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The Pattern of Schistosome Egg Distribution in a Micturition Flow

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INTRODUCTION

Despite the importance of knowing how schistosome eggs are distributed in the urinary stream in an act of micturition, there appears to have been little study of the subject. It has been well recognised for years that in an infected subject schistosome eggs could be most easily found in the terminal portion of the micturition stream. There is, however, surprisingly little proof that the assumption is well founded. There are a number of references, particularly by Egyptian workers, that all that is necessary to establish a diagnosis of urinary bilharziasis is to take the last drop of urine adhering to the external meatus of the penis on to a microscope slide and examine it directly. Manson-Bahr (1941) states, "But even when eggs are very few, they may be found in the last drop or two of urine passed." The Joint OIHP/WHO Study group on bilharziasis in Africa (1950) states that "Specimens of urine to be examined should be taken from the last part of micturition, following physical exercise." The Expert Committee on Bilharziasis (1953) in its first report stated, "The eggs filtering through the wall of the bladder are usually passed towards the end of the period of micturition and, together with cellular debris and salt crystals, rapidly settle towards the bottom of the container into which the patient urinates." The Third Report of the WHO Expert Committee on Bilharziasis (1965) drew attention to the importance of the time of sampling of urine and advised that this should be standardised to be taken between 1100 and 1500 hours. They also advised that urine volumes should be measured accurately. In field epidemiological surveys, however, accurate urine volume measurements are generally not practicable. The committee did not consider egg count studies based on urine samples passed after a fixed time, generally one hour from the previous micturition, of any value in assessing intensity of infection in epidemiological surveys. In fact, they seemed to favour reliance on counting the eggs from a 10 or 15 ml. sample of urine taken during the period of peak egg production. Sometimes the results of the sample were related to the volume of the total urine sample passed, but were not related to the time in which the urine had been in the bladder.

Bennie (1949) showed clearly in a detailed study of a subject suffering from urinary bilharziasis due to *Schistosoma haematobium* that the average number of eggs counted in an early morning urine sample was only 11, while the afternoon samples averaged 446 eggs. She also noted that when the subject engaged in increased manual labour, particularly exercise such as hoeing, involving the musculature of the hips and lower abdomen, higher egg counts resulted.

Gove (1962) conducted a series of experiments on persons with urinary infections with *S. haematobium*. He collected urine directly into a series of test tubes each of capacity of approximately 6.5 ml. In one instance a patient filled a series of 16 such tubes. The author does not explain how he was able to get patients to micturate into a series of small test tubes, 6 cm. long and with an internal tube diameter of only 12 mm., without considerable spillage and the need to use the urethral sphincter to cut off the urine flow while the subject moved from one tube to the next. On a rather inadequately described trial the author concluded that in heavy and light infections with *S. haematobium*, ova are distributed throughout the urinary stream and that at times when the urine contains very few ova they may be passed at any time in the stream. He sums up by stating, "There seems to be little justification for the practice, when looking for ova, of examining only the last specimen of urine voided." Unfortunately this author's paper has been read rather uncritically and Davis (1966), for example, states, "It is doubtful whether exercise increases egg output and, contrary to the belief held for some years, there is little evidence of a concentration of eggs in the terminal urinary stream (Gove, 1962)."

The chief difficulty in undertaking a study of schistosome egg distribution in a micturition stream is to devise a satisfactory method of fractionating the stream in such a way that there is no halt in the passage of urine from the bladder; from the act of relaxing the internal urethral sphincter to the final act of forcible expression of the last portion of urine in the bladder by voluntary muscular action. It is considered that the inability of Gove to solve this problem detracts from his observations.

Apparatus and Method

The ideal receptacle for the apparatus is the square sectioned clear hard plastic covers provided by Ortho Pharmaceuticals for the containers for their immunological reagents. The containers are 7 cm. in height and have an outside measurement of 3.2 cm. square at the top of the container and 2.8 cm. at the base. Each container can hold 45 ml. of fluid. Six of these containers were fitted into a simple wooden rack to form a row. Because of their square section, the containers fitted snugly together to provide an area of 20 cm. long and 3 cm. wide over which the subject could urinate. The subject, after practice, had no difficulty in urinating into the series of containers without spilling a drop. Further, the act of micturition could be performed without any interruption in the flow.

Our volunteer subject, a male African of 23 years of age, comes from an area of Rhodesia where the prevalence of urinary bilharziasis is low. He came to work in Salisbury about four years ago and visited friends in a rural area near the city. While it is not possible to be dogmatic on this matter, the skin test, fluorescent antibody test and signs and symptoms indicate that the disease is of relatively recent acquisition. His micturition technique with the rack of containers was to hold the rack between his legs, with the first tube in the series identified by the letter "S" (for start) facing forwards. As micturition proceeded he moved the rack forwards from between his legs as the successive tubes received their quota of urine. He was able to gauge very accurately how much urine was in his bladder and distribute this over the six containers. An analysis of micturitions in which urine was passed into all six tubes of the rack showed that in a large series of observations involving the passage of 10.5 litres of urine, he averaged the following percentage of the total volume of each micturition in the series of tubes Nos. 1 to 6: 13.8, 16.3, 17.9, 16.6, 15.7 and 19.7 per cent. respectively. This shows a high degree of accuracy and indicates only a tendency to move a little fast from No. 1 to No. 2 and to find the need to put more in tube No. 6. As a tribute to his accuracy we quote his performance on 11th October, 1966: the urine volumes in the tubes were 14, 14, 16, 16, 14 and 16 ml. with a total micturition of 90 ml. of urine.

