The Distribution of Viable and Non-Viable Eggs of Schistosoma Haematobium in the Urine

BY

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Introduction

In the study of intestinal bilharziasis caused by *Schistosoma mansoni*, a method has been developed by Pereira and Barretto (1949) by which it is now possible to estimate the age of an egg since it was laid by the female worm, and by this to assess the efficacy of a therapeutic agent. This method is referred to as the oogram. Weber *et al.* (1967) studied the distribution of eggs of *S. haematobium* in the urine at different

times of the day and in different fractions of the micturition stream. In this paper they reported in detail the methods by which the eggs were collected, concentrated and examined. In the course of this study the eggs that were seen and counted were grouped into one of four categories:

(1) Normal mature eggs with few, if any, red blood cells adherent to the egg shell; these will be referred to as "normal" eggs.

(2) Blood-encrusted eggs (Be eggs) with six or more red blood cells adherent to the egg shell. Some of these eggs may have been so heavily encrusted that it was difficult to see the spine or make out the details of the contained miracidium.

(3) Dead eggs. Each containing a dead miracidium, generally in a shrivelled condition. The miracidial body is well separated by a clear zone from the egg shell.

(4) Black eggs. Eggs with no discernible miracidial structure, of an even, dense, black appearance with no clear zone within the egg. These eggs have a clean-cut appearance and never have adherent red blood cells. Occasionally eggs of this type may be found lying close together in a deposit, sometimes held together in a tiny fragment of epithelial tissue.

It was expected that in urine specimens containing large quantities of blood that the ratio of blood-encrusted eggs to normal eggs would have been high; however, this was not observed, and in fact in such urine specimens the ratio of normal eggs to Be eggs was at its highest, while the proportion of Be eggs tended to be greatest in urine specimens which were relatively free of visible blood. It should be stressed that in this particular study urine samples were processed and examined with the utmost despatch.

Diurnal Variations in the Categories of Eggs

Over a period of four months specimens of urine were collected from a volunteer at different times of the day. At 0600 hours he collected his early morning urine specimen in bottles provided and brought these with him to work. From 0800 hours to 1600 hours he passed specimens of urine to a predetermined programme at hourly, two-hourly or, on some occasions, three-hourly intervals. Table I sets out the number and percentage of each category of eggs at different times of the day. It will be noted that the percentage of Be eggs is at its lowest at the beginning of the daily study period and at its highest shortly after noon. There was an indication of a decreasing

Table I

DISTRIBUTION OF CATEGORIES OF S. HAEMATOBIUM EGGS IN URINE SAMPLES PASSED AT VARIOUS TIMES OF THE DAY

Hour of Sample	Number of Observations	Normal Eggs	Be Eggs	Dead Eggs	Black Eggs	Total
0600	13	1,660	190	40	70	1,960
		84.7	9.7	2.0	3.6	100.0
0800	19	2,950	800	30	90	3,870
		76.2	20.7	0.8	2.3	100.0
0900	12	1,620	450	50	220	2,340
		69.2	19.2	2.2	9.4	100.0
1000	15	4,020	1,040	20	40	5,120
		78.5	20.3	0.4	0.8	100.0
1100	9	4,450	940	0	50	5,440
		81.8	17.3	0.0	0.9	100.0
1200	16	10,950	2,790	100	260	14,100
		77.7	19.8	0.7	1.8	100.0
1300	10	7,550	1,670	10	320	9,550
		79.0	17.5	0.1	3.4	100.0
1400	13	15,190	4,250	120	210	19,770
		76.8	21.5	0.6	1.1	100.0
1500	14	11,130	3,360	70	30	14,590
		76.3	23.0	0.5	0.2	100.0
1600	14	8,080	1,480	140	280	9,980
		81.0	14.8	1.4	2.8	100.0
TOTAL		67,600	16,970	580	1,570	86,720
		78.0	19.5	0.7	1.8	100.0

percentage of Be eggs after this time. Dead eggs were few in number (less than 1 per cent. of the total), but they showed a tendency to a higher average in the early hours of the morning. Black eggs were more variable in their percentage of the total eggs, but they showed the same tendency to higher proportions in the early morning.

Variations in Percentages of Eggs of the Different Categories in the First and Terminal Fractions of the Micturition Stream

Weber *et al.* (1967, *op. cit.*) described the distribution of the *S. haematobium* eggs in serial fractions of the micturition stream. The methods and techniques were described in detail and they have been followed in this study; however, for this study the results for the first five fractions have been consolidated into one total for comparison with the number of eggs found in the terminal fraction. Similarly, for this study urine specimens were only examined at three times of the day: 0800, 1200 and 1600 hours. The terminal portion of a urine specimen represents under one-fifth of the total volume of that specimen, but it was observed that more than half of the eggs passed were to be found in this terminal portion. This was particularly obvious in the midday specimen, where not only were the total numbers of eggs passed much greater, but the eggs found in the terminal specimen represented two-thirds of the total numbers (see Table II). This increase was largely due to an increase of eggs classed as normal eggs.

