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STUDIES ON SCHISTOSOMIASIS. 9. PATHOLOGY OF THE BOVINE URINARY TRACT*

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ABSTRACT

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Pathological findings in the urinary tract of 40 head of cattle, experimentally or naturally infested with *Schistosoma mattheei*, are presented. Lesions of the ureter found in 57,5% of the cases consisted of linear granulomata with associated haemorrhages or granular patches. The segmental hydro-ureter caused by disruption of the muscular tunic by a granular patch type of lesion was a prominent finding.

Bilharzial bladder lesions observed in 75% of the animals were of 4 distinct types: granulomatous foci 1–2 mm in diameter, granular patches, polypoid patches and polyps. In all cases with bladder lesions the fundus was affected; neck and trigonal lesions tended to occur in the more severely affected bladders.

The Hoeppli phenomenon was more common than in other species and was associated with the subacute stage of lesion. Urinary bladders with ovum granulomata throughout the lamina had more severe microscopic lesions than those with ovum granulomata in the submucosa only. The stage of development of the bladder lesion was related directly to the length of infestation of the animal. There was an inverse ratio between the percentage of live ova in the bladders and increased chronicity of the lesions.

Since the effects of reinfestation on the pathology were quantitative, a larger percentage of the reinfested animals had bladder and ureteric lesions, which tended to be more severe and, in the bladder, more chronic than in singly infested animals.

A significant rank correlation was observed between the W–D index (relating the worm burdens and the duration of infestation) and the severity of macroscopic and microscopic bladder lesions ($P < 0,001$).

The duration of infestation and the W–D index were significantly greater in animals with bladder lesions than in those without ($P < 0,000\,1$) and no lesions of the bladders and ureters were found in animals infested less than 185 days, indicating an extended prepatent period in bovine urinary schistosomiasis. On the other hand, there were no significant differences between the worm burdens of animals with or without bladder lesions, suggesting that the first lesions appear between 70 and 185 days after infestation, regardless of the number of worms present.

Possible migration routes to the urinary tract are discussed.

The bilharzial granulomata observed in the renal pelvis of 20% of the animals is the first reported incidence of bilharzial lesions of the renal pelvis.

The incidence of renal parenchymal lesions in infested animals was more than double that in uninfested controls.

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Résumé

ETUDES SUR LA SCHISTOSOMIASE. 9. LA PATHOLOGIE DU TRACTUS URINAIRE

L'anatomo-pathologie du tractus urinaire est décrite d'après 40 *boeufs infestés soit expérimentalement soit dans la nature avec* Schistosoma mattheei. *Les lésions de* 57,5% *de ces animaux au niveau de l'uretère étaient des granulomes linéaires ou en taches, parfois accompagnés d'hemorragies. Un effet exceptionnel de ces lésions était la segmentation de l'uretère à cause de la rupture de sa couche musculaire.*

On a pu distinguer 4 *types distincts de lésions au niveau de la vessie chez* 75% *des animaux: des foyers granulomateux de* 1–2 *mm de diamètre, plaques granuleuses, plaques polypeuses et polypes. Le fond de la vessie était constamment atteint, tandis que le col et le trigone vésical n'étaient atteints que pour les vessies gravement affectées.*

Le phenomène de Hoeppli, plus marqué que chez les autres espèces, s'est associé avec les lésions subaiguës. Les lésions microscopiques étaient plus marquées en tant que les granulomes paraissaient dans toute la paroi de la vessie que dans la sousmuqueuse seule. Le développement des lésions cystiques était en rapport direct avec la durée de l'infestation de l'animal. D'autre part, le pourcentage d'oeufs viables dans la paroi vésicale était en rapport inverse avec la chronicité des lésions.

Comme l'effet d'une réinfestation sur la pathogénie était quantitatif, davantage d'animaux réinfestés montraient des lésions au niveau de la vessie et de 13 *uretère avec une tendance à être plus importantes et plus chroniques (au niveau de la vessie), que chez les animaux infestés une seule fois.*

Un rapport significatif entre l'index W–D (rapport entre le nombre de parasites et la durée de l'infestation) et l'importance des lésions macroscopiques et microscopiques de la vessie a pu être mis en évidence ($P < 0{,}001$).

La durée de l'infestation et l'index W–D était plus important chez les animaux avec lésions cystiques que chez ceux sans lésions ($P < 0{,}000\,1$). *Les vessies et les uretères des animaux infestés au-delà de* 185 *jours étaient indemnes de lésions ce qui montre une période de prépatence prolongée dans le cas de la schistosomiase urinaire des boeufs. D'autre part il n'y avait aucune différence entre le nombre de parasites recueillis chez des animaux avec ou sans lésions de la vessie, ce qui suggère l'apparition des premières lésions à partir de* 70 *jusqu'à* 185 *jours après l'infestation sans égard au nombre de parasites présents.*

Introduction

Although the pathology of schistosomiasis has been extensively studied in humans and some experimental animals, information on the pathology of the disease in cattle is limited. In man, urinary schistosomiasis has received a great deal of attention, but in cattle this aspect is largely unreported or undescribed.

Schistosoma mattheei infestation of ruminants was first described after an investigation of sheep deaths on the farm of Mr S. W. Matthee in the Humansdorp district of the Cape Province (Veglia & Le Roux, 1929). In his report on the pathological findings of this outbreak, Le Roux (1929) observed that a comparative study of spontaneous animal and human schistosomiasis would have elucidated various points associated with human schistosomiasis which have puzzled man for years, but his comment has largely gone unheeded.

Despite reports on the high incidence of the disease, descriptions of urinary tract lesions in cattle are uncommon and concern only the bladder. Hussein (1968; 1971) and Malek (1969) observed no lesions of the urinary tract in naturally or experimentally infested cattle. Condy (1960) reported that 3% of 1 000 urinary bladders from Rhodesian abattoirs contained schistosome ova. In a similar survey Lawrence & McKenzie (1972) found that 10,5% of 869 bovine urinary bladders contained ova of *S. mattheei*. McCully & Kruger (1969) found gross lesions of the urinary bladder in cattle and detected ova in 7 of 9 bladders examined microscopically. Neither gross nor microscopic involvement of the ureters or kidneys in bovine bilharziasis has been reported.

This study on urinary involvement in bovine bilharziasis was undertaken for the following reasons:

1. A systematic, morphological study of frequency, distribution and pathogenesis of the lesions in the urinary tract is fundamental to a full understanding of the disease in cattle.

2. A comparison of the pathology of experimental and spontaneous infestations in cattle should provide a basis for future studies of the host-parasite relationship.

3. A study emphasizing the comparative pathology and pathogenesis of urinary lesions in *S. mattheei* infestation of cattle with similar lesions in man may lead to a better understanding of human urinary bilharziasis.

