

Investigations into the Recovery of Viable Schistosome Eggs from Human Tissue

BY

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The extraction of viable schistosome eggs from tissues has been achieved in the past by (1) a digestion method; (2) a sedimentation method; and (3) a saline flotation method. This last technique was worked out using animal tissues and is a simple and relatively quick procedure. The present study was performed as a preliminary to further pathological studies in man, so it was decided to investigate the application of this method to human tissue and in particular to liver. Since none of the papers describing the above methods indicated the percentage viability of the eggs obtained, it was thought that some of the conditions encountered in extraction might affect egg viability. The purpose of this study was therefore to find a method of isolating schistosome eggs from human tissue in a state where viability remained unchanged.

EXPERIMENT I.—ISOLATION METHODS

Method

An aspirator was fitted to a diffusing head by rubber tubing. The diffusing head consisted of a glass funnel glued to a base of sintered glass. The aspirator, containing saline of predetermined concentration, was placed at a level which gave enough pressure on the diffusing head to allow rapid flow of the saline. The flow was then regulated to a slower rate using a stopcock on the tubing to the diffusing head. A portion of liver was chopped into small pieces and homogenised in normal saline in a Waring blender for five minutes, and the mixture obtained was passed through a sieve of mesh 100 (British SWG) and/or a household sieve into a 1 litre beaker. The diffusing head was placed into the homogenate until it was almost at the bottom of the beaker. It was also positioned as near as possible to the centre of the beaker so that there was minimal disturbance during flow. The stopcock was released, and as the saline diffused through the homogenate the latter became layered on top of the saline. All of the homogenate was allowed to flow over the sides of the beaker into a container. The clear saline (solution) obtained contained eggs which had sedimented from the homogenate. The beaker was left to stand until the eggs had fallen, and

then the greater portion of the saline solution was removed by gentle suction. The remainder was transferred to a urine flask. When the eggs had settled, the greater part of the solution was again suctioned off and the eggs were transferred to a centrifuge tube, centrifuged and examined for viability. Flame cell activity was used as the criterion of viability in this study.

Results

A. Using a Household Sieve Only

I. *Using 2 per cent. Saline as the Flotation Solution*

Samples of different weights were taken from a total of six livers and suspended in different volumes of saline.

- (a) 50 gm. homogenised in 300 ml. normal saline—gave incomplete layering.
- (b) 25 gm. in 250 ml. normal saline—layering well defined at first; later tissue pieces began to sediment.
- (c) 10 gm. in 250 ml. normal saline—as for (b), but with less tissue sedimenting.
- (d) 5 gm. in (1) 250 ml. (2) 350 ml. normal saline—smaller amount of tissue sedimenting than in (c).

II. *Using 3 per cent. Saline*

Samples were taken from one liver.

- (a) 25 gm. in 250 ml. normal saline resulted in slightly less tissue sedimenting than samples of similar size using 2 per cent. saline.
- (b) 10 gm. in 150 ml. normal saline resulted in slightly less tissue sedimenting than samples of similar size using 2 per cent. saline.

III. *Using 4 per cent. Saline*

Samples were taken from a total of two livers.

- (a) 25 gm. in 250 ml. normal saline.
- (b) 10 gm. in 150 ml. normal saline.
- (c) 5 gm. in 150 ml. normal saline.

All gave good layering with negligible sedimenting of tissue. A viability count on these eggs was performed and, of 1,000 eggs counted, none was viable.

B. Using a Household Sieve and a Sieve of Mesh 100

Using 2 per cent. saline as the flotation solution. Samples were taken from one liver.

- (a) 50 gm. in 250 ml. normal saline—layering was incomplete.
- (b) 25 gm. in 250 ml. normal saline—layered well, although the 2 per cent. saline was

left slightly cloudy after flotation. On examination, the eggs were clearly outlined and free from any tissue, except for one group of 30 eggs found entangled in a piece of tissue. A total of 1,700 eggs were counted.

