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Circadian variation of ova excretion, proteinuria, hematuria, and leukocyturia in urinary schistosomiasis

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Circadian variation of ova excretion, proteinuria, hematuria, and leukocyturia in urinary schistosomiasis. Urine samples from five boys (7 to 9 years) with urinary schistosomiasis were collected at 6 A.M. and thereafter at 3-hr intervals until 9 P.M. on 5 consecutive days. Ova excretion in the urine, proteinuria (PU), erythrocyturia (EU), and leucocyturia (LU) were assessed quantitatively. Egg excretion followed a circadian rhythm with a peak at 12 noon and was paralleled closely by pathological PU. Maximal erythrocyturia occurred at 6 P.M., whereas leucocyturia revealed two distinct peak times. Taking the congruent patterns of egg excretion and PU together with the results of qualitative urinary protein analysis into account, it was concluded that PU was linked causally to ova excretion and could be explained by bleeding and exudation of serum proteins during penetration of ova through the bladder mucosa. In contrast, EU and LU seemed to be caused by different pathological mechanisms. EU followed a time-delayed circadian rhythm, possibly induced by persistent bleeding, whereas LU may have indicated a concomitant inflammatory component of the bladder.

Variation circadienne de l'excrétion d'oeufs, de la protéinurie, de l'hématurie et de la leucocyturie au cours de la schistosomiase urinaire. Des échantillons urinaires de cinq garcons (7 à 9 ans) atteints de schistosomiase urinaire ont été collectés à 6 A.M. du matin, puis toutes les trois heures jusqu'à 9 P.M. du soir pendant 5 jours consécutifs. L'excrétion d'oeufs dans les urines, la protéinurie (PU), l'érythrocyturie (EU), et la leucocyturie (LU) ont été déterminées quantitativement. L'excrétion des oeufs suivait un rythme circadien avec un pic à midi et était parallèle étroitement à une PU pathologique. L'érythrocyturie maximale survenait à 6 P.M. du soir, tandis que la leucocyturie présentait deux pics temporels distincts. En prenant en compte les aspects concordants de l'excrétion d'oeufs et la PU en même temps que les résultats de l'analyse qualitative des protéines urinaires, on a conclu que le PU était liée de facon causale à l'excrétion d'oeufs et qu'elle pouvait être expliquée par un saignement et une exsudation de protéines sériques pendant la pénétration des oeufs à travers la muqueuse vésicale. A l'opposé, EU et LU semblaient dues à des mécanismes pathologiques différents. EU suivait un rythme circadien décalé dans le temps, peut-être induit par un saignement persistant, alors que LU aurait pu indiquer une composante inflammatoire vésical concomitante.

Bennie [1] first described that urine specimens from patients with urinary schistosomiasis taken in the afternoon contained more ova than those taken in the morning. Stimmel and Scott [2] then postulated a circadian rhythm of urinary egg excretion. Their findings were confirmed subsequently by several authors [3–8].

Typical symptoms of urinary schistosomiasis are proteinuria (PU), erythrocyturia (EU), and leukocyturia (LU). It has been reported in recent investigations that these urinary findings, assessed by test reagent strips, correlated well with the amount of schistosome ova excreted in the urine [9–12]. However, the etiology of the associated urinary findings remains controversial. In the case of PU, for example, renal lesions have been held responsible [13]. On the other hand, bladder disease has been said by other investigators to be a source of PU [14, 15].

The present investigations were performed to assess the origin of PU, EU, and LU.

On the basis of the characteristic circadian variation of ova excretion, the temporal relation between egg output and urinary findings was investigated quantitatively.

Methods

Study area and patients

The investigations were carried out in Heibeika, a village in the Gezira irrigated area of the Central Sudan, which is known to be hyperendemic for urinary and intestinal schistosomiasis [16]. Five boys aged between 7 and 9 years who presented a wide range of intensity of infection were selected for the study. The estimated duration of infection in these patients was 3 to 5 years, as concluded from a parallel investigation including all age groups. The patients were not enuretic according to statements from their parents. All patients received a specific treatment with Praziquantel (40 mg/kg body wt) as well as a complete tetanus vaccination at the end of the study.

The patients rose at 5.30 A.M. and were instructed to pass their first urine sample of the day 30 min later. Visual inspection of the patient beds was done every morning to determine enuresis.

Subsequently, micturitions took place every 3 hr until 9 P.M. During the whole day for 5 consecutive days, the patients were closely observed by one investigator who made certain no urine was passed between the fixed times.