Two types of study of the subject's micturition pattern were carried out: the first a series of micturitions carried out at fixed times during the working hours of the day, commencing at 0800 hours and going through to 1600 hours; and another series in which he was invited to pass urine into the special apparatus at odd times during the day. On the day prior to which a series of micturitions at fixed times was to be done, the subject was provided with three screw-topped numbered bottles each of 150 c.c. capacity. On rising at about 0600 hours he passed urine into the first numbered bottle, and on filling it carried on to pass urine into the second bottle. On only one occasion did he find it necessary to make use of a third bottle. These bottles of urine he brought with him to work in the morning. On arriving at the laboratory at 0800 hours he passed urine again, using the urine container rack. As soon as the containers had been used they were taken out of the rack and placed in order on the bench, a few inches apart. The volume of urine in each container was estimated by comparison with water in another plastic container filled up to the level of the container of urine. This could be done quite accurately using a plastic squeeze bottle. The water in the measuring container was poured into a 50 ml. measuring cylinder and the volume read off. This was repeated in turn for each of the six containers of urine. After the containers had stood for 30 minutes on the bench to allow the eggs to settle, the supernatant urine was sucked off carefully to 15 ml. with a bulb pipette. The contents of each container were gently agitated and the urine sediment poured into centrifuge tubes. As the containers were of plastic material the contents were easily poured off, leaving the container dry. The centrifuge tubes of urine were spun at 1,000 r.p.m. for exactly 90 seconds and the supernatant removed with a water suction pump and glass pipette to leave 0.5 ml. of sediment.

In preparation of a sample for microscopic examination and egg counting, the centrifuge tube was flicked vigorously to re-suspend any sediment in the 0.5 ml. and 0.05 ml. taken up with a fine bulb pipette and placed on a 76 x 26 mm. microscope slide—the drop then being covered by a 22 x 22 mm. micro cover glass. The urine sample under the micro cover glass was tracked and scanned carefully and all eggs counted. The number of eggs in a particular specimen was taken to be ten times the number of eggs observed in the micro cover glass preparation. In addition to the total count of eggs, a record was kept of the number of "black eggs," eggs with dead and shrivelled miracidia and eggs which were classed as "rough eggs." "Rough eggs" are those having adherent red blood cells on the egg shell.

On a very few occasions in the microscopic preparations small flakes of bladder mucosa were noted containing numbers of dead eggs. On one occasion a flake of tissue was found to be crowded with 101 dead black eggs. None of the eggs trapped in tissue fragments were included in the egg count.

It is interesting to record that over 800 microscopic examinations of the subject's urine were made from 6th October, 1966, to 10th February, 1967, each examination involving the scanning of the area of a cover slip preparation; on only two occasions were eggs of *S. mansoni* seen. A number of stool examinations failed to show any evidence of intestinal bilharziasis.

Results

1. *The Pattern of Egg Production Within the Micturition Stream*

The most important trend which was easily apparent was the characteristic "J"-shaped graph as illustrated in Fig. 1. In the great majority of specimens examined from either a series of children of Hatcliffe School or from the subject in the laboratory, there was a pattern of moderate egg numbers in the first portion of urine, very few, if any, eggs in the second, third, fourth and fifth portions, and usually large numbers in the last portion. The results given in Table I show this pattern of egg production and they are taken from 23 random samples of urine from the subject; these samples were passed at the request of the authors—the subject being given no prior warning. The same trend is apparent in Table II, which gives the results of egg passage patterns from urine specimens from each of six children from Hatcliffe School. It will be seen that some of these six children were unable to pass urine into each of the six containers in the rack given to them. This was possibly due to the fact that the children were asked to pass these specimens at the end of the mid-morning "break," and it is probable that little time had elapsed since the last emptying of their bladders. This is shown by the fact that only two of the six children were able to pass more than 40 ml. of urine.

It was interesting to note that the extent of blood in the urine fractions also followed a similar pattern within the urine stream. In fact, blood was visible to the naked eye only in the last fraction, although microscopic examinations revealed quantities of blood in the first and last fractions, with only scattered cells in the mid-stream portions.

2. *Comparison of Patterns of Egg Release*

The patterns of egg passage were studied in three series when the subject was made to micturate every hour from 0800 hours to 1600 hours, every two hours through the same period and every three hours from 0900 hours to 1500 hours. These three series entailed passing nine specimens during the series of hourly specimens, five during the series of two-hourly specimens, and only two during the series of three-hourly specimens (see Tables III, IV and V and Figs. 2 and 3).

During the third series, in addition to the three-hourly specimens, the subject was made to micturate at 0800, 0900 and at 1600 hours and the egg counts from these specimens were included when comparing the three series.

Comparisons of the results gave the following information:

(i) There was no significant difference in the diurnal patterns of egg production in the three series

(ii) There was no significant difference in the patterns of egg production within the micturition stream. However, there was an observed tendency in the one-hour series towards higher egg numbers in the first portions of the urine. This showed itself as a U-shaped graph rather than the typical J graph of all other specimens.

(iii) There was a similarity in the total numbers of eggs passed per day in the

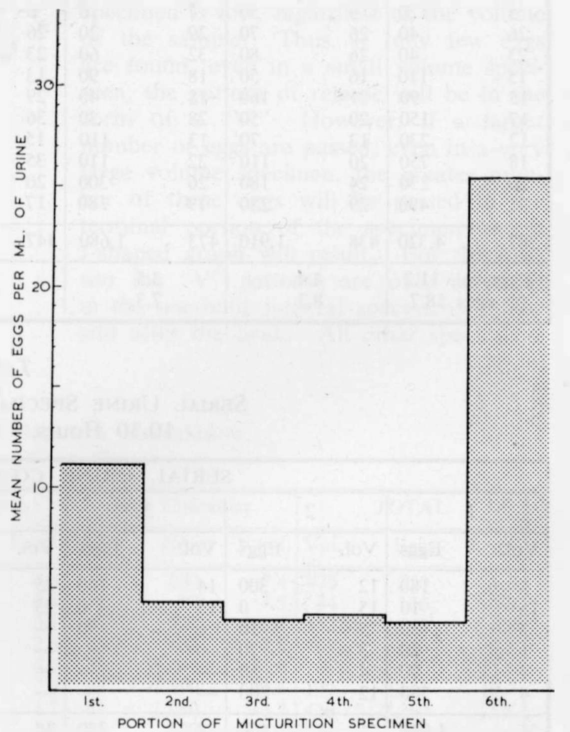

Fig. 1—Eggs in fractions of the micturition stream.

three series (Table VI), and this similarity was observed regardless of the number of micturitions which contributed to the total (Table VII).

3. *The Effects of Exercise on the Pattern of Egg Release*

It has been widely believed that exercise immediately prior to micturition has the effect of increasing the number of eggs recovered. If this is true it might also affect the pattern of egg passage within the micturition stream.

On six occasions, therefore, the subject was made to do 50 rapid "knees-up" exercises prior to urinating into the serial containers. The results are presented in Table VIII, which should be compared with Table I.

