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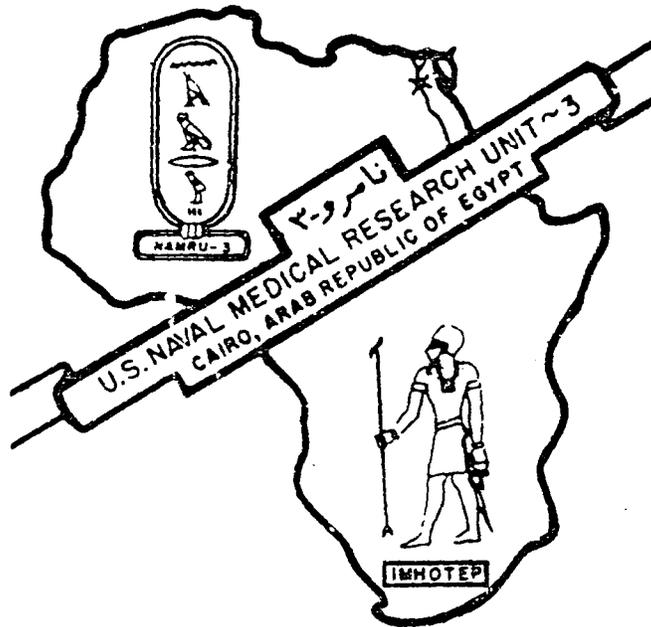
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CALCIFICATION OF SCHISTOSOMA HAEMATOBIIUM EGGS:  
RELATION OF RADIOLOGICALLY DEMONSTRABLE CALCIFICATION  
TO EGGS IN TISSUES AND PASSAGE OF EGGS IN URINE

By

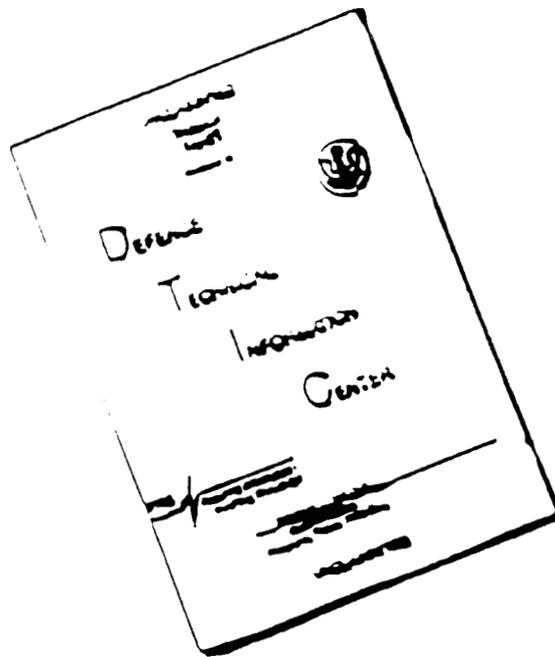
Allen W. Cheever, Stuart W. Young and  
Ayoub Shehata

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**CALCIFICATION OF SCHISTOSOMA HAEMATOBIIUM EGGS: RELATION OF RADIOLOGICALLY DEMONSTRABLE CALCIFICATION TO EGGS IN TISSUES AND PASSAGE OF EGGS IN URINE**

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**Summary**

The extent of calcification of the bladder in schistosome infected patients is roughly correlated with the number of calcified schistosome eggs present in the bladder. The focal concentration of eggs in the bladder appears to be one major variable, and we estimate that as few as 100,000 eggs per cm<sup>2</sup> might be detected in a clinical radiograph. The excretion of calcified eggs in the urine was related to the activity of the schistosome infection and was unaffected by the presence or extent of bladder calcification.

**Introduction**

Calcification of the bladder and ureters in schistosomiasis is caused by the accumulation of large numbers of calcified *Schistosoma haematobium* eggs in the tissues rather than by calcification of the host tissues (BUCHANAN and GELFAND, 1970). It should thus be possible to correlate calcification observed radiologically with the number of *Schistosoma haematobium* eggs present in the tissues. In the present study, we have compared the degree of calcification seen on radiological examination with the number of eggs in the bladder of patients undergoing cystectomy for carcinoma of the bladder. The passage of calcified eggs in the urine was examined in a second group of patients who showed variable degrees of bladder calcification but did not have carcinoma of the bladder.

**Materials and methods**

Preoperative X-rays were examined when available. In the remaining cystectomy cases, X-rays were taken of the excised bladder with and without submersion of the bladder in water. Paraffin embedded tissues of selected cases were X-rayed following preparation of histological sections. In all of the above material, calcification was classified as minimal (just visible), moderate or heavy (as dense as pubic bone).

After removal of blocks for histological examination, the remaining tissues were digested for 18 hours in 4% KOH at 37 C. (for unfixed tissues) or 56 C. (for formalin fixed tissues) and the number of schistosome eggs counted (CHEEVER, 1970). In several cases, calcified areas were dissected free of the remaining tissue in the specimen or paraffin block and digested separately. Predetermined numbers of calcified eggs from digested tissues were also imbedded in paraffin and examined radiologically. In digested specimens, black eggs were considered to be calcified. When placed in 1 N HCl, these gave off gas, became light brown in colour and frequently showed remnants of miracidial structure.

Urine was collected for each of 3 or more consecutive 24 hour periods in 500 ml. of neutral phosphate buffered formalin. After mixing, 200 ml. was homogenized for 30 seconds at half speed in a Waring blender. After adding 5 drops of octanol to disperse the foam, 3 one ml. samples were examined in Sedgwick-Rafter chambers<sup>†</sup>. Two 50 ml. samples were concentrated in vertical-walled centrifuge tubes and the

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<sup>†</sup>Obtained from A. H. Thomas Co., Philadelphia, Pa., U.S.A.

TABLE I. Relation of radiological calcification to macroscopic sandy plaques and the number of eggs in the bladder.

Case No.	Radiological calcification	Gross sandy plaques	Eggs per gramme urinary bladder (without tumour) in thousands**	Total eggs in urinary bladder plus tumour in millions**
S73-59	0*	0	0.1	0.6
S71-346	0	0	16	0.3
S71-413	0	0	17	2.8
S73-170	0	1	2	0.3
S73-57	0*	1	10	0.6
S73-58	0*	1	194	8.7†
S72-151	1	1	12	1.3
S73-161	1	1	91	1.3
S73-171	1*	1	36	3.2
S71-345	1	2	43	2.5
S71-347	2	1	41	1.0
S72-169	2	2	51	10.2
S71-421	2	2	186	12.5
S73-172	3*	2	17	3.7
S72-43	3	1	32	4.8
S72-82	ND	3	26	5.6
S72-313	ND	3	120	14.4
S73-63	3*	3	328	21.2
S73-168	3*	2	182	23.9
S72-392	ND	3	552	42.0

\*Indicates that the radiographs were taken *ex situ* after surgical removal of the bladder.

ND Indicates that calcification was noted on the X-ray but was not graded.

†In this case moderate numbers of calcified eggs were present in the submucosa but these could not be detected by X-rays of the paraffin blocks. Many eggs were also scattered through the tumour.

\*\*Over 95% of the eggs were calcified.

### Results

There was a general correlation between the severity of calcification noted radiologically, the extent of sandy deposits noted grossly and the numbers of eggs counted after digestion of the bladder (TABLE I). On microscopic examination, these areas showed closely packed calcified eggs, without evident calcification of bladder tissue (Fig. 1).

When dissected free of the remaining tissue, macroscopically calcified or sandy areas contained between 504,000 and 1,031,000 *S. haematobium* eggs per gramme tissue (TABLE II). This corresponded to between 260,000 and 710,000 eggs per cm<sup>2</sup> of radiologically observed calcification. When calcified eggs were placed in paraffin, 40,000 eggs per cm<sup>2</sup> were barely visible radiologically and 100,000 eggs per cm<sup>2</sup> were clearly visible.

Almost no calcified eggs were passed in the urine of patients without active schistosome infection, regardless of whether or not the bladder was calcified (Table III). Patients with active infections excreted moderate numbers of calcified eggs, but the number of calcified eggs excreted was related to the activity of the infection rather than to the extent of calcification of the bladder. The number of eggs in the urine judged to be calcified on microscopic examination was not affected by acidification or alkalinization of the urine.

### Discussion

Our data indicate the approximate numbers of calcified schistosome eggs corresponding to different degrees of calcification detected radiologically. It is obvious, on purely theoretical grounds, that the radiological detection of calcified eggs should depend upon their concentration in the tissues. Thus a large number of calcified eggs may be present in whole bladders and not be detected radiologically if the eggs

Insignificant numbers of dead and calcified eggs were passed in the urine of patients without active *S. haematobium* infection. In active infections, the passage of dead and calcified eggs was related to the number of embryonated eggs in the urine and was not influenced by the presence or absence of bladder calcification, suggesting that passage of calcified eggs is not influenced by the large reservoir of calcified eggs observed radiologically. The calcified eggs being passed in the urine appear to be part of a dynamic pool, and one presumes that in patients with active infection, calcification of the bladder is increasing rather than decreasing. Urine pH did not influence the passage of eggs or the classification of eggs as calcified or noncalcified.

Partial or complete resolution of bladder calcification sometimes occurs (FORSYTH and HUGHES, 1973; YOUNG et al., 1973). Our present data suggest that resorption of calcified eggs *in situ* may be responsible for the disappearance of calcification, and that passage of calcified eggs in the urine is less important. However, we studied patients over a brief period and the passage of calcified eggs may be irregular (BLAIR et al., 1969). Furthermore, we did not study patients known to be resolving their calcification. In one patient in whom heavy calcification of the bladder resolved over a 5 year period, 2 routine quantitative examinations were done using a modified BELL (1963) technique. These showed 4,000 eggs per day in the urine at the time of discharge from the ward after treatment and 7,000 eggs per day on re-examination 5 years later; none of these eggs were live (YOUNG et al., unpublished observations). If representative, this indicates excretion of about 2 million eggs per year, a number sufficient to produce decalcification. The relative roles of egg destruction and egg passage in the resolution of calcification are thus uncertain.

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