C to 120°C for 1 hour showed little effect on the antigenicity of the egg antigens. Incubation at temperatures of 120°C for 24 hours and 150°C and 200°C decreases the antigenicity significantly as shown in table 2. In addition, the length of precipitin at 120°C for 24 hours became shorter, and the majority of positive eggs (56.5 %) indicated the Type II precipitin reported by YOKOGAWA et al. (1967), which was the same as that obtained at 150°C incubation for 1 and 6 hours. No Type III precipitin was observed at incubations of 150°C for 12 hours and 200°C for 1 hour. Moreover, there was no precipitin observed at 200°C for 6 hours incubation.

DISCUSSION

It has been well known that COPT is of good diagnostic value for schistosomiasis in the respect of sensitivity, specificity and stability of the test.^{1,3,4,9,13)} Few studies, however, have been carried out on the nature of the egg antigen as related to storage and other conditions, although it has been recognized that ambient conditions may affect antigenicity. In this study prolonged preservation at 4°C least affected the antigenicity. The stable temperature, which was maintained even up to 8 months of incubation at 60°C, did not rapidly decrease the antigenicity. However, the eggs purified from the intestines of mice with 6 week infections showed decreased antigenicity after

8 month incubation. The eggs, however, still showed antigenicity after incubation at 120°C for 24 hours and 150°C for 12 hours and 200°C for 1 hour. These results suggested that the antigens involved in COP reaction might be stable polysaccharides or glycoproteins.^{5,7)} Since the varied temperatures induced the decrease in antigenicity gradually, the author concluded that lyophilized eggs should be preserved under stable temperature conditions to prevent the decline of antigenicity. It was also suggested that ultraviolet rays may impair lyophilized egg antigenicity.

In conclusion, present result have demonstrated that the lyophilized egg antigen is heat stable in nature, and the author thus recommend that for practical and other reasons, the COPT be used in field studies of schistosomiasis conducted in the tropics. Further studies are needed to characterize more particularly the egg antigens of *S. japonicum*.

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EXPLANATION OF PLATE

PLATE

Figures 1-4, 5-6 and 7-8 show COP reaction of the egg incubated at 150°C for 1 hour, 150°C for 12 hours, and 200°C for 1 hour, respectively. The Olympus differential interference microscope, model BH-NIC, was used for the observation of COP reaction. The reading criteria for COP by YOKOGAWA et al. (1967) was employed.

- Fig. 1 Segmented Type III precipitin with many vacuolations
- Fig. 2 Strongly reacted precipitin with many vacuolations
- Fig. 3 Slender segmented Type III precipitin and precipitin between the vitelline membrane and miracidia in the egg (↑)
- Fig. 4 Type I or II precipitin of ruptured egg (↑)
- Fig. 5 Type II precipitin with a big vacuolation
- Fig. 6 Small Type I or II precipitin at the portion ruptured
- Fig. 7 The typical Type I precipitin (↑)
- Fig. 8 Type II precipitin of the shrunken egg with large vacuolations