Table I

RANDOM SELECTION OF 23 SAMPLES OF URINE TO SHOW EGG PASSAGE IN THE MICTURITION STREAM

SERIAL URINE CONTAINERS 1		2		3		4		5		6		TOTAL	
Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs
36	210	18	120	23	40	23	20	9	230	15	780	124	1,400
14	560	14	80	16	120	16	100	14	90	16	260	90	1,210
17	90	19	70	17	40	14	50	17	80	25	690	109	1,020
10	310	14	140	13	130	10	90	16	100	8	1,030	71	1,800
6	140	9	110	13	100	8	120	6	60	16	240	58	770
13	160	18	40	22	60	21	20	20	20	23	160	107	460
19	330	22	160	25	30	16	80	22	40	30	120	134	760
18	60	15	20	18	10	20	0	22	0	26	200	119	290
9	30	15	10	20	40	18	40	14	10	28	130	104	260
18	10	22	20	22	40	22	10	16	30	18	170	118	280
13	90	15	30	16	50	18	90	16	20	25	280	103	560
26	40	21	10	44	70	28	20	31	80	22	200	182	420
16	40	22	30	12	0	13	30	19	40	22	180	104	340
5	120	9	50	7	10	6	100	6	30	13	280	46	590
26	40	26	70	29	20	26	50	19	0	14	310	140	490
23	40	26	80	32	60	23	0	23	110	36	1,340	163	1,630
15	110	16	50	18	90	13	100	8	50	10	670	80	1,070
15	90	31	160	18	40	29	160	18	70	18	1,080	129	1,600
17	150	20	50	28	30	36	10	41	40	24	260	166	540
12	230	13	70	13	110	15	40	16	60	23	1,360	92	1,870
18	750	20	110	22	110	35	260	28	90	35	900	158	2,220
20	230	24	180	26	300	20	200	14	60	7	620	111	1,590
21	490	29	250	19	180	17	120	22	100	20	840	128	1,980
387	4,320	438	1,910	473	1,680	447	1,710	417	1,410	474	12,100	2,636	23,130
Eggs/ml.	11.2		4.4		3.5		3.8		3.4		25.5		8.8
% eggs	18.7		8.2		7.3		7.4		6.1		52.3		100.0

Table II

SERIAL URINE SPECIMENS: HATCLIFFE SCHOOL
10.30 Hours, 18th October, 1966

SERIAL URINE CONTAINERS 1		2		3		4		5		6		TOTAL	
Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs
5	180	12	300	14	140	25	120	17	120	13	960	86	1,820
15	10	15	0	13	0	23	10	24	10	33	10	123	40
5	180	5	10	6	90	—	—	6	80	1	80	23	440
1	20	3	70	—	—	—	—	1	30	3	250	8	370
4	70	4	50	—	—	—	—	—	—	2	590	10	710
4	580	12	200	—	—	—	—	16	300	3	1,480	35	2,560
34	1,040	51	630	33	230	48	130	64	540	55	3,370	285	5,940

The results indicate that such exercise immediately prior to micturition has no influence on the total numbers of eggs recovered from the specimens, but it has an influence on the pattern of egg release within the urine stream. It would seem that the violent exercise does stimulate more eggs to pass through the bladder wall into all levels of urine in the bladder, thus leaving a smaller proportion of eggs to be passed in the final container.

4. *Diurnal Patterns of Egg Release*

As stated previously, the pattern of egg passage within the micturition specimen was characteristic in that more eggs were found in the first and, particularly, the last fractions of the specimen than in the intermediate portions.

Comparisons were made of these patterns in urine samples taken at different times of the day, and after varying periods between micturitions. The results have already been presented in Tables III, IV and V and Figs. 2 and 3.

Table II and Fig. 2 give the results of serial fractions of urine specimens taken each hour of the day from 0800 to 1600 hours. When providing specimens hourly, the high frequency of micturition and the consequent low volume of each specimen made it difficult for the subject to divide his specimen over all six containers. Therefore, regardless of the number of containers used for each specimen, the first and last fractions were retained as separate counts, and the counts of all mid-stream fractions were combined. Thus three counts were recorded for each micturition—the first fraction, the mid-stream fraction and the terminal fraction.

Table IV and Fig. 3 present the results of urine specimens taken at two-hour intervals from 0800 to 1600 hours, and Table V presents the three-hour intervals from 0900 to 1500 hours. Since the volume of each specimen was sufficient for the subject to distribute the fractions of each specimen over all six containers on most occasions, all egg counts are given.

The following conclusions are apparent from these results:

(i) Regardless of the frequency of micturition, there is a characteristic pattern of egg production through the day, with the peak of production around midday (see Table IX and Fig. 4). This confirms the reports of Bennie (1949) and Jordan (1963).

(ii) The patterns of egg release within the individual urine specimen vary during the day. This is best illustrated in Fig. 2, where it is seen that, before the peak of total egg production at midday, the number of eggs in the first fraction tends to exceed the numbers in the last fraction of each specimen. However, at the peak at midday the last portion exceeds the first in egg numbers. The V-shaped figure representing the early samples (as opposed to the J-shape of the graphs of the later samples) only appears to occur when the total number of eggs in a specimen is low, regardless of the volume of the sample. Thus, if only few eggs are found, even in a small volume specimen, the pattern of release will be in the form of a "V." However, if a larger number of eggs are passed, even in a very large volume specimen, the greater number of those eggs will be passed in the terminal portion of the specimen and a J-shaped graph will result. For this reason the "V" patterns are only apparent in the one-hour interval specimens before and after the peak. All other specimens,

Table III

Pattern of Egg Output: Hourly Specimens
0800 to 1600 Hours (Four Days)

