APPLICATION OF ALCOHOL- OR ACETONE-FIXED *SCHISTOSOMA* EGGS AS AN ALTERNATIVE TO LYOPHILIZED EGGS FOR THE CIRCUMOVAL PRECIPITIN TEST

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Abstract. Antigenicity of *Schistosoma mansoni* and *S. japonicum* eggs preserved in ethanol or acetone were assessed in a circumoval precipitin (COP) assay. The egg antigens were found to retain sufficiently their COP reactivity for the diagnosis of both schistosomiasis mansoni and japonica, although their reactivity became lower than that of lyophilized eggs. These alternative preparations for COP tests have advantages, such as keeping eggs directly in fixatives soon after the egg-purification process. Furthermore, evaporation-process may cause eggshell cleavages which facilitate the reaction. The possible usefulness of those eggs in COP assays in local endemic areas is discussed.

INTRODUCTION

Circumoval precipitin (COP) tests for schistosomiasis can be easily performed with only minimal equipment and also expense (Lewert and Yogore, 1969; Yogore *et al.* 1979; Mott *et al,* 1987). COP tests, therefore, should be most appropriate serodiagnostic tools in endemic areas of the disease. Although lyophilized eggs have been exclusively used in most laboratories for the routine COP assay, the preparation of those eggs is not necessarily easy in the local endemic areas, where there is a great need for the COP test (Kamiya *et al,* 1980). From this point of view, the usefulness of air-dried or formalin-fixed eggs with sonication treatment in COP tests was demonstrated in our previous reports (Kamiya, 1983; Kamiya *et al,* 1985). In the present study, we investigated the possible employment of schistosome eggs preserved in alcohol or acetone to establish a more practical COP method, which is applicable in the field in endemic areas.

MATERIALS AND METHODS

Egg preparation: Eggs of *Schistosoma japonicum* (Philippine strain) and *S. mansoni* (Puerto Rican strain) were harvested at 8 weeks postinfection from livers of male ddY mice and Mongolian gerbils, respectively. The eggs were collected according to the digestion procedure of Kamiya *et al* (1980), then preserved in 95% ethanol or acetone and kept in a refrigerator until use. At the time of assay, a drop of the above-mentioned egg suspension containing approximately 200 eggs was put on a clean glass microscope slide. Then it was left for one hour prior to use for evaporation of fixatives.

Serum samples: The serum samples from schistosomiasis japonica patients in Leyte, Philippines, all proven by stool and/or COP test using lyophilized eggs were employed. Thirty sera from normal Japanese volunteers were used as controls. Six serum pools (serum pool nos. 1 to 6) from male ddY mice, 10 to 13 weeks after infection with *S. mansoni* were used. Serum pool nos. 1 to 4 were collected from 10 infected mice each and the remaining two pools from 5 mice each. The mean number of paired worms recovered from each infected mouse group, used for obtaining the serum pools above, were also recorded. Ten serum samples from age and sex matched naive mice were used as controls.

COP test: The test was carried out as described elsewhere (Yokogawa *et al,* 1967; Yogore *et al,* 1968), and the mean % of COP positive eggs was calculated from the data of duplicate tests.

RESULTS

Schistosomiasis mansoni: The COP reactivity of eggs preserved in ethanol or acetone is shown in Table 1, compared with the reactivity of lyophilized eggs. Typical COP reaction products of the eggs preserved in ethanol and also acetone are shown in Fig 1. Eggs preserved in ethanol for 3 months showed higher reactivity than the eggs preserved in acetone for 8 months. The lowest percentage of COP positive eggs was exhibited against the serum pool no. 1. However, as can be seen in Table 1, there was little relationship between intensity of infection and % of COP positive eggs in the assays, although the proportion of eggs with long precipitates seemingly increased with intensity of infection assessed by mean number of paired worms recovered. Eggs preserved in both fixatives had sufficient reactivity for application to the COP assay, although those eggs exhibited lower reactivity than that of the lyophilized eggs, which were liable to rupture in the serum samples and therefore, appeared to increase their reactivity considerably. No COP reaction product was recognized in any of 10 normal mouse sera.

Schistosomiasis japonica: Eggs preserved in ethanol or acetone for approximately 1 month were used. Characteristics of COP reaction products were the same as those of *S. mansoni* eggs (Fig 2). At the point of eggshell cleavage, COP was also seen (Fig 3). Acetone-fixed eggs showed higher reactivity than the ethanol-fixed eggs (Table 2). No COP reaction product was detected in 30 normal human sera.

DISCUSSION

The circumoval precipitin (COP) test is a useful serodiagnostic tool, using lyophilized eggs in schistosomiasis endemic areas (Hillyer *et al,* 1979;

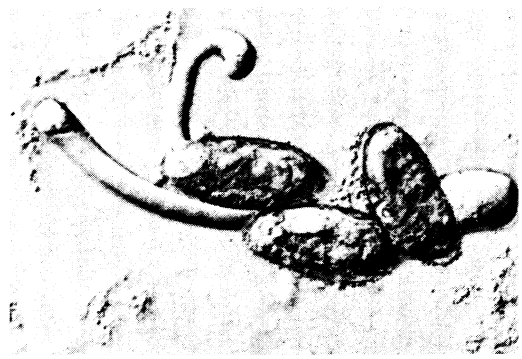

Fig 1—Typical circumoval precipitin (COP) on ethanol-fixed *S. mansoni* eggs. Differential interference microphotograph (DIM) × 140.