The spontaneous activity during the day was probably somewhat reduced because the patients had to stay together to be observed at all times of the day. However, they participated in cooking activities and a daily soccer game around late afternoon as is the custom after a sleep period between 12 noon and 3 P.M. in that age group in the Sudan.

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Clinical and hematological investigations

The patients underwent a complete physical examination. A thick blood smear was taken for the purpose of testing for exclusion of malaria. The hematocrit count was determined using a battery-operated microhematocrit centrifuge (Compur, Munich, Federal Republic of Germany).

Parasitological technique

The number of schistosome ova in the urine was assessed by the filtration trypan blue staining technique [17]. If initially more than 500 ova per 10 ml of urine were found, only 5 ml were filtered subsequently and counted. In all other cases at least 10 ml of urine were processed.

Informed consent

During an introductory assembly the informed consent for the protocol was obtained in the Arabic language from the parents or family representatives.

Urine examinations

Erythrocytes and leukocytes in freshly voided, native urine samples were counted in a chamber (Neubauer). A phase contrast microscope was used to assess erythrocyte morphology according to the criteria published by Fairley and Birch [18]. Concurrently, urinary test reagent strip analysis was performed (Nephur®; Boehringer, Mannheim, Federal Republic of Germany). This included testing for PU, EU, LU, and nitrite.

The proportion of urine already filtered for the parasitological investigation through polycarbonate membranes (Nuclepore, Pleasanton, California, USA) was centrifuged. A 2-ml sample was deep-frozen and kept at −20°C until processed in Germany 2 weeks later. PU was determined quantitatively by the Coomassie blue dye binding test [19]. Qualitative characterization of proteins according to their molecular weight was achieved by the SDS polyacrylamide gel electrophoresis [20]. Urinary creatinine was measured using an analyser (no. 2, Beckman Ltd., Munich, Federal Republic of Germany) and a modified Jaffé technique.

Proteinuria in excess of 100 mg/liter was considered pathological. Accordingly, more than 5 erythrocytes and 20 leukocytes per microliter of urine were regarded as pathological.

Statistical evaluation

Egg counts were calculated as ova per 10 ml of urine. Daily urinary excretion of ova, PU, EU, and LU was determined by multiplying the different parameters with the corresponding micturition volumes of a sample and adding up the six periods of examination.

To assess the circadian variation of the four parameters, medians of all five patients over all days were formed for each of the six periods examined (that is, 6 A.M., 9 A.M., 12 noon, 3 P.M., 6 P.M., and 9 P.M.). Because these data combined dependent and independent values, the significance of difference was calculated for every individual patient. Accordingly, one-way analysis of variance was carried out. Thereafter, subsequent multiple testing according to the least significant difference was performed after transformation of raw data according to the formula $x' = \text{Log}(x + 1)$.

Table 1. Median ova excretion, proteinuria, erythrocyturia, leucocyturia per day for every individual patient as well as patient age, weight, and height

Patient no.	1	2	3	4	5
Age in years	8	8	9	9	7
Weight, *kg*	27	24	32	31	26
Height, *cm*	136	130	142	140	126
Ova excretion per day	675	28,841	15,152	597	2,043
Proteinuria, *mg/day*	21	152	139	33	129
Erythrocyturia, $\times 10^3$ *per day*	28,990	921,780	992,480	9,440	188,394
Leucocyturia, $\times 10^3$ *per day*	27,550	293,880	188,040	19,715	144,628

Results

Clinical and laboratory findings

Physical examination revealed no pathologic findings; above all, no enlargement of spleen or liver or, on palpation, increased consistency of the liver were found. No malaria parasites were detected in the blood, and the patients were not anemic. Also, there was no nitrite excretion in the urine at any timepoint.

Erythrocyturia exhibited the typical appearance of postrenal blood loss under phase contrast microscopy. Deformed erythrocytes indicative of transglomerular blood loss were not found.

Data analysis of individual patients

The age, weight, and height as well as parasitologic and urine analysis data of individual patients are summarized in Table 1. The median of ova output varied from 597 to 28,841 eggs per day. It can be seen that median PU, erythrocyte, and leukocyte excretion was highest in heavily infected patients.

Patterns of circadian variation

Median ova excretion, PU, EU, and LU of all five patients per time point of examination over 5 days were calculated. Ova excretion followed a circadian rhythm with a peak at 12 noon (Fig. 1). Egg counts in the afternoon were similar to those in the midmorning (9 A.M.) but only about one third when compared with the egg count at noon.

When the total number of ova excreted every 3 hr and during the night period were calculated, the peak at noon became even more pronounced (Fig. 2). From 9 A.M. to 12 noon egg excretion was 1,210 as compared to 974 during the rest of the day.

The results of one-way variance analysis and subsequent multiple testing revealed significant circadian variations of ova output, PU, EU, and LU in each individual patient ($P < 0.05$).

Median PU at 6 A.M. and 9 P.M. showed physiological values (Fig. 3), although median ova excretion was 9.2 and 23 per 10 ml, respectively. At all other periods there was a markedly pathologic PU which closely paralleled the circadian rhythm of ova output.

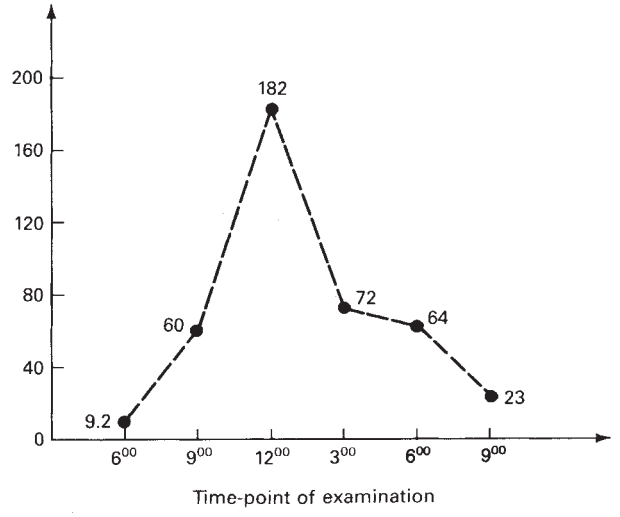

Fig. 1. *Polygone indicative of the median number of ova output per 10 ml of all five patients at each period of examination over 5 days.* Ova excretion per 10 ml of urine is symbolized by ●---●.

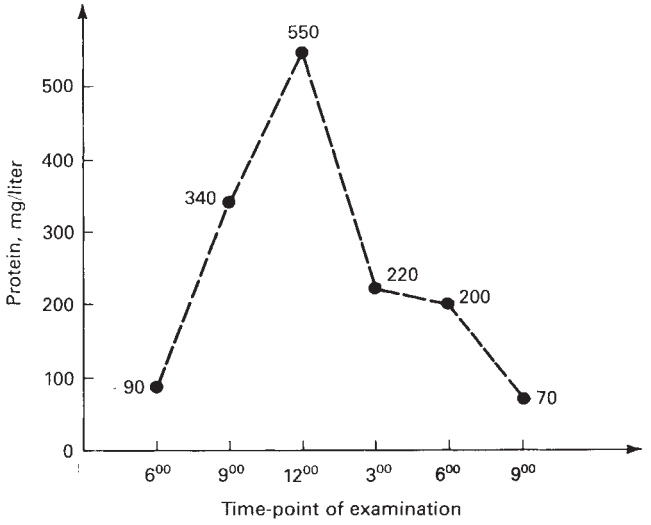

Fig. 3. *Circadian rhythm of proteinuria (PU) as medians of all five patients at each period of examination for 5 days.*

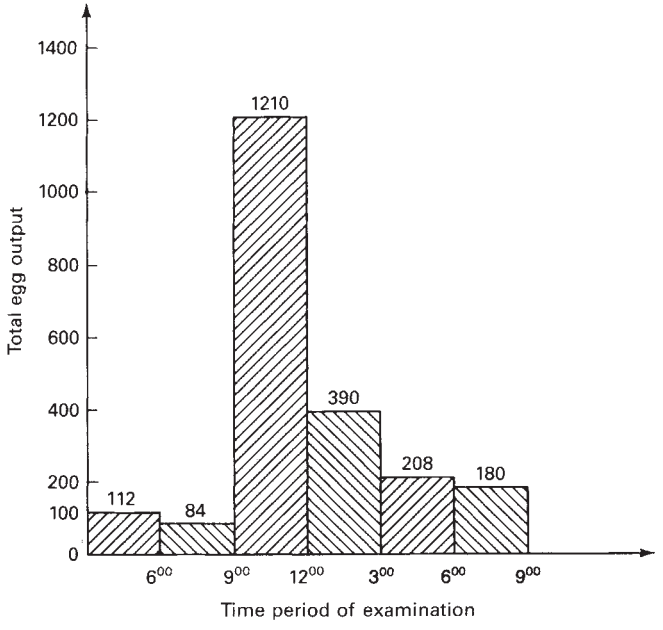

Fig. 2. *Total amount of ova excreted over the night between 9 P.M. and 6 A.M. as well as at every consecutive 3-hr period over the day.* The *bars* represent the median number of ova per collection period for all five patients for 5 days.

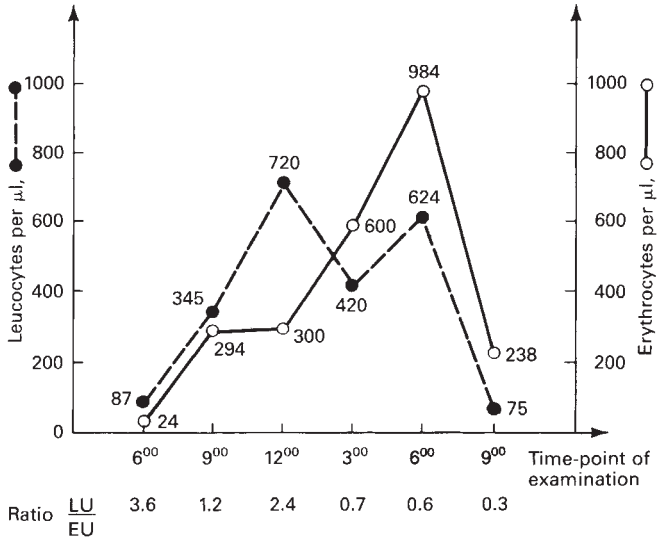

Fig. 4. *Circadian variations of erythrocyturia (EU) and leucocyturia (LU) as medians of all five patients per microliter of urine for 5 days.* The ratio between LU and EU is given for each period examined.

SDS polyacrylamide gel electrophoresis showed a protein pattern consisting mainly of albumin, transferrin, and IgG as it is typically found in the serum. No low-molecular proteins such as β_2 microglobulin were detected. This pattern remained unchanged during all periods of the day.

The circadian variations of EU and LU are depicted in Figure 4. LU exceeded EU in the first half of the day (until 12 noon). In contrast, the reverse was found to be the case in the afternoon and evening with EU exceeding LU. Maximal erythrocyte excretion occurred at 6 P.M.; it was three times higher than at 12 noon. Accordingly, the ratio between LU and EU except for 12 A.M. constantly decreased over the day from 3.6 at 6 A.M. to 0.3 at 9 P.M.

The circadian variation of LU showed two peaks, one at 12 noon and the other at 6 P.M. However, the increase of LU in the morning and the decrease in the evening were similar to the diurnal variations in egg excretion.

Influence of urine concentration

To indicate the degree of urine concentration, the median micturition volume of samples and median urinary creatinine concentration at given periods was calculated (Fig. 5). In the early morning the micturition volume was 195 ml (22 ml/hr) after a 9-hr sleep. Correspondingly, the creatinine concentration was low. During the day an almost constant urine volume of about 40 ml per voiding (13 ml/hr) was reached. This was

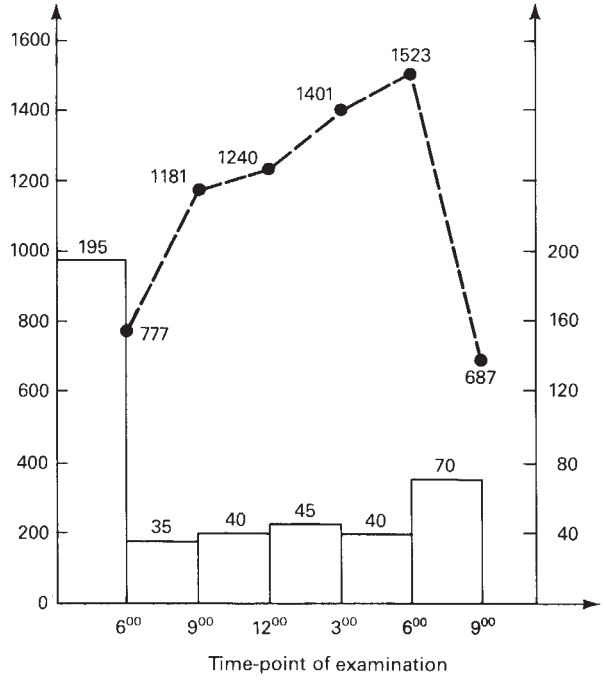

Fig. 5. *Median urine volume of micturition samples and creatinine concentration of each sample from five patients for five days.* Symbols are: □, milliliter of urine and ●----● for micromoles per liter of creatinine.

paralleled by a steady increase in urinary creatinine concentration until 6 P.M.

Discussion

The circadian rhythm of schistosome ova excretion in the urine, as previously reported from different geographical areas, for example, Britain [21], Egypt [2], and East and West Africa [22, 23] was confirmed clearly by our data. Various hypotheses have been offered to explain the diurnal variation of egg excretion in urinary schistosomiasis. Most authors agree that physical activity of the host [6, 21] rather than an intrinsic rhythm of the parasite may be causative.

With ambient noon temperatures of about 40°C at the time when the present experiments were conducted, it seems conceivable that peak ova concentration at noon was a mere expression of antidiuresis. However, egg excretion increased fifteenfold between 6 A.M. to 12 noon, whereas only a twofold increase in urinary creatinine concentration was observed during the same period. Thus, our data prove that the circadian variation in ova excretion cannot merely be explained by an effect of urinary concentration around midday, which agrees with findings by Dukes and Davidson [5].

Most authors have agreed previously that maximal egg concentration occurs between 12 noon and 3 P.M. [2, 5, 24, 25]. According to our findings reported here and elsewhere [8], maximal ova output was found around 12 noon. The varying results could be explained by the fact that we examined boys between 6 and 9 years of age, and that peak ova output in older patients may take place later in the afternoon as previously suggested by Bradley [23].

It is still a controversial matter as to whether PU in urinary schistosomiasis indicates a glomerular lesion [13, 26], nephritic disease [27–29], or merely a protein loss through the bladder wall [14, 15]. As discussed in more detail elsewhere (Doehring, Ehrich, Vester, Feldmeier, and Brodehl, unpublished observations), the qualitative determination of urinary proteins according to their molecular weight revealed a pattern consistent with postrenal PU. This, in combination with the synchronous diurnal variations of ova discharge and PU, favors the hypothesis that in urinary schistosomiasis PU is caused by ova excretion. In fact, in patients with urinary schistosomiasis, the bladder has been compared with a burned epithelial surface with high exudative qualities [30]. Thus, PU can be held to originate mainly from the secretion of serum proteins into the bladder lumen and partly from bleeding of the bladder mucosa.

In contrast to the almost congruent diurnal variations of egg excretion and PU, EU and LU exhibited markedly different and individual characteristics. EU resembled egg excretion in a time-delayed pattern, reaching its maximum at 6 P.M. This finding agrees with results obtained by Weber, Blair, and Clarke [4] who found that the urine of infected patients was heavily bloodstained from 4 P.M. on, but not in the morning. It can be speculated that this phenomenon is due to persistent capillary leakage, thus, perpetuating microbleedings even after ova have penetrated the bladder mucosa.

Leukocyturia had a bimodel distribution over the day. The relationship between leukocytes and erythrocytes in the urine was much in excess of that in the circulatory blood. Thus, LU did not originate solely from bleeding into the urine and might therefore indicate concomitant bacterial infection of the bladder. The association between urinary schistosomiasis and lower urinary tract infection is still controversial and not yet well established [31–34]. For technical reasons, no bacteriological investigations were carried out in this study. However, concomitant urinary tract infection in our patients is most unlikely for several reasons: none of the five patients had nitrite excretion in the urine at any of the thirty examinations as assessed by test reagent strips. A parallel study (Doehring, Ehrich, Vester, Feldmeier, and Brodehl, unpublished observations) on 272 boys from the same school revealed nitrite excretion indicative of urinary tract infection in two patients only. Because about 50% of urinary tract infections can be detected by test reagent strips [35], the prevalence of urinary tract infection in this area can be considered to be low. Antischistosomal treatment of boys infected with urinary schistosomiasis reverted LU to normal within 1 month after therapy (Doehring, Ehrich, Vester, Feldmeier, and Brodehl, unpublished observations).

Therefore, it seems more likely that, besides the almost insignificant amount of LU contributed by bleeding, an active process of leucocyte excretion must be operative. Lymphocytes surrounding ova granuloma may drain into the urine as ova penetrate the mucosa. In addition, schistosome eggs have been reported to release chemotactic substances [36] for eosinophiles which may thus initiate an accumulation of the white blood cells in the bladder wall.

The different circadian maxima of EU and LU have to be considered when test reagent strips are used as a screening tool for infection with urinary schistosomiasis, which has been described by Feldmeier et al [9], Mott et al [11], Doehring et al [37], and others. Whereas PU and LU may be found best at

noon, maximal hematuria is to be expected in the late afternoon. If the amount of urinary protein loss in urinary schistosomiasis is to be estimated, 24-hr urine specimens instead of spontaneously voided urine specimens, must be used.

In conclusion, urinary schistosomiasis features unique patterns of PU, EU, and LU, a circadian variation for which parameters have not been described in any other renal or urological disease. The different diurnal variations have to be explained by different underlying pathologic mechanisms.

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