Hour	No. of Samples	First Container			Middle Containers			Last Container			TOTAL		
		Vol.	Eggs	E/ml.	Vol.	Eggs	E/ml.	Vol.	Eggs	E/ml.	Vol.	Eggs	E/ml.
0800	4	65	20	0.3	293	80	0.3	117	630	5.4	475	730	1.5
0900	1	7	60	8.6	25	10	0.4	12	90	7.5	44	160	3.6
1000	4	38	350	9.2	52	160	3.1	62	520	8.4	152	1,030	6.8
1100	3	22	230	10.5	104	260	2.5	41	400	9.8	167	890	5.3
1200	3	26	570	21.9	116	480	4.1	38	1,010	26.6	180	2,060	11.4
1300	3	38	570	15.0	104	270	2.6	57	1,290	22.6	199	2,130	10.7
1400	4	65	950	14.6	273	330	1.2	82	1,080	13.2	420	2,360	5.6
1500	4	41	460	11.2	173	190	1.1	70	670	9.6	284	1,320	4.6
1600	3	31	210	6.8	126	180	1.4	60	370	6.2	217	760	3.5

Table IV

Pattern of Egg Output: Two-Hourly Specimens 0800 to 1600 Hours (Five Days)

	Serial Urine Containers												Total		
Hour	1		2		3		4		5		6				
	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	E/ml.
0800	64	210	81	120	74	20	79	70	70	40	127	420	495	880	1.8
1000	50	270	70	130	47	120	44	90	37	50	55	640	303	1,300	4.3
1200	57	550	77	280	60	90	57	150	62	250	88	2,260	401	3,580	8.9
1400	71	1,250	110	310	107	240	96	280	96	210	105	3,550	585	5,840	10.0
1600	52	1,200	74	350	65	130	56	250	65	230	86	1,950	398	4,100	10.3
Total	294	3,480	412	1,190	353	600	332	840	330	780	461	8,820	2,182	15,700	7.2
Eggs/ml.	11.8		2.9		1.7		2.5		2.4		19.1		7.2		

Table V

Pattern of Egg Output: Three-Hourly Specimens (Three Days)

	Serial Urine Containers												Total		
Hour	1		2		3		4		5		6				
	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	E/ml.
0800	36	110	47	60	56	70	35	40	50	70	55	140	279	490	1.8
0900	22	110	24	50	—	—	—	—	6	20	41	230	93	410	4.0
1200	65	390	67	190	66	180	71	110	62	100	96	2,370	427	3,340	7.8
1500	53	490	54	250	65	180	59	150	63	190	80	2,400	374	3,660	9.8
1600	29	740	51	120	59	130	38	90	18	70	54	520	249	1,670	6.7
Total	205	1,840	243	670	246	560	203	390	199	450	326	5,660	1,422	9,570	6.7
Eggs/ml.	9.0		2.8		2.3		1.9		2.3		17.4		6.7		

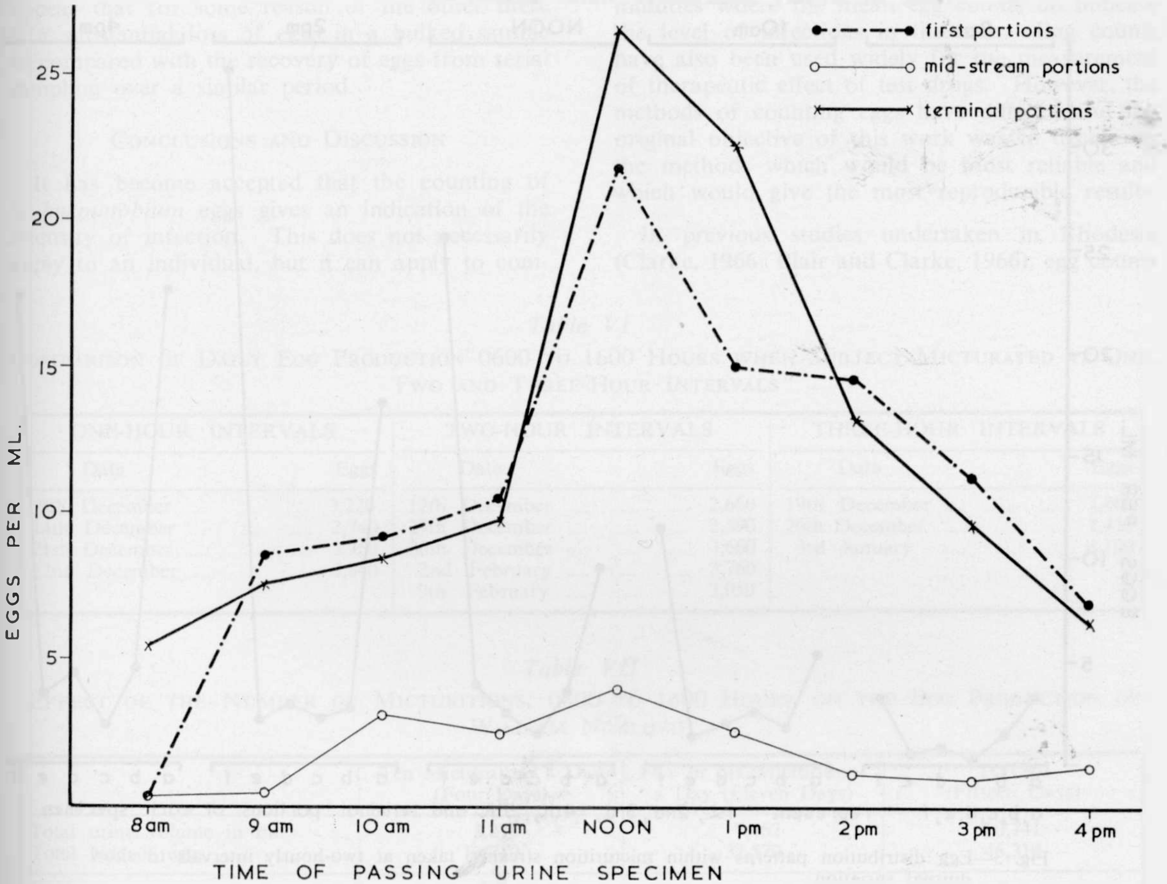

Fig. 2—Egg distribution patterns within micturition streams, taken at one-hourly intervals, comparing first, mid-stream and terminal portions to show diurnal variations.

including the peak one-hour specimens, all two and all three-hour specimens, and all random specimens, show the "J" pattern of distribution.

(iii) The increased number of eggs passed in the midday hours are released, particularly in the terminal portion of the urine.

(iv) In the volunteer subject there is a diurnal pattern of terminal haematuria which did not appear to be directly related to the numbers of eggs passed. It was observed that frank haematuria occurred only in the afternoons, and it even increased in extent as the egg output decreased. Only in the specimens passed at one-hour intervals was frank haematuria observed at midday. It was never seen in the mornings.