Table 1

Circumoval precipitin (COP) reaction of *S. mansoni* eggs preserved in ethanol or acetone.

No. pooled sera of infected mice	Mean no. of paired worms recovered	% COP positive eggs		
		Lyophilized*	Preserved in	
			95 % ethanol	Acetone
1	3.8	46.5	23.0	17.4
2	12.9	50.6	31.3	21.6
3	23.0	51.0	32.2	23.4
4	33.7	50.0	37.7	23.2
5	42.4	50.4	34.4	24.3
6	65.2	51.7	33.8	25.8
Mean		50.0	32.1	22.6

*Lyophilized eggs yielded in infected Mongolian gerbils.

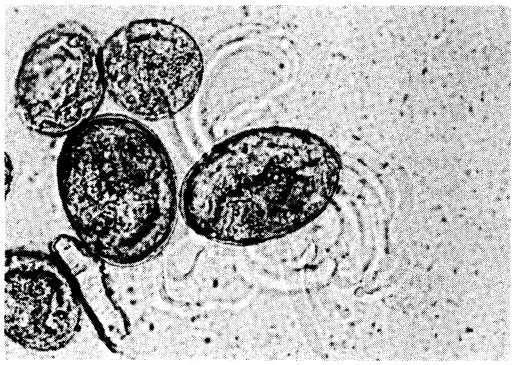

Fig 2—Many COP reaction products on an ethanol-fixed *S. japonicum* egg. × 250.

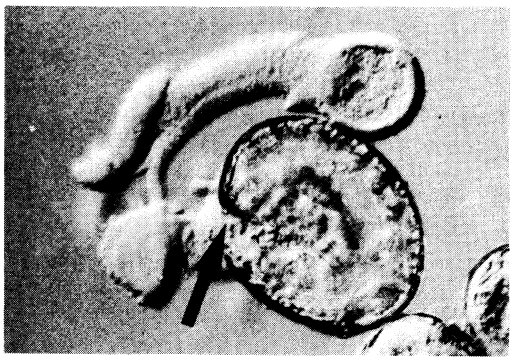

Fig 3—COP at the point of cleavage on an acetone-fixed *S. japonicum* egg (↗), DIM × 350.

Yogore *et al,* 1979). However, unavailability of the eggs makes difficult the frequent employment of the COP test in those areas. From this point of view, the introduction of air-dried (Kamiya, 1983; Hirata *et al,* 1985), paraformaldehyde (PFA)-fixed (Kamiya *et al,* 1985) and frozen (Ismail *et al,* 1983; Oliver-González and Vázquez, 1983) eggs represent a contribution to the solution of this problem. Especially PFA-fixed *S. mansoni* and *S. japonicum* eggs treated by sonication for making artificial eggshell cleavages have markedly increased their COP reactivity. Those eggs, however, have to be washed to get rid of the fixative and also to be sonicated prior to use. Therefore, the present COP test using ethanol- or acetone-preserved eggs is much more simple than the previous assays.

It is thought that heat-stable antigenic substances which localize between vitelline membrane and miracidia in the eggs (Kamiya, 1980) and are also involved in the COP reaction, indirect fluorescent antibody test (Kamiya *et al,* 1982), indirect immunoperoxidase test (Kamiya *et al,* 1981) and intraoval precipitin reaction (Kamiya, 1981), come out through the pore of the eggshell in the COP assay (Race *et al,* 1971; Sakumoto *et al,* 1972; Ford *et al,* 1980; Demaree and Hillyer, 1981). Therefore, it might seem reasonable to surmise that the rapid evaporation of ethanol or acetone from the eggs should contribute to artificial cleavages of the eggshell, which is composed of the protein (Byram and Senft, 1979).

Table 2

Circumoval precipitin (COP) reaction of *S. japonicum* eggs lyophilized or preserved in ethanol or acetone for 1 month against schistosomiasis japonica patient sera.

Patient serum No.	% COP positive eggs		
	Lyophilized*	Preserved in	
		95% ethanol	Acetone
1	40.9	12.4	25.3
2	37.1	10.4	20.9
3	27.9	12.0	18.4
4	27.6	11.4	13.6
5	26.2	8.2	11.4
6	24.9	11.0	12.1
7	24.6	7.2	15.7
8	22.7	2.3	11.1
9	20.7	10.9	10.8
10	18.9	6.5	11.2
Mean	27.2	9.2	15.1

* : Lyophilized eggs yielded in infected ddY mice.

It also appears that there was little relationship between the intensity of infection measured by mean number of paired worms recovered and % of COP positive eggs in murine schistosomiasis mansoni, as shown in Table 1. This result shows that the test is only qualitative in nature, although many attempts have been made to quantitate the COP test (Bruijing, 1964; Tanaka, 1976; Yogore *et al,* 1978; Hillyer *et al,* 1979; Ruiz Tiben *et al,* 1979).

It should be stressed that eggs preserved in alcohol or acetone still have antigenicity after more than 6 years' preservation (data not shown),

which should greatly facilitate carrying out the COP test in endemic areas.

Further studies are required to evaluate the present COP assay using the alcohol- or acetone-fixed eggs in endemic areas, where those COP egg antigens are most required.

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