- (c) 15 gm. in 250 ml. normal saline—layered well with only a trace of cloudiness after flotation. A total of 700 eggs were counted and all were clearly outlined and free from tissue.
- (d) 5 gm. in 250 ml. normal saline—layered well, leaving a clear solution after flotation. A total of 500 eggs were counted; all were free from tissue.

Discussion

Reference to the method used by Ritchie and Beinos-Duran (1961) suggested that sieves were not found to be critical for complete layering to occur. In the first trials of this present method only a household sieve was used, but this was not successful. A sieve of mesh 100 was added in the second trials and this gave successful layering. Although frothing of the mixture occurred, it did not disturb layering significantly.

The first trials proved 2 per cent. saline to be insufficient in preventing tissue pieces from sedimenting. It was thought that an increased saline concentration would eliminate tissue sedimentation as effectively, and with less trouble, than the use of a fine sieve. The saline concentration was therefore increased through 3 per cent. to 4 per cent. before tissue sedimentation was stopped altogether. However, at this concentration no viable eggs were found. As a result of this finding it was decided to investigate some of the factors affecting egg viability.

The second trials showed that, provided the sieve was fine enough, 2 per cent. saline was sufficient as the flotation solution. Of the successful samples, the 25 gm. sample gave a good egg supply, almost completely free of tissue, and thus seemed to be the most suitable size.

EXPERIMENT II.—INVESTIGATION INTO SOME OF THE FACTORS AFFECTING EGG VIABILITY

These factors are:

- (1) The technique used to separate the eggs from the tissues, including the effect of different saline concentrations on egg viability.
- (2) The effect of various temperatures on egg viability. The egg is subjected to—

- (a) body temperature during life;
- (b) room temperature at death;
- (c) refrigeration after death until *post-mortem*.

The effects of all three of these temperatures were observed.

Methods

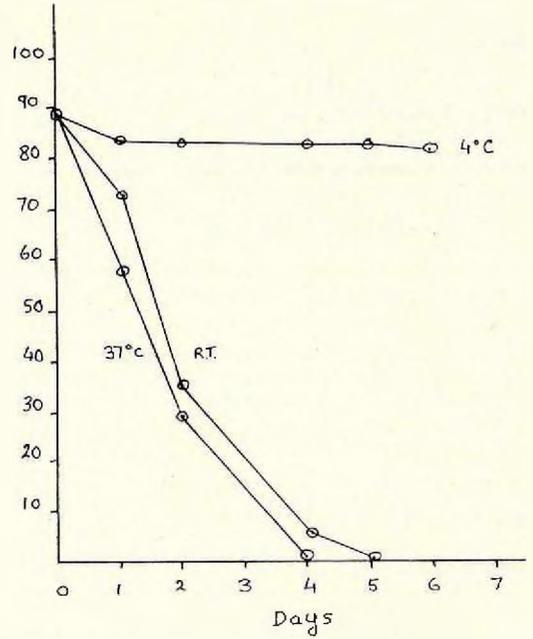
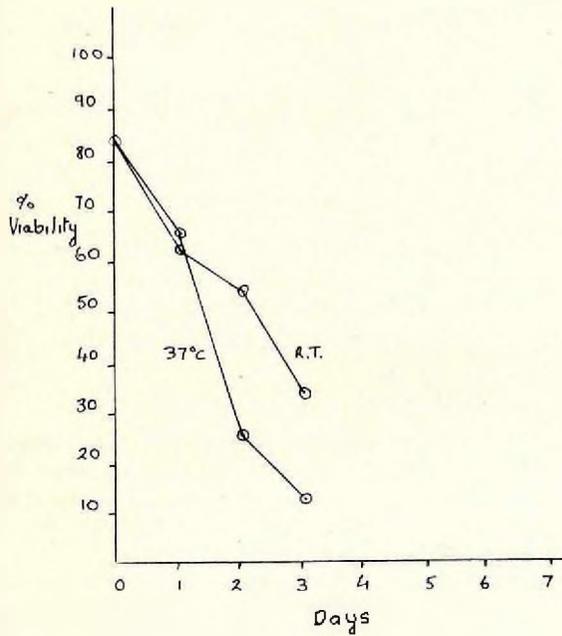
Eggs voided in the urine of untreated patients suffering from bilharziasis were used.

- (a) Effect of different saline concentrations at room temperature. The urine sample was poured into five tubes, centrifuged and the supernatant was poured off four of these tubes and replaced with 4 per cent., 3 per cent., 2 per cent. and 1 per cent. saline solutions. The eggs in the remaining tube were left in the urine supernatant. The tubes were placed in the dark at room temperature. After five hours, viability counts were performed.
- (b) Effect of different temperatures at constant saline concentration. The urine sample was poured into 25 tubes which were centrifuged and the supernatant removed. 0.9 per cent. saline was added to 24 of the tubes, and eight of these were incubated at 37° C., eight were left at room temperature and eight were refrigerated at 4° C. A viability count was performed on the remaining tube. All the tubes were left in the dark to prevent hatching, and viability counts were done on each every 24 hours. The complete procedure was repeated four times. The first two trials were done on patient A, in whose urine the percentage of *S. mansoni* eggs was 0.57, the remainder being terminal-spined eggs. The next two trials were done on patient B, who had terminal-spined eggs only.
- (c) Effect of different saline concentrations acting for different times at 4° C. The urine sample was poured into seven tubes which were centrifuged and the supernatant was removed. A viability count was done on the eggs in one tube. To the three pairs of tubes remaining were added 2 per cent., 3 per cent., 4 per cent. saline solutions at 4° C. The tubes were maintained at 4° C. in the dark. After three hours a viability count was done on the eggs from one tube of each pair.

I

PATIENT A

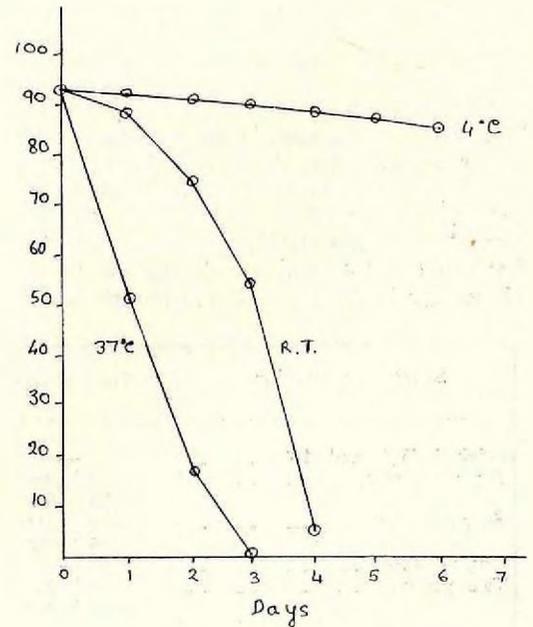
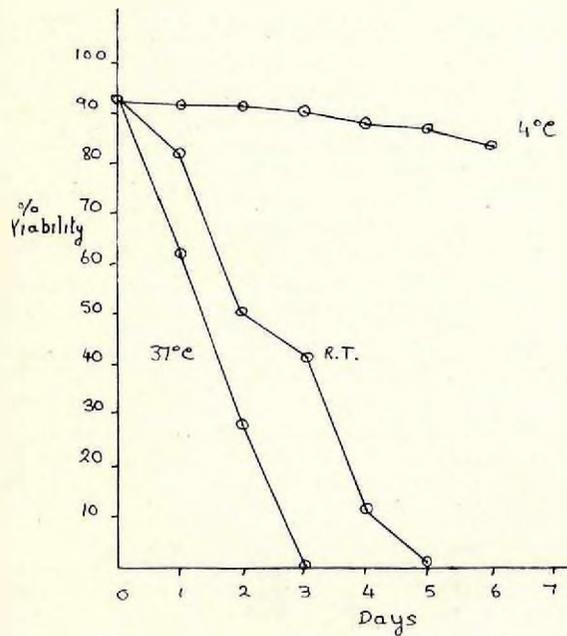
II



PATIENT B

III

IV



After 24 hours a viability count was done on the eggs in the remaining tube of each pair.

Results

(a) Effect of different saline concentrations at room temperature:

Saline Concentration	Total Egg No.	Viable	
		No.	%
PATIENT A			
Urine	100	48	48
4 per cent.	100	0	0
3 per cent.	100	0	0
2 per cent.	100	10	10
1 per cent.	100	46	46
PATIENT B			
Urine	472	431	91.2
4 per cent.	100	0	0
3 per cent.	100	0	0
2 per cent.	76	37	48.4

(b) Effect of different temperatures at constant saline concentration:

(c) Effect of different saline concentrations acting for different times at 4° C.:

DISCUSSION

After a five-hour interval and taking the viability count of the eggs in the urine as a control, results showed that those eggs in 3 per cent. and 4 per cent. saline lost their viability completely, whereas those in 2 per cent. saline showed a reduced viability of 38 per cent. in the first count and 43 per cent. in the second count. Those eggs in the 1 per cent. saline showed only

a slight difference in viability from those of the control.

Results showed that refrigeration will preserve viable eggs over a reasonable period. After a three-day period of refrigeration, three sets of eggs showed a 5 per cent., 1 per cent. and 1 per cent. decrease respectively from the original viability. After six days the viability decrease amounted to 6 per cent., 7 per cent., 6 per cent. (Figs. 2, 3 and 4). Those eggs incubated at 37° C. and taken from patient A showed no viability after four days; those taken from patient B showed no viability after three days. The eggs kept at room temperature lost their viability after five days in the case of patient A and after six days in the case of patient B, although the viability of the latter group at five days was only about 2 per cent.

These results suggest that the viability of eggs in the body will not deteriorate significantly if the body is kept under refrigeration from the time of death. Those eggs kept at 37° C. were very nearly consistent in their decline in viability, whereas those at room temperature varied somewhat. This could be a result of a steady temperature of 37° C. on the one hand and an ever-changing room temperature on the other. The increased time of viability of the eggs kept at room temperature could suggest that the optimal conditions for miracidia (as yet unhatched) approximate more to room temperature than to 37° C. It seems probable, therefore, that living at room temperature and still under the protection of the shell, the miracidium is capable of surviving longer than at 37° C.

Relating to the isolation technique, these results have shown that, provided the body is refrigerated soon after death, the viability of the isolated eggs will be unchanged, i.e., if the technique itself does not interfere with egg viability.

The viability of the eggs in 3 per cent. and 4 per cent. saline were both 3.8 per cent. after

Saline Concentration	Time Interval	Total No.	Viable	
			No.	%
Original (3% and 4%)	—	472	431	91.2
Three per cent.	3 hours	208	8	3.8
	24 hours	200	0	0
Four per cent.	3 hours	104	4	3.8
	24 hours	200	0	0
Original (2%)	—	326	300	92.1
Two per cent.	3 hours	314	283	91.8
	24 hours	545	500	91.3

three hours. The viability of the eggs in 2 per cent. saline was unchanged from the original after three hours, and after 24 hours it had dropped by 1 per cent. This suggests that 2 per cent. saline maintained at 4° C. is sufficient to keep the viability of the isolated eggs from deteriorating. If this modification is applied to the isolation technique it might be expected that the viability of the eggs would be maintained at the level present at the time of death of the host.

SUMMARY

(1) A flotation method for isolating eggs from human tissue, which also preserves the original viability at death of the body, has been investigated.

(2) Two per cent. saline caused satisfactory separation between eggs and tissue in homogenised material which had previously been put through a fine sieve.

(3) At room temperature 4 per cent., 3 per cent., 2 per cent. saline solutions caused a marked decrease in the original viability of eggs voided in urine.

(4) At 4° C., 37° C. and room temperature, experiments were performed on egg viability.

(5) At 4° C. the egg viability decreased only slightly after six days.

(6) Eggs subjected to 2 per cent. saline at 4° C. for three hours maintained their original viability; after 24 hours the loss of viability was only 1 per cent. of the original.

(7) Thus it is suggested that 2 per cent. saline at 4° C. is necessary to isolate eggs satisfactorily so that a true viability count is obtained.

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