(v) In the specimens passed early in the morning (about 0600 hours) it was noted that, although very few eggs were passed, the majority of these were found in the first portion of the urine.

5. *The Comparison of Egg Recoveries from Bulked and Serial Sampled Urines*

There are many people who believe that the examination of a single micturition specimen, even when taken under optimum conditions at the period of maximum egg output, about midday, is of less value in establishing a diagnosis than the examination of a 24-hour sample of urine. Twenty-four-hour sampling of urine is particularly popular in the more sophisticated hospital practice where patients are admitted to the wards in order to undergo a battery of diagnostic procedures. On the face of it, it is rea-

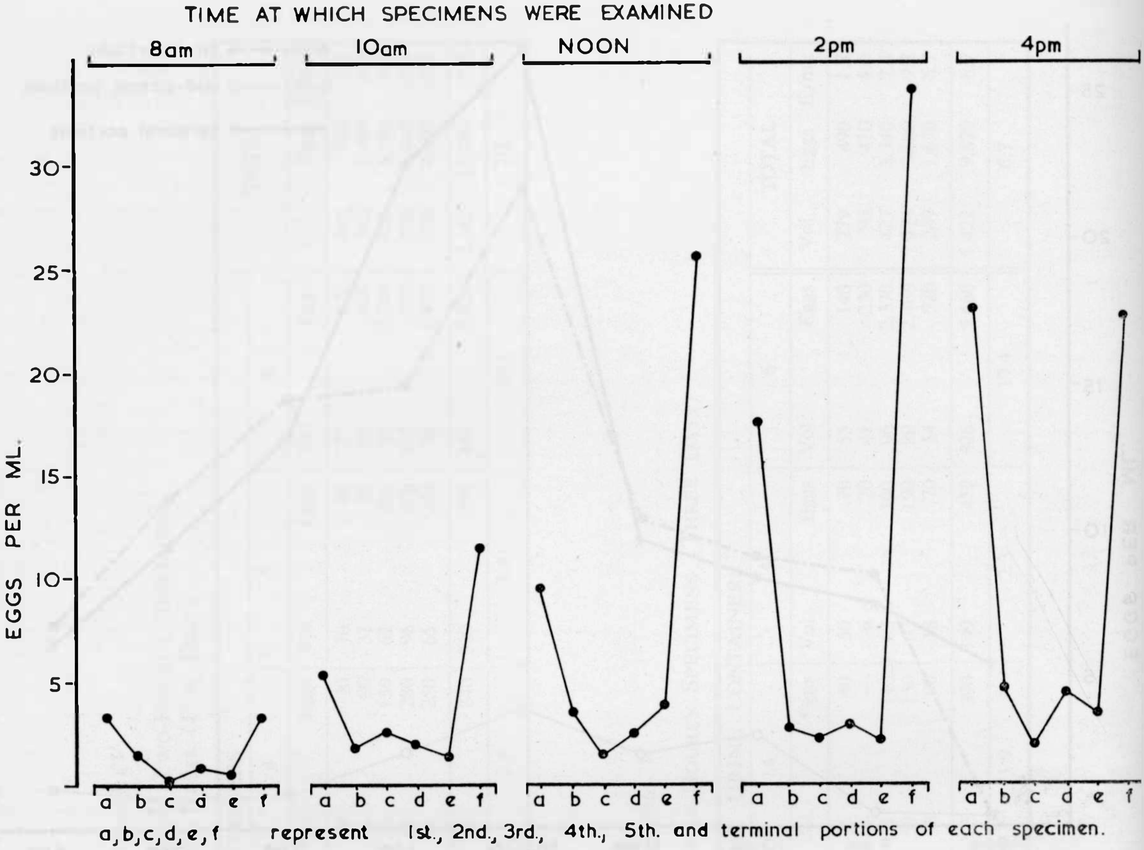

Fig. 3—Egg distribution patterns within micturition streams, taken at two-hourly intervals to show diurnal variation.

sonable to suppose that if a patient provides a 24-hour specimen of urine it should be possible to recover for microscopic examination all the schistosome eggs in the sample.

To test this view the volunteer was asked to bring with him to work the usual 0600 hours urine sample which was put into a screwcapped wide-mouth jar. Whenever he wished to pass urine he did this directly into the jar and noted the time on the label. At about 1600 hours he was requested to empty his bladder into the jar, the whole contents of which were poured into a 1-litre measuring cylinder, and the total bulked volume of urine was measured. The screw-capped jar was rinsed out with physiological saline and the washings added to the measuring cylinder; it was then left overnight to sediment. In some instances 15 ml. samples were withdrawn by pipette from various levels of the urine column. These samples showed that no eggs were remaining in the upper levels of the column.

In all bulked samples the supernatant urine was drawn off to leave 100 ml. containing the sediment in the bottom of the cylinder. This was agitated and transferred to a smaller bottle and left for a further 30 minutes to sediment. Thereafter this remaining urine was treated in the same way as were the 0600-hour and serial samples described previously. During the weeks when bulk samples were being taken, on occasional days two-hourly serial samples were carried out in order to be certain that the egg output was not showing marked variations from day to day. The results of this study are set out in Table X. This shows a marked discrepancy between the number of eggs recovered from bulked urines and the sum of the number of eggs recovered from the individual serial two-hour urine studies. Recoveries of eggs from the sum of a day of serial urines was three to ten times the number of eggs recovered from the bulked urines of the same hours of the day. The significance of these observations will be discussed later, but it would appear that for some reason or the other there is a substantial loss of eggs in a bulked sample as compared with the recovery of eggs from serial sampling over a similar period.

CONCLUSIONS AND DISCUSSION

It has become accepted that the counting of *S. haematobium* eggs gives an indication of the intensity of infection. This does not necessarily apply to an individual, but it can apply to communities where the mean egg counts do indicate the level of infections in the area. Egg counts have also been used widely for the measurement of therapeutic effect of test drugs. However, the methods of counting eggs have differed and the original objective of this work was to decide on the methods which would be most reliable and which would give the most reproducible results.