It will be seen that the percentages of normal and Be eggs are essentially similar in the first and terminal portions of the micturition stream taken at 0800 hours. However, in the terminal portions of micturition stream taken at 1200 and 1600 hours the percentage of normal eggs increases as the percentage of Be eggs decreases.

The total numbers of dead eggs found in these series is low and represents only 1 per cent. of the total eggs seen; however, both the number and percentage of dead eggs in the first portion of the urine were usually higher, being nearly double the numbers and percentages found in the terminal portion of urine. The differences in numbers and percentages of black eggs in the two fractions of the micturition sample were small.

DISCUSSION

It was surprising that great numbers of normal eggs were seen in urine containing large numbers of red blood cells; equally so, it was surprising that Be eggs were found in numbers in urine which was virtually devoid of free red blood cells. It seems, therefore, that with blood-encrusted eggs

Table II

DISTRIBUTION OF CATEGORIES OF S. HAEMATOBIUM EGGS IN FIRST PORTIONS AND THE TERMINAL FRACTION OF THE MICTURITION STREAM

Hour	First Portions, Fractions Nos. 1-5					Terminal Fraction (6)					Total				
	N	Be	D	B	T	N	Be	D	B	T	N	Be	D	B	T
0800	1,400	370	20	50	1,840	1,550	430	10	40	2,030	2,950	800	30	90	3,870
	76.1	20.1	1.1	2.7	100.0	76.3	21.2	0.5	2.0	100.0	76.2	20.7	0.8	2.3	100.0
1200	3,330	1,300	60	120	4,810	7,620	1,490	40	140	9,290	10,950	2,790	100	260	14,100
	69.2	27.0	1.3	2.5	100.0	82.1	16.0	0.4	1.5	100.0	77.7	19.8	0.7	1.8	100.0
1600	3,850	900	90	150	4,990	4,230	580	50	130	4,990	8,080	1,480	140	280	9,980
	77.2	18.0	1.8	3.0	100.0	84.8	11.6	1.0	2.6	100.0	81.0	14.8	1.4	2.8	100.0
Total	8,580	2,570	170	320	11,640	13,400	2,500	100	310	16,310	21,980	5,070	270	630	27,950
	73.7	22.1	1.5	2.7	100.0	82.2	15.3	0.6	1.9	100.0	78.6	18.1	1.0	2.3	100.0

N = Normal eggs.
Be = Blood-encrusted eggs.
D = Dead eggs.
B = Black eggs.
T = Total eggs in sample.

the red blood cells adhere to the egg while it is still in the tissues and not when it is free in the urine in the lumen of the bladder.

From tissue digest studies it is known that black eggs can be found in many of the organs of the body, often in enormous numbers. It is our experience that dead and black eggs are not found in the urine of persons who have recently been infected with the parasite, and possibly the presence of these forms in high proportions in the urine is an indication of the duration of infection. The dead eggs, small and shrivelled as they are, may be the product of female schistosome worms which are ageing or under stress as the result of host resistance. Similarly, experience indicates that after specific therapy numbers of eggs of this category increase in urine, and this is presumably an indication of the effect of the drug on the sexual apparatus of the female worms. Black eggs are presumably the remains of mature eggs which have died in the tissues. The output of these black eggs into the urine is generally intermittent and it may occur in showers sometimes lasting for several days, at times in clusters in fragments of bladder epithelial tissue. Generally, however, the black eggs are free from tissue fragments or adherent blood cells.

The relationship of the remaining classifications of schistosome eggs, normal and Be eggs, which together constituted over 97 per cent. of the total eggs seen in this series, is of interest. It is our suggestion that the adherence of red blood cells to the schistosome egg could be the result of a host response, possibly the adherence being the result of an antigen antibody reaction on the surface of the egg shell. Normal eggs could thus indicate a relatively new infection where the host has had insufficient time to develop the partial resistance and the antibody reaction required to cause the adherence of the red cells to the eggs. Alternatively, it is possible that the adherence of red cells occurs on eggs which have passed their peak of maturity because of time taken to pass from the site of egg-laying to the site of extrusion into the bladder, thus giving more time for a weak antigen antibody reaction to take place. Whatever the mechanism resulting in this adherence, the relative proportions of these two categories of eggs in the urine of a patient may well indicate the duration of the infection in that patient.

Summary

The ratio of *S. haematobium* eggs in different categories in urine specimens passed at different times of the day has been determined. Similarly, these ratios have been studied in the first portion and terminal fraction of the micturition stream.

It is suggested that the ratio of the different egg categories may indicate the duration of the infection, since the category of the egg may indicate its age.

References

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