4. A comparative investigation may help to elucidate the poorly documented lesions of the bovine urinary tract.

Materials and Methods

Forty head of cattle infested with *S. mattheei* were utilized for pathological studies. Thirty of these were experimentally infested (Van Wyk, unpublished data, 1971) and 10 were naturally-infested field cases (Van Wyk, Bartsch, Van Rensburg, Heitmann & Goosen, 1974). With the exception of 5 bulls and 1 heifer in the experimental group and 1 bull in the naturally-infested group, all the animals were steers. The experimental animals were maintained at Onderstepoort in pens containing shelters and were fed dry lucerne hay *ad libitum* with concentrate and mineral supplement. Five of the naturally-infested animals, which originated from the farm "Otthilie" near Tolwe in the north western Transvaal, were treated with trichlorphon as a vermicide (Van Wyk *et al.*, 1974).

The experimental animals were from several experiments, but only those data pertinent to the pathological findings of the urinary tract will be presented here.

The experimental design is summarized in Table 1. Details of the dates and numbers of cercariae given to individual animals in each of the 5 experimental groups will be published separately.

Groups VI and VII consisted of field cases. The cattle in Group VI were untreated while those in Group VII were treated with various doses of injectable trichlorfon*.

The techniques used for the maintenance of *S. mattheei* in the laboratory and for infesting cattle were those described by Van Wyk & Groeneveld (1973) and Van Wyk (1973).

* Dylox Injectable, Bayer Agrochem

TABLE 1 Experimental design

Group	No. of cattle	Mean age at death (months)	Primary infestation**	Interim	Secondary infestation**	Interim before death	Remarks
I.....	10	25	31 913	—	—	16–18 m	Killed for worm recovery
II.....	3	21	146 741 (439/kg)	—	—	56–59 d	Died or killed *in extremis*
III.....	6	25	31 813	10 m	123 844 (437/kg)	6 m	Killed for worm recovery
IV.....	6	32	9 882	15 m	142 787 (436/kg)	4–6 m	Oxen E20 & E24 killed *in extremis*. Others sacrificed for worm recovery
V.....	5	20	9 548	—	—	2–6 m	Killed for worm recovery
VI.....	5	±24	Not known	Not known	Not known	> 6 m*	Field cases (untreated)
VII.....	5	±24	Not known	Not known	Not known	> 6 m*	Field cases (treated)

* See van Wyk *et al.*, 1974 for origin of estimate
** Mean number of cercariae

With the exception of Calf E12, which died naturally, the animals were injected intravenously with 50 000 units of heparin before being killed. Calves E11, E13, E20, E24, N31 and N39 were killed *in extremis*, all the rest being killed for worm recovery at the termination of the various experiments. Animals E3, E11, E13, E24, E25 and N31 were killed by severing the spinal cord at the atlanto-occipital joint; N36 and N37 were shot and exsanguinated, and E30 was anaesthetized with sodium pentobarbitone* before exsanguination.

Visceral perfusion and schistosome collections were performed *in situ* by adapting the techniques used by McCully & Kruger (1969) for perfusing sheep. With the exception of 3 field cases of bilharzia where aliquot counts were used for estimating the worm burden (Van Wyk *et al.*, 1974), a total count was made of the worms collected from the mesenteric and gastric radicles of the portal vein, intra-hepatic portal vessels and the pulmonary arteries of the remaining cases. Only the total number of worms collected is recorded in Table 2; the distribution of parasites will be dealt with in a later publication.

After the parasites had been collected from the mesentery, necropsies usually commenced within an hour of death.

Classification of bladder lesions

The appearance, location, severity and chronicity of the lesions were recorded semi-quantitatively and/or qualitatively.

Macroscopic

(a) *Types of granulomata:* The granulomata were classified into 4 types, ranging from individual small foci to conglomerations of granulomata and polyps (see Results).

(b) *Severity and frequency:* Lesions of the urinary bladder were evaluated according to the fraction of the mucosal surface involved: up to 1/10th involvement was marked slight or 1; from 1/10th to 1/5th, mild or 2; 1/5th to 1/3rd, moderate or 3; 1/3rd or greater, severe or 4.

(c) *Location:* e.g. fundus, neck or trigone of bladder.

* Sagatal (May & Baker)

Microscopic

(a) *Severity and frequency of the lesions:* The lesions were classified as follows:

1. slight or infrequently observed lesions;
2. mild or occasional lesions;
3. moderate or frequently observed lesions; and
4. severe or very frequent lesions.

(b) *Stage of development of the lesion* (*stage of lesion*): The lesions were divided into subacute, chronic active and chronic stages (see Results).

(c) *Grade* (*laminar distribution*) *of lesion:* Laminar distribution of ovum granulomata was graded as follows: In Grade 1, granulomata occurred only in the submucosa; in Grade 2, in the submucosa and superficial muscularis and in Grade 3, throughout the bladder wall.

(d) *Ova:* The number of ova per mm^2 as well as the frequency of necrotic and calcified ova and of the Hoeppli phenomenon (see pp. 81 and 88) were determined in representative sections.

Uninfested control animals

Kidney specimens from 25 head of randomly selected, apparently healthy slaughter cattle were collected for histological comparison with the kidneys of the infested animals. The control animals were 2–3 years old, not infested with schistosomes and moreover originated from areas in which schistosomiasis is not enzootic.

Processing of specimens for histopathology

Tissue specimens for histopathological examination were collected in 10% buffered formalin and paraffin sections prepared. Besides haematoxylin and eosin (H & E), the following special stains were used: Giemsa, Masson's trichrome, Schmorrl's method for lipo-fuchsin, the PAS reaction, Perl's method for iron, Pickworth's benzidine method for haemoglobin, crystal violet for amyloid, oil red 0 on frozen sections for lipids, Mayer's mucicarmine and Fraser-Lendrum stain for fibrin.

Macroscopic and microscopic post mortem findings of individuals and groups were compared and correlated with the number of worms found post mortem and with the duration of infestation.

Statistical Evaluation

1. Differences in experimental values between groups of cattle or between treatments (e.g. single or reinfestation) and lesions were examined for statistical significance (5% level of significance) by means of the Kruskal-Wallis one-way analysis of variance by ranks test (Siegel, 1956) and the Nemenyi multiple-comparison test (Miller, 1966) when more than 2 groups were compared simultaneously, or by the Mann-Whitney U test (Siegel, 1956) when 2 groups were compared at a time.

2. The various values per animal were tested for significant correlations (5% level of significance) by either Pearson's coefficient of correlation (Downie & Heath, 1965) or Kendall's coefficient of correlation by ranks (Siegel, 1956).