In previous studies undertaken in Rhodesia (Clarke, 1966; Blair and Clarke, 1966), egg counts

Table VI

COMPARISON OF DAILY EGG PRODUCTION 0600 TO 1600 HOURS WHEN SUBJECT MICTURATED AT ONE, TWO AND THREE-HOUR INTERVALS

ONE-HOUR INTERVALS		TWO-HOUR INTERVALS		THREE-HOUR INTERVALS	
Date	Eggs	Date	Eggs	Date	Eggs
9th December	3,220	12th December	2,660	19th December	3,060
14th December	2,740	29th December	2,590	20th December	2,410
21st December	3,350	30th December	3,660	3rd January	4,100
22nd December	3,840	2nd February	3,760		
		9th February	3,050		

Table VII

EFFECT OF THE NUMBER OF MICTURITIONS, 0600 TO 1600 HOURS, ON THE EGG PRODUCTION OF WILLIAM NDHLOVU

	Ten Micturitions a Day (Four Days)	Five or Six Micturitions a Day (Eleven Days)	TOTAL (Fifteen Days)
Total urine volume in ml.	3,279	7,462	10,741
Total No. of eggs	13,740	32,570	46,310
Eggs/ml.	4.2	4.4	4.3
Average daily volume of urine in ml.	820	678	716
Average daily egg output	3,435	2,961	3,087

Table VIII

EGG OUTPUT PATTERN IN SIX SEPARATE MICTURITIONS AFTER VIOLENT EXERCISE (FIFTY "KNEES-UP")

SERIAL URINE CONTAINERS													
1		2		3		4		5		6		TOTAL	
Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs	Vol.	Eggs
13	180	25	260	20	130	25	220	6	160	14	140	103	1,090
9	160	6	100	15	280	15	100	12	100	20	280	77	1,020
27	260	27	80	27	100	29	10	37	160	27	280	174	890
12	170	20	180	21	250	24	280	24	180	30	1,280	131	2,340
15	110	18	40	22	190	20	20	26	30	25	630	126	1,020
15	100	18	20	24	40	24	30	25	40	32	360	138	530
91	980	114	680	129	990	137	660	130	670	148	2,970	749	6,890
Eggs/ml.	10.8		6.0		7.7		4.8		5.2		20.1		9.2
% Eggs	19.8		11.0		14.1		8.8		9.5		36.8		100.0

were related to the time that urine was retained in the bladder, the volume of urine being ignored. Thus a patient was made first to empty his bladder and then a second urine specimen was collected for examination after exactly one hour. This method was selected because it was believed that the rate of release of eggs into urine of the bladder is not governed in any way by the degree of renal activity. Workers, particularly in East Africa, have used a different method. A 10 ml. sample of the urine has been examined and the egg count, from this sample, is accepted for comparative purposes; alternatively, this count has been related to the total volume of the specimen as an aliquot. By using this technique the inference is drawn that the number of eggs released into the lumen of the bladder is directly related to the volume of urine passed. In both cases the assumption is made that, within reasonable limits, the total egg output is constant from day to day.

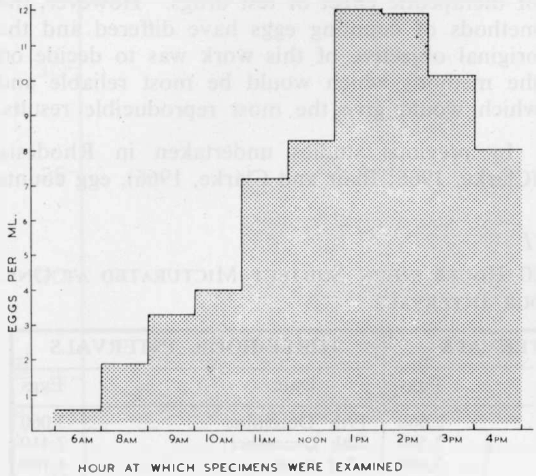

Fig. 4—Diurnal pattern of egg passage.

The results demonstrate that the most reliable and reproducible method of egg counting is by taking a specimen of known duration. The total urine output from day to day was found to be

Table IX

DIURNAL PATTERN OF EGG PRODUCTION

Hour of Passing Specimen	No. of Observations	Total Volume of Urine in ml.	Total No. of Eggs	Average per Specimen		Average Eggs per ml.
				Vol.	Eggs	
0600	15	3,521	2,260	235	151	0.6
0800	21	2,140	4,170	102	198	1.9
0900	12	701	2,340	58	195	3.3
1000	17	1,421	5,710	84	336	4.0
1100	9	753	5,440	84	604	7.2
1200	18	1,859	15,440	103	858	8.3
1300	10	797	9,550	80	955	12.0
1400	15	1,785	21,310	119	1,421	11.9
1500	14	1,565	15,850	112	1,132	10.1
1600	16	1,445	11,530	90	721	8.0

This table reflects the results of all observations; serial urine studies and random samples obtained at various times during the day from 30th October, 1966, to 3rd January, 1967.

Table X

EGG OUTPUT ON BULKED URINE (0600 TO 1600 HOURS) COMPARED WITH DAILY EGG COUNTS BASED ON INDIVIDUALLY EXAMINED TWO-HOURLY SAMPLES

Date	Bulk *or* 2-hour Samples	No. of Micturitions	Urine Volume in ml.	Total Egg Count	Eggs per ml.
24th Jan.	Bulk	4	540	480	0.9
26th Jan.	Bulk	4	640	840	1.3
30th Jan.	Bulk	5	950	470	0.5
*31st Jan.	*2-hourly*	6	524	1,470	*2.8*
2nd Feb.	*2-hourly*	6	536	3,880	*7.2*
3rd Feb.	Bulk	5	610	410	0.7
7th Feb.	Bulk	4	560	250	0.4
9th Feb.	*2-hourly*	6	560	3,160	*5.6*

* Patient had headache and pains in joints.
Mean daily egg count—bulked urine = 490
Mean daily egg count—2-hourly urine = 2,837

Table XI

Pattern of Egg Output: 0600 to 1600 Hours
(Fifteen Days Over a Two-Months Period)

Date	0600 Hours		0800-1600 Hours		0600-1600 Hours		No. of Micturitions	Average Eggs/ml.
	Vol. ml.	Eggs	Vol. ml.	Eggs	Vol. ml.	Eggs		
1966								
9th Dec.	205	100	323	3,220	528	3,320	10	6.3
12th ,,	281	260	489	2,660	770	2,920	6	3.8
13th ,,	207	290	512	2,460	719	2,750	5	3.8
14th ,,	292	320	613	2,740	905	3,060	10	3.4
16th ,,	132	80	410	1,700	542	1,780	5	3.3
19th ,,	303	310	487	3,060	790	3,370	6	4.3
20th ,,	253	60	494	2,410	747	2,470	6	3.3
21st ,,	294	120	702	3,350	996	3,470	10	3.5
22nd ,,	168	60	682	3,840	850	3,900	10	4.6
29th ,,	312	100	582	2,590	894	2,690	6	3.0
30th ,,	109	130	470	3,660	579	3,790	6	6.5
1967								
3rd Jan.	360	180	441	4,100	801	4,280	6	5.3
31st Jan.	96	0	428	1,470	524	1,470	6	2.8
2nd Feb.	165	120	371	3,760	536	3,880	6	7.2
9th Feb.	300	110	260	3,050	560	3,160	6	5.6
Total (15 days)	3,477	2,240	7,264	44,070	10,741	46,310	—	4.3

variable and it is dependent on such factors as fluid intake, climate, extent of physical exercise. In these trials it was found that the total daily egg production was remarkably constant (Table XI). There are grounds for the belief that the numbers of eggs released daily from the bladder follow a cyclical pattern with large daily counts for periods of several weeks, followed by similar periods of counts which are much lower. However, from day to day the counts were remarkably constant, which is not in agreement with the findings of Gerritsen *et al.* (1953), who found that the number of eggs in consecutive 12-hour collections were subject to enormous variation. In a series of examinations one of their patients passed 85,000 eggs in the first 12-hour period, but during the second, sixth and eighteenth 12-hour periods was almost egg-free. The authors, however, do not state whether the majority of eggs seen were living or dead. It is well known that a patient suffering from a chronic infection may at times shed enormous numbers of dead, generally black, eggs into the urine, which at times quite overshadow the much smaller and steadier number of viable (and hatchable) eggs that the patient may be passing at the same time.

It is seen that even the taking of aliquot samples is unreliable if they have to be related to urine volume. The acceptance of an egg count from a 10 ml. sample which is not related to total volume is even less acceptable.

Thus some elucidation of the problems which were the primary objective of the investigations was possible. However, the greater part of the interest of the work was additional to this information.

The pattern of egg production in the micturition stream is of great importance in individual diagnosis. The results showed that over 50 per cent. of eggs passed by the subject were passed in the terminal portion of the urine, a portion amounting to less than one-fifth of the total specimen. It is seen, therefore, that it would be of greater diagnostic significance to examine the terminal portion than to examine the total specimen if the standard techniques of examination are employed. This is because the terminal portion will have an egg density which is much higher than the egg density of the total specimen.

If the terminal portion is examined, and if the specimen is passed between the hours of 1100 and 1300 hours, the best diagnostic result will be obtained.

It seems useful to speculate on an explanation for the J or V distribution of eggs in the serial fractions of a single micturition stream. These eggs do not seem to make their way into the urine at a steady rate. If this was so, as the bladder filled with urine so would all layers of urine in the bladder have their quota of eggs. Attention has been drawn to the fact that the 0600 hours urination contains twice the number of eggs per ml. in the first urine passed as compared with the second portion. It may be that these few eggs represent the eggs in the bladder residual urine remaining from the usual middle of the night micturition. At 0800 hours there are few eggs in the first portion, just as there were few in the last portion of the 0600 hours specimen. During the morning, eggs in the submucosa are expelled by muscular action, at the end of each micturition, into the urine. Most of these eggs pass out of the bladder into the last of the serial containers, but some remain in the small amount of urine remaining inside the internal urethral sphincter. The gradual expansion of the bladder to accommodate the urine coming down steadily from the kidneys does not seem in any way to encourage the eggs to pass out of the submucosa into the urine during the filling process. Presumably the bladder is accommodated to the volume of urine present, so there is little mixing of the layers of urine as the bladder fills. When required, he starts the act of micturition. The first urine to pass out is the residual urine containing eggs and red blood cells from the previous micturition, which remained as dregs in the bladder. The next portions of urine, those usually collected in second to fifth containers, are from the main body of the bladder. This urine contains only few eggs and red blood cells. When the bladder has been nearly emptied by involuntary muscle action, the voluntary musculature of the lower abdomen and perineum comes into action to express the last portion of urine in the bladder. It appears that when these forces come into play, large numbers of eggs are squeezed from the mucosa into the lumen of the bladder.

A study of the results of serial urine specimens taken after violent exercise does seem to show that it is possible to force eggs into the urine in a full bladder at all levels. When the bladder is emptied these eggs pass out in their particular urine level, leaving a lesser number of eggs remaining in the submucosa to be expressed into the final container. A comparison of the proportions of eggs passed into the six containers, with and without prior exercise (Table I with Table VIII) shows that there is a close approximation of the proportion of eggs in first containers; that 20 per cent. of the eggs in the ordinary urinations passed out in mid-stream, whereas after violent exercise 43 per cent. of the total eggs were in this portion; and that after exercise only 37 per cent. were passed out in the final containers as compared with 52 per cent. in the ordinary samples taken without previous exercise.

A study of the quantities of blood in the urine, both macroscopic and microscopic, showed that the quantity of blood shed into the urine was not closely related to the number of eggs passed. In the case of the volunteer subject studied, it was clear that the amount of blood in micturitions before midday was much less than in samples passed in the afternoon. In fact, red blood-stained urine was rarely observed before midday, except on a few occasions when he was passing urine at hourly intervals. At 1600 hours the terminal urine was generally heavily blood-stained, even in cases where the number of eggs seen was much less than in the first portion passed.

The comparison of egg counts in bulked urine as compared with the immediate processing and examination of each specimen of urine when passed produced most surprising results. These are set out in Tables X and XII. Table X shows that on days when the urine was bulked and examined as one examination, the eggs counted were less than one-fifth the number seen on each of three days during the same period when two-hourly serial sampling was carried out. On 31st January, 1967, the subject was not feeling well and only 1,470 eggs (2.8 eggs per ml.) were recovered in the two-hour serial sampling, but even this was more than twice as many eggs per ml. than were found in any of the bulked urine samples. The reason for this great discrepancy is far from clear. It seems, however, that the subject did produce the eggs in the urine of the

Table XII

Egg Output on Bulked Urine 0600 to 1600 Hours Compared with Expected Output on Study of Individual Serial Samples

Date	Urine Volume in ml.	BULKED URINE		SERIAL SAMPLE URINE	
		Eggs	Eggs/ml.	Eggs	Eggs/ml.
February, 1967					
13th	619	510	0.8	1,770	2.9
16th	589	390	0.7	1,740	3.0
17th	531	380	0.7	2,300	4.3
TOTAL	1,739	1,280	0.7	5,810	3.3

bulked sample, but for some reason the examination of the bulked urine does not recover all the eggs. Table XII sets out the results of a trial to compare egg output in bulked urine with the expected output of eggs on a study of individual serial samples. On 13th, 16th and 17th February the volunteer passed urine at his own volition into the serial containers. Egg counts were made in the usual fashion, but instead of discarding supernatant urine from containers and centrifuge tubes, everything other than the 0.05 ml. of urine sediment on which the egg count was carried out was put into the bulked urine container.

At first it was thought that perhaps sedimentation of eggs in a one-litre measuring cylinder in a column of urine about 20 cm. deep might result in eggs being held up in the upper layers of urine. Withdrawal of 15 ml. samples of urine from the measuring cylinder at various levels showed, however, that no eggs were to be found in the upper layers. Saline washings of the measuring cylinder to dislodge any eggs which might have become stuck to the side walls of the cylinder gave no evidence of such adherence. To test this possible explanation further, on three other days the urine passed was bulked in a one-litre measuring cylinder and allowed to sediment overnight. Next morning the urine was drawn off carefully to 100 ml. levels and each 100 ml. of urine placed in a jar to sediment for a further 30 minutes. This procedure went further than taking a 15 ml. sample at each level and counting the eggs in the aliquot sample. The results of this are shown in Table XIII, which shows that in a tall column of urine sedimentation of eggs may be slow. However, even if the eggs counted in levels above 100 ml. are included, the average eggs recovered in the bulked samples were 0.9, 0.8 and 0.7 eggs per ml. respectively—far short of the egg recoveries achieved in a single specimen of urine taken at the peak of diurnal egg production.

Bulked urine samples in wide, shallow containers demonstrated a similar loss in egg recovery.

Another possibility was that urine passed at different times of the day was of varying specific gravity, and eggs passed in the first half of the period in urine of higher specific gravity were hatched by the subsequent admixture with urine of lower specific gravity. The evidence of miracidia and empty shells, though looked for very carefully, may have been destroyed by the time the bulked urine was processed and examined. Yet another possibility was that the subject's urine contained considerable numbers of red blood cells which must encourage bacterial growth in the urine. This might have led to the dissolution of the eggs in the bulked urine.

Whatever the reason for the lack of recovery of eggs in the bulked sample, it is true to say that on each of the three days named in Table XII the subject passed twice as many eggs in the terminal portion of urine at or near midday than were found in the whole bulked 10 hours of urine production. This problem remains under investigation, but the results already demonstrate the lack of value of the favoured "24-hour specimen" in individual diagnoses.

Summary

(1) An apparatus is described which allows the study of schistosome eggs in serial fractions of a micturition stream, which can be passed without any interruption of the stream.

(2) Studies have been undertaken on the pattern of egg release in specimens taken at different times of the day, at differing intervals between micturitions and with or without physical exertion prior to micturition.

(3) Within any one micturition stream eggs are released in a characteristic pattern, with the majority of the eggs in the first and particularly the last fractions of urine and few in the intermediate fractions.

(4) The number of eggs in the first fraction of a micturition stream is apparently related to the number of eggs in the last fraction of the previous micturition.

Table XIII

Sedimentation of Eggs in Bulked Urine Samples in a One-Litre Measuring Cylinder

Date	Total Volume of Urine in ml.	EGGS COUNTED IN 100 ML. LEVELS				
		Bottom 100 ml.	101-200 ml. Level	201-300 ml. Level	301-400 ml. Level	401 ml. Level and Above
22.2.67	905	380	140	180	100	0
23.2.67	726	370	210	30	0	0
24.2.67	970	580	130	0	0	0

(5) The diurnal pattern of egg release into the urine is clearly shown.

(6) The daily egg output remained remarkably constant over a period of four months.

(7) The presence of frank blood in the urine is only evident in the last fraction of a micturition stream and then only in urine passed after midday. The amount of blood in any specimen is not directly related to the number of eggs released.

(8) More frequent micturitions through the day does not seem to increase the total number of eggs released as compared with less frequent micturition schedules.

(9) Violent exercise involving the muscles of the hips and lower abdomen carried out immediately before a micturition does not increase the total number of eggs in the urine, but does tend to distribute a proportion of the eggs normally found in the last fraction into the intermediate fractions. It would appear, therefore, that exercise prior to micturition does not assist in achieving a higher egg recovery for diagnosis.

(10) Comparison of egg recoveries from bulked urine (0600 to 1600 hours) with eggs seen in examinations of fractionated portions of an individual micturition over the same time period show that over three-quarters of the eggs are lost in the bulked sample. In fact, a single specimen passed between 1200 and 1400 hours will produce far more eggs than can be recovered from the whole bulk sample. Serious doubts are cast on the value of the common diagnostic practice of examinations of urinary deposits from bulked 24-hour specimens of urine.

(11) Certain speculations are advanced on the importance of the voluntary muscular action of the perineal and abdominal muscles in expressing schistosome eggs from the bladder wall into the terminal fraction of urine of a micturition.

(12) The suggestion is made that a more accurate diagnosis of urinary bilharziasis can be made and the intensity of the infection better estimated by examining a specimen of urine collected between 1100 and 1400 hours, after the bladder has been emptied of urine one hour previously. This results in the maximum number of eggs in a small volume of urine. The uselessness of "early morning" specimens for diagnosis of bilharziasis is demonstrated.

(13) The abstraction of a 10 or 15 ml. sample from a urine specimen, sedimenting such a sample and examining the deposit for eggs, is a most ineffective method of examination and produces much less consistent results than the study of the sedimentation of a one-hour specimen of urine taken at or near peak of egg production.

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