Worm-Day Index (W–D Index)

A worm/day index was calculated to obtain an indication of a possible cumulative effect of the worm burden and the duration of the infestation of the animal host (Table 2):

$$\text{(a) W–D index} = \frac{Wa \times (Da-45) + Wp \times (Dp-45)}{10^5}$$

where Wa = the number of worms recovered at autopsy,

Wp = the estimated primary worm burden at the time of reinfestion (from Lawrence, 1973),

Da = the number of days between reinfestation and the autopsy, and

Dp = the number of days between the primary infestation and reinfestation.

Deposition of ova by the worm is generally accepted to be responsible for most of the pathology in the host (Warren, 1973). Hence the prepatent period (45 days; Van Wyk, unpublished data, 1971) was subtracted from the duration of infestation for both the primary and challenge infestations.

The W–D index was also calculated in 3 other ways:

(b) As for (a), but the worm burdens were multiplied with the duration of infestation (in days) instead of the patent period;

(c) An approximate mean worm burden was calculated, taking into account an expected mean development of 60% at 60 days after infestation, diminishing to 12% of this total by 12 months after infestation (Lawrence, 1973).

This mean worm burden was substituted for the worm burdens in the 2 formulae above for obtaining the remaining 2 W–D indices.

As the 4 values for each animal correlated highly significantly with one another, the minimum r-value being 0,839 4 ($P<0,000\,1$), only the first method of calculating the W–D index is described and this index is listed in Table 2 for each animal.

Results

I. Urinary Bladder Pathology

A. *Macroscopic pathology*

Macroscopic granulomata were observed in the mucosa of the urinary bladder in 75% (30) of the cattle.

1. *Types of granulomata*

The following 4 types of granulomata were recognized (Table 3):

(a) The smallest lesions were grey-white granulomata, slightly raised, and 1–2 mm in diameter (Fig. 1). These were generally found scattered over areas up to 5–10 cm in diameter.

(b) More densely aggregated granulomata, called granular patches, formed slightly raised foci 1–2 cm in diameter; these lesions were also grey-white and haemorrhagic, presenting a rough granular surface with poorly demarcated borders (Fig. 1).

(c) Polypoid patches were 1–3 cm in diameter and raised up to 1 cm. These conglomerations of granulomata, also grey-white in colour or haemorrhagic, with a rough surface, were sharply demarcated from the surrounding mucosa and somewhat oedematous on the cut surface (Fig. 2).

(d) Finally, one pedunculated polyp 4 cm long with a pedicle 1 cm in diameter was observed in the bladder of Animal E10; it was haemorrhagic with a rough surface and oedematous on incision (Fig. 3).

In all 4 types of lesion petechiae and ecchymoses were commonly observed within the granulomatous areas.

In lightly affected bladders there were granulomata mostly 1–2 mm in diameter, or a few granular patches, or else a combination of the two types of lesions. The 1–2 mm foci occurred in 12 of 30 cases (40,0%) and appeared to be the initial gross lesions, progressing by confluence and aggregation into the larger granular patches which occurred in 25 of 30 cases (83,3%). The types of granulomata correlated highly significantly with the macroscopic ($r=0,656\,5$; $P<0,001$) as well as the microscopic severity of the lesion ($r=0,824\,7$; $P<0,001$), and significantly ($r=0,252\,8$; $P<0,05$) with the grade (laminar distribution) of lesion (below).

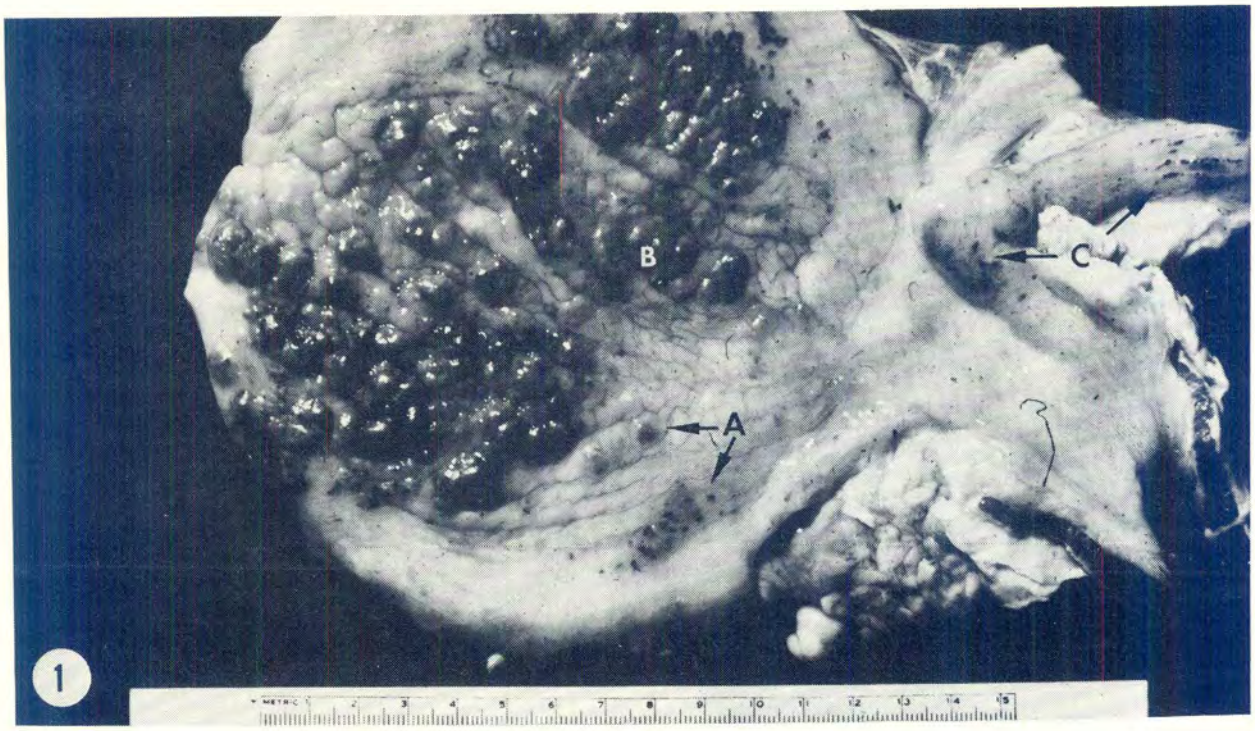
More severely affected cases had a higher incidence of lesions in the neck of the bladder; granulomata were found here in only 20% of lightly affected bladders, compared with 33% in mild, 67% in moderate and 50% in severe cases.

2. *Severity and frequency of gross lesions* (Table 2).

The mean severity of gross lesions was 2,3 in Group I; 0,0 in Group II; 2,5 in Group III; 2,7 in Group IV; 0,2 in Group V; 3,6 in Group VI and 0,6 in Group VII. The lesions varied from slight in 5 cattle to severe in 10.

There was a highly significant negative rank correlation between the gross severity of the lesions and the percentage of live ova ($r=-0,500\,9$; $P<0,001$), as well as a positive rank correlation with the W–D index ($r=0,532\,4$; $P<0,001$) and the severity of the microscopic lesions ($r=0,771\,1$; $P<0,001$). A significant negative rank correlation was found with the percentage of ova showing the Hoeppli phenomenon ($r=-0,323\,8$; $P<0,05$).

In one animal (E21) severe discrete polypoid lesions 1 cm in diameter, confluent at times, involved 80% of the bladder mucosa. There were raised foci of haemorrhage and oedema 1 cm in diameter scattered over the trigonal region. The bladder of another animal (N35) contained confluent, villous, haemorrhagic granular and polypoid patches involving most of the fundus (Fig. 2).



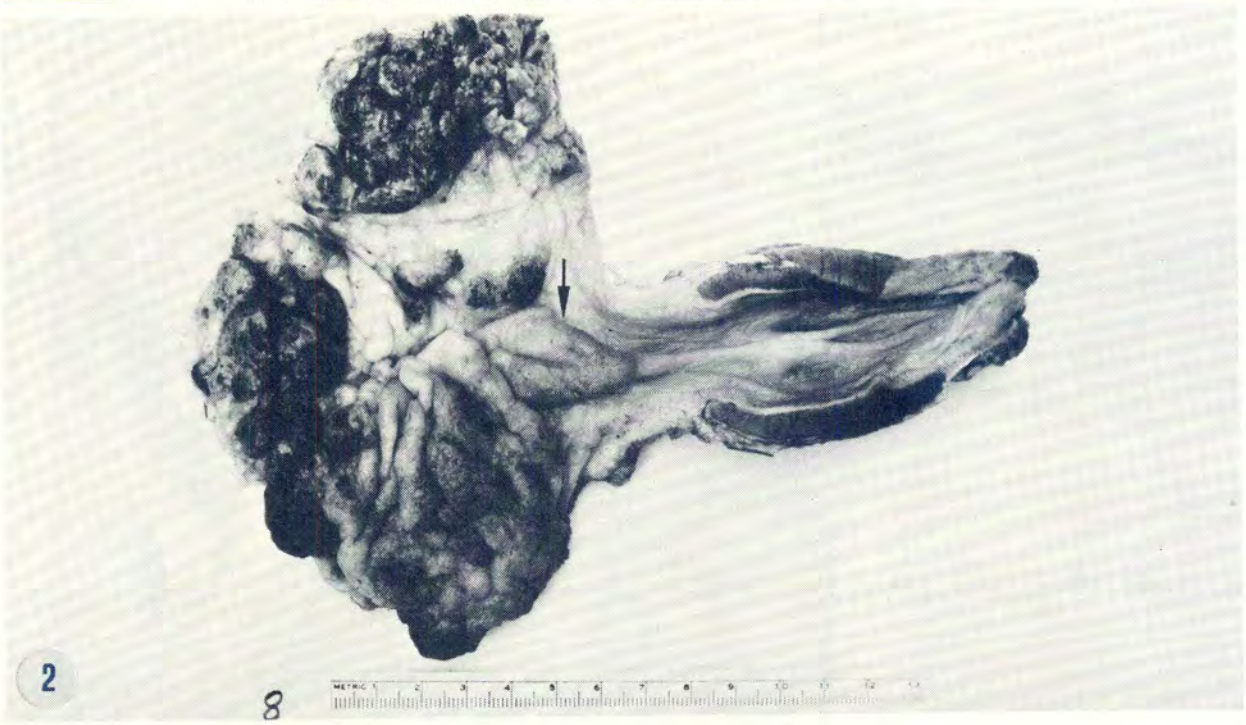


FIG. 1 Urinary bladder showing 1 to 2 mm focal granulomata and haemorrhage (A) and the larger granular patches (B). Not haemorrhages and granulomata at trigone and distal ureters (C)

FIG. 2 Severely affected bladder containing many polypoid patches throughout fundus. Note involvement of the neck and trigonal area (arrow)

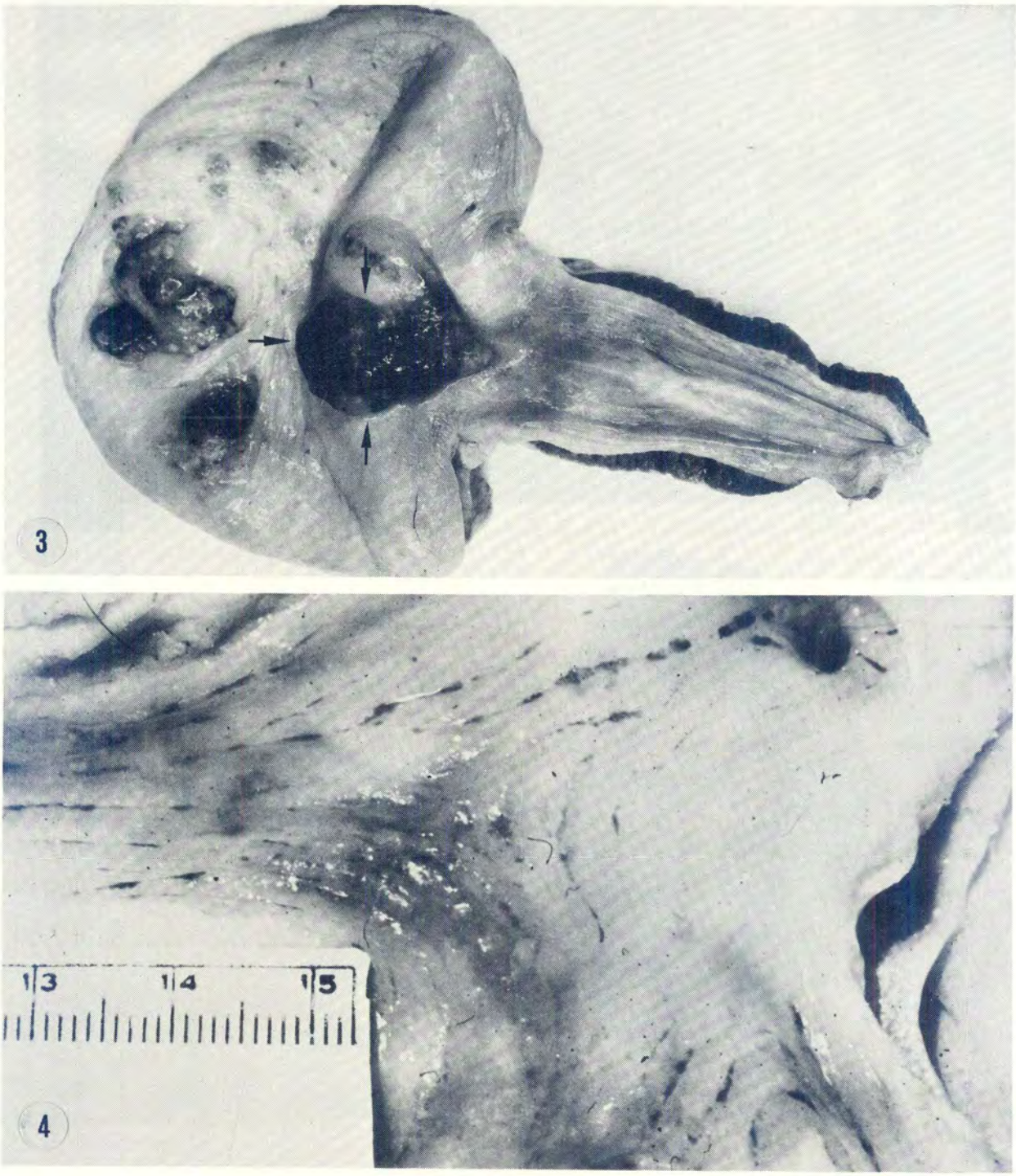


FIG. 3 Urinary bladder with a large polyp (arrow) originating from the neck and trigone region. Note the polypoid patches in the fundus

FIG. 4 Close-up of the renal pelvis showing the linear relationship of the granulomata and haemorrhages

TABLE 2 Number of worms recovered and a summary of the pathological data

Group	Animal	No. of worms	Worms/kg (Imm. before death)	Classification of bladder lesions					Number of ova			W-D Index
				Macroscopic		Microscopic			/mm²	Live (%)	With Hoeppli reaction (%)	
				Severity*	Type of Granuloma††	Severity**	Stage***	Grade†				
I.....	E1	8 381	20,69	4	1,5	4	1	3	0,55	24	6	35,20
	2	10 104	24,35	2	2,5	4	1	3	0,74	53	10	47,39
	3	3 757	8,84	2	1,5	2	2	2	0,12	95	0	16,68
	4	9 208	21,07	3	1,5	1	1	2	0,05	56	0	41,71
	5	4 144	11,58	2	1,0	1	1	1	0,02	60	0	18,69
	6	6 604	22,39	2	2,0	4	1	3	0,16	42	5	29,65
	7	2 796	7,36	1	1,5	2	1	3	0,22	43	2	12,86
	8	1 751	5,27	2	2,0	3	1	3	0,06	33	11	8,28
	9	2 767	8,76	2	1,5	2	1	1	0,15	60	0	13,92
	10	2 542	5,22	3	2,3	4	1	3	0,30	50	3	11,85
	MEAN	**5 205**	·	**2,3**	**1,73**	**2,7**	**1,1**	**2,4**	**0,24**	**51,6**	**3,7**	**23,62**
II.....	E11	102 467	334,86	0	0	0	a—	—	—	—	—	12,30
	12	80 476	268,25	0	0	0	—	—	—	—	—	9,66
	13	61 814	219,20	0	0	0	—	—	—	—	—	8,65
	MEAN	**81 586**	·	**0,0**	**0,0**	**0,0**	b ·	·	·	·	·	**10,20**
III.....	E14	32 306	98,48	1	2,0	1	3	2	—	—	—	71,71
	15	33 673	107,93	2	1,5	2	3	2	—	—	—	65,28
	16	95 162	280,71	2	2,0	3	2	3	0,42	48	0	160,96
	17	17 812	44,53	4	2,0	4	2	3	0,24	22	1	37,17
	18	34 350	81,59	2	2,0	4	2	3	0,97	23	0	85,79
	19	65 420	161,93	4	2,0	4	2	2	0,76	29	0	113,23
	MEAN	**46 454**	·	**2,5**	**1,91**	**3,0**	**2,3**	**2,5**	**0,60**	**30,5**	**0,25**	**89,02**
IV.....	E20	25 728	74,79	4	1,0	2	2	1	2,04	33	0	34,01
	21	31 752	94,78	4	2,5	4	2	1	0,18	18	0	51,25
	22	36 637	87,65	4	2,0	4	3	3	0,12	5	0	55,01
	23	46 568	92,95	3	2,0	3	3	3	0,05	3	0	88,16
	24	39 542	136,82	1	2,0	3	2	3	1,36	22	0	32,16
	25	31 960	85,68	2	1,0	3	2	3	0,06	13	12	57,95
	MEAN	**35 365**	·	**2,7**	**1,75**	**3,2**	**2,3**	**2,3**	**0,64**	**15,7**	**2,0**	**53,09**
V.....	E26	6 149	·	0	0	0	—	—	—	—	—	0,74
	27	6 203	·	0	0	0	—	—	—	—	—	0,74
	28	6 087	·	0	0	0	—	—	—	—	—	1,58
	29	4 059	·	1	1	1	—	—	—	—	—	5,72
	30	5 961	·	0	0	0	—	—	—	—	—	8,41
	MEAN	**5 692**	·	**0,2**	**0,2**	**0,2**	·	·	·	·	·	**3,44**
VI.....	N31	41 654	·	4	2,0	4	3	3	3,98	5	0	·
	32	56 833	231,97	4	2,5	4	3	1	4,94	9	0	·
	33	64 434	241,33	4	2,5	4	2	3	0,24	31	0	·
	34	54 603	280,02	2	1,0	2	3	2	0,72	35	0	·
	35	71 083	·	4	2,5	4	2	3	0,50	22	0,3	·
	MEAN	**57 721**	·	**3,6**	**2,1**	**3,6**	**2,6**	**2,8**	**2,08**	**20,4**	**0,06**	·
VII.....	N36	1 815	·	0	0	0	0	—	—	—	—	·
	37	46 342	·	0	0	0	—	—	—	—	—	·
	38	24 751	·	2	2,5	4	1	3	1,30	21	5	·
	39	15 896	·	0	0	0	—	—	—	—	—	·
	40	1 517	·	1	2,0	3	3	—	—	—	—	·
	MEAN	**18 064**	·	**0,6**	**0,7**	**1,4**	**1,0**	**3,0**	**1,30**	**21,0**	**5,0**	·

Key to Table

Classification	Macroscopic severity of lesions*	Types of granulomata††	Microscopic severity of lesions**	Stage of lesion***	Grade (laminar distribution) of lesions†
0.....	No lesions (included in mean values)	No lesions (included in mean values)	No lesions (included in mean values)	—	—
1.....	< 1/10 of bladder mucosa involved	Foci 1–2 mm diam.	Slight or infrequent	Subacute.....	Lesions in submucosa only
2.....	1/10–1/5th of mucosa involved	Granular patches 1–2 cm diam.	Mild or occasional..	Chronic active.....	In submucosa + superficial muscularis
3.....	1/5th–1/3rd of mucosa involved	Polypoid patches 1–3 cm diam.	Moderate or frequent	Chronic.....	Throughout bladder wall
4.....	> 1/3rd of mucosa involved	Pedunculated polyps	Severe or very frequent	—	—

a — Not applicable or no bladder lesions or too few eggs for classification
b · Unknown or not calculated

TABLE 3 Severity of the gross bladder lesions and the type and location of the lesions

Severity of lesion	Animal number	Type of lesion				Location of lesion		
		1–2 mm foci	1–2 cm granular patches	Polypoid patches	Pedunculated polyps	Fundus	Neck	Trigone
1.....	E 7	+	+	—	—	+	+	—
	E14	—	+	—	—	+	—	—
	E24	—	+	—	—	+	—	—
	E29	+	—	—	—	+	—	—
	N40	—	+	—	—	+	—	—
2.....	E 2	—	+	+	—	+	—	—
	E 3	+	+	—	—	+	+	—
	E 5	+	—	—	—	+	—	—
	E 6	—	+	—	—	+	+	+
	E 8	—	+	—	—	+	—	—
	E 9	+	+	—	—	+	—	—
	E15	+	+	—	—	+	—	—
	E16	—	+	—	—	+	+	—
	E18	—	+	—	—	+	—	—
	E25	+	—	—	—	+	+	—
	N34	+	—	—	—	+	—	—
	N38	—	+	+	—	+	—	—
3.....	E 4	+	+	—	—	+	+	—
	E10	+	+	—	+	+	—	—
	E23	—	+	—	—	+	+	—
4.....	E 1	+	+	—	—	+	—	—
	E17	—	+	—	—	+	+	—
	E19	—	+	—	—	+	+	+
	E20	+	—	—	—	+	—	—
	E21	—	+	+	—	+	+	+
	E22	—	+	—	—	+	+	—
	N31	—	+	—	—	+	—	—
	N32	—	+	+	—	+	—	—
	N33	—	+	+	—	+	+	—
	N35	—	+	+	—	+	—	—

3. *Location of lesions* (Table 3)

In all 30 cases with bladder lesions the fundus was involved. In addition 12 animals had lesions of the bladder neck and in 3 cases the trigone was enlarged, oedematous and haemorrhagic and contained granulomatous foci.

4. *Relationship between gross lesions and infestation*

The W–D index of animals with gross bladder lesions was significantly higher ($P<0,000\,1$) than those without lesions. While the period of infestation of animals with bladder lesions was significantly longer ($P<0,000\,1$) than in those without, there were no significant differences between the worm burdens of animals with or without bladder lesions, indicating that the first lesions appear between 70 and 185 days after infestation, regardless of the number of worms present. Note that there were no observations in these investigations for the period between 70 and 185 days.

All reinfested cattle had bladder lesions compared with only 73% of the singly infested animals. However, if one disregards the singly infested animals infested for 70 days or less, there were bladder lesions in 11 of 12, or 92%. On the other hand the reinfested animals had more serious lesions than the singly infested animals (the mean severity of the former group being 3,0 and the latter 1,33; $P<0,01$). The grade of lesions (laminar distribution) of the reinfested animals did not differ significantly from that of the singly infested cattle ($P>0,4$).

B. *Microscopic pathology of the urinary bladder*

1. *General*

Microscopic ovum granulomata were present in 30 of the 40 urinary bladders (75,0%) and were not observed in bladders free of gross lesions.

(a) *Pathogenesis of the ovum granuloma*

The complete pathogenesis of the ovum granuloma could not be determined because the experiments were not specifically planned for this purpose. Ova were mostly deposited in "convoys", which complicated elucidation of the pathogenesis of single granulomata, since most ova were located in areas of diffuse inflammatory reaction. However, a general progression of events became evident after examination of the large number of bladder and ureter histological sections. It is possible that the earliest host reaction to the bilharzia ovum escaped detection, but the first apparent reaction was histiocyte infiltration. Apparently viable ova were often accompanied by histiocytes and few or no eosinophils, while eosinophils were never found in the absence of histiocytes around these ova. It appears, therefore, that histiocytes, followed closely by eosinophils, were the earliest inflammatory cells to respond to the presence of the foreign protein. This pattern seemed constant in all 3 groups irrespective of previous immunological challenge. If the ovum did not pass unimpeded into the lumen of the bladder or ureter, it became necrosed. At about this time most ova were engulfed by one or more foreign-body giant cells interspersed with, or

immediately surrounded by, eosinophils. A small number of plasma cells, lymphocytes and histiocytes formed the peripheral layer of reactive cells.

The cell reaction became strongly eosinophilic following necrosis of the contents of the ovum. At this stage of granuloma formation, the ovum was often immediately surrounded by a Hoeppli reaction (an acidophilic, crystalline coating radiating from the ovum cuticle in a stellate pattern—Von Lichtenberg, Smith & Cheever, 1966), then by several layers of eosinophils, followed by a layer of giant cells and histiocytes and finally a few layers of plasma cells, lymphocytes and eosinophils.

Once the ovum had undergone necrosis the pathogenesis followed 1 of 2 pathways. If the ovum shell remained intact, a gradual diminution of the exudative inflammatory process and Hoeppli reaction was noticed as the cell contents of the ovum progressed from pyknosis to dissolution and loss of detail and finally to a homogeneous acidophilic mass within the shell. By this time the ovum had usually been engulfed by a giant cell surrounded by a few histiocytes, eosinophils, lymphocytes and plasma cells. Calcification of the ovum and its remaining contents appeared to be the next change and was associated with the loss of all inflammatory response. The final stage of the granuloma was a calcified focus, usually surrounded by collagen and with a few occasional lymphocytes nearby.

Alternatively, some ova became ruptured and the contents pyknotic. The lesion in this case was characterized by a marked eosinophil infiltration called an "eosinophilic granuloma". Usually the large numbers of eosinophils were surrounded by histiocytes, giant cells, lymphocytes and plasma cells. The extent of the eosinophilic granuloma decreased with the progressive removal of the ovum contents until only fragments of the shell remained. These were engulfed by a foreign-body giant cell and surrounded by lymphocytes, plasma cells, histiocytes and a few eosinophils. Many ovum lesions were observed in this stage. The inflammatory process decreased progressively until finally the fragmented shell was associated with an occasional histiocyte or lymphocyte. Both calcified and non-calcified shell fragments were seen.

Fibroplasia did not become evident until late in the exudative stage of the granuloma. As the number of epithelioid cells, plasma cells and eosinophils decreased, they were gradually replaced by newly formed fibrous tissue. Eventually at the final stage of the granuloma, concentric rings of connective tissue formed around the remnants of the ovum.

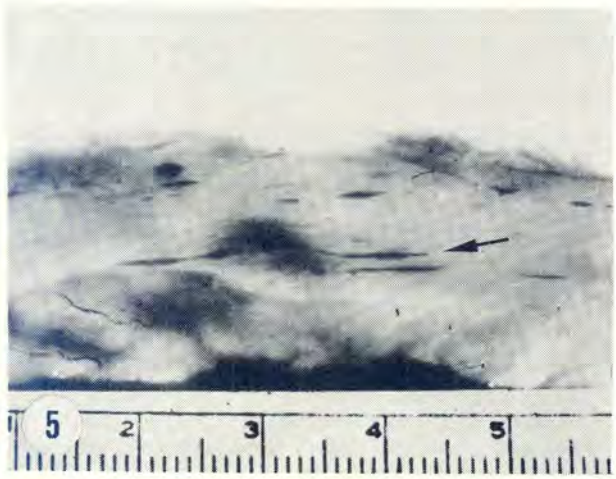
(b) *The Hoeppli reaction:* (Tables 2 and 4)

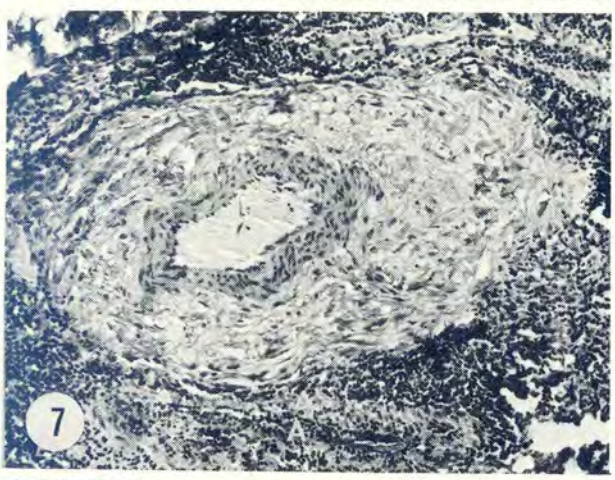
Hoeppli reactions surrounded some ova in 15 of the 30 (50%) affected bladders. They occurred in 9 of 18 (50%) singly infested animals, 4 of 12 (33%) reinfested ones and 2 of 10 (20%) field cases. Furthermore, a highly significant negative rank correlation between the percentage of ova showing the Hoeppli phenomenon and the stage of lesion ($r=-0,507\,3$; $P<0,001$) was observed most frequently in the subacute cases, and least often in the chronic cases. Seven of 10 (70%) subacute cases listed in Table 2, compared with 3 of 11 (27,2%) of chronic active cases, had Hoeppli reactions. Table 2 lists only 10 cases with Hoeppli phenomena. The additional 5 animals mentioned above which had Hoeppli reactions contained fewer than 3 ova in the bladder sections examined and were deleted from the percentage calculations of Table 2.

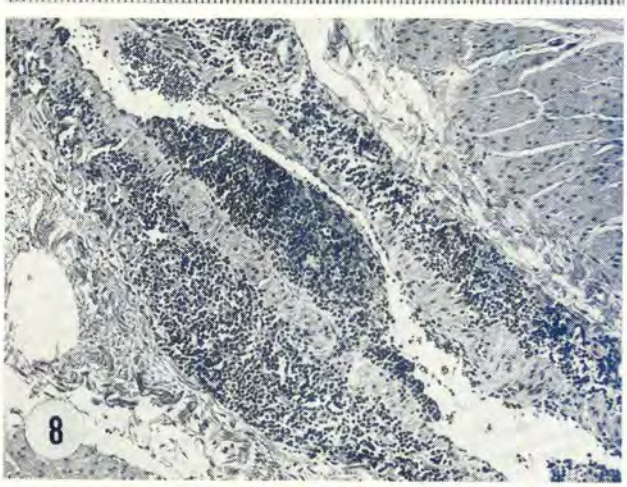
The Hoeppli reaction always appeared to be associated with necrotic or ruptured ova. The reaction was not found surrounding empty shell fragments or calcified ova.

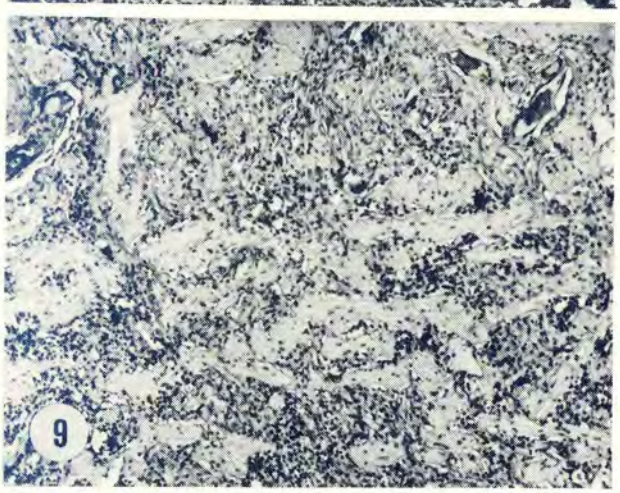
TABLE 4 The stage of lesion, number of ova/mm² of bladder section, percentage live ova and percentage of ova with Hoeppli reactions

Stage of lesion	Animal number	Number of ova counted	Ova/mm²	Percentage live ova	Percentage ova with Hoeppli reaction
Sub-acute	E 1	71	0,55	24	6
	E 2	119	0,74	53	10
	E 4	11	0,05	56	0
	E 5	5	0,02	60	0
	E 6	62	0,16	42	5
	E 7	38	0,22	43	2
	E 8	9	0,06	33	11
	E 9	59	0,15	60	0
	E10	83	0,30	50	3
	N38	221	1,30	21	5
	MEAN	**67,8**	**0,36**	**44,2**	**4,2**
Chronic active	E 3	17	0,12	95	0
	E16	32	0,42	48	0
	E17	90	0,24	22	1
	E18	171	0,97	23	0
	E19	130	0,76	29	0
	E20	276	2,04	33	0
	E24	233	1,36	22	0
	E25	8	0,06	13	12
	E21	54	0,18	18	0
	N33	56	0,24	31	0
	N35	241	0,50	22	0,3
	MEAN	**118,9**	**0,63**	**32,4**	**1,2**
Chronic	E22	21	0,12	5	0
	E23	18	0,05	3	0
	N31	1 288	3,98	5	0
	N32	889	4,94	9	0
	N34	104	0,72	35	0
	MEAN	**464,0**	**1,96**	**11,4**	**0,0**









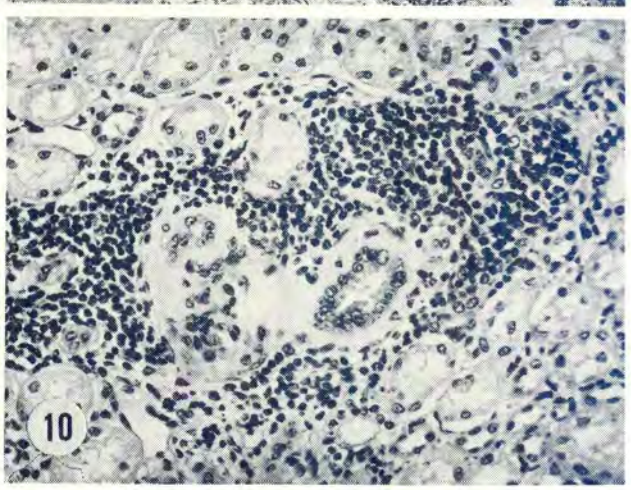


FIG. 5 Ureter affected with linear granulomata and associated haemorrhages (arrow)

FIG. 6 The ureter below contains a large granular patch type of lesion and is markedly dilated compared to the normal ureter above

FIG. 7 Photomicrograph of a submucosal venule from a urinary bladder. There is severe adventitial oedema and marked contiguous inflammatory reaction. The small arteriole shows severe vasculitis. ×75. H & E

FIG. 8 Photomicrograph of a submcousal venule of a urinary bladder. Note the severe intimal and perivascular infiltrates. The infiltrating cells are predominantly eosinophils. The tunica media beneath the severe intimal infiltrate is hypertrophic. ×75. H & E

FIG. 9 Photomicrograph of the muscularis of a ureter displaying marked separation and disorientation of the bundles of smooth muscle fibres by inflammation. There are several ova scattered throughout the field. ×75. H & E

FIG. 10 Photomicrograph of a kidney showing the interstitial infiltration of lymphocytes and plasma cells surrounding an atrophying glomerulus (left) and tubule (right). ×200. H & E

(c) *Vascular and perivascular lesions*

Lesions involving blood vessels were observed in all 3 groups.

Perivascular cuffing and inflammation of the adventitia of small submucosal arterioles and venules were quite frequently found adjacent to the granulomatous inflammatory reactions. The composition and extent of perivascular cuffs varied directly with the stage of the submucosal lesion. Cuffs associated with exudative lesions generally consisted of eosinophils with occasional plasma cells and lymphocytes, the numbers usually varying with the intensity of the inflammatory response. Submucosal lesions of cases in the chronic stage commonly contained vessels cuffed by a number of plasma cells, lymphocytes and, rarely, eosinophils.

The adventitia of submucosal arterioles and venules of 9 cases was markedly thickened by loose connective tissue interspersed with slightly basophilic material (Fig. 7). No inflammatory reaction was noticed in the adventitia of these vessels and the thickening did not appear to involve the tunica media. Special stains did not assist in the identification of this material. Mayer's mucicarmine, the Fraser-Lendrum method for fibrin and the PAS reaction were all negative. However, the affected vessels were contiguous with, or in close proximity to, granulomatous inflammatory responses and this was thought to be an oedematous response resulting from the nearby inflammation.

Vasculitis was encountered in 4 cases (E2, Group I; E17 & 18, Group III; E21, Group IV—Fig. 7 & 8). The severity varied from infiltration of the tunica by eosinophils and occasional lymphocytes to eosinophil and histiocyte-rich granulomata of the intima and adventitia. Several thrombosed veins were observed in 1 case (E2) in conjunction with severe vasculitis, ovum granulomata and necrotic worms. Hypertrophy of the tunica media was noted in the submucosa of only 1 urinary bladder (E17—Fig. 8). The thickened portion of the arteriolar wall was contiguous with an eosinophilic granuloma of the interna. Mild endothelial hyperplasia was observed in the 4 cases with vasculitis. Endothelial hyperplasia was characterized by slight intimal thickening which led to formation of small villi in the endothelium.

Neovascularity of the vesicular submucosa, consistently found in all cases displaying schistosomal lesions, was characterized by a marked increase in the number of arterioles and capillaries and occurred in all 3 stages of lesion (discussed under severity and frequency of microscopic lesions).

(d) *Lesions of the laminae of the bladder*

The *mucosa* of most bladders with moderate or severe submucosal lesions was markedly hyperplastic and characterized by the development of folds and papillary structures. In many instances circumscribed pockets of epithelial cells (epithelial nests of Von Brunn) were observed in the submucosa at varying depths. Small ulcers with associated haemorrhages were often noticed in areas of hyperplasia adjacent to ovum granulomata.

Submucosal thickening in bladders with active lesions varied directly with the extent of inflammation and granulomatous response. The most extreme examples of inflammatory reaction were found in Animals E21 and N38 where extensive oedema, fibrin accumulation, haemorrhagic foci and very severe diffuse eosinophilic infiltration of the submucosa occurred.

Marked fibrous thickening of the submucosa was encountered in 8 cases, the submucosa showing increased vascularity and resembling granulation tissue. The lesions in all but 3 (E25 in Group IV and N33 and N35 in Group VI) were in the chronic stage. By comparison, thickening of the submucosa of bladders in the chronic active stage was due to both exudative inflammatory reaction and active fibroplasia.

Usually the few inflammatory cells in the submucosa of areas without ovum granulomata consisted of eosinophils and plasma cells accompanied by mild perivascular plasma cell and lymphocyte cuffing. In the chronic stage, submucosal areas free from ovum granulomata showed varying degrees of infiltration (Kloetzel, 1967) pigment-laden histiocytes, lymphocytes and, infrequently, eosinophils.

In many bladders foci of closely packed lymphocytes were found near areas of ovum granulomata. These foci occurred in the submucosa of bladders in all 3 stages as well as in bladders without granulomata, but they were smaller in the latter. The aggregates of lymphocytes were usually found near a prominent capillary or arteriole. Submucosal connective tissue adjacent to granulomatous areas was usually oedematous and was infiltrated by varying numbers of eosinophils, plasma cells and lymphocytes.

Granulomatous lesions of the *lamina muscularis* and *serosa* were similar to those of the submucosa. However, ovum granulomata of the muscularis contained a markedly higher proportion of histiocytes than those found elswhere. Moreover, most of the ovum granulomata in the muscularis occurred close to blood vessels, suggesting that the eggs are laid in this area and break through the venules of the lamina muscularis on their way to the bladder lumen. Organization and fibrosis of granulomata in the muscularis cause separation of the smooth muscle bundles.

2. *Severity and frequency of microscopic lesions of the urinary bladder*

The microscopic lesions varied greatly in severity. Several bladders contained only a few ovum granulomata with minor associated changes, some were severely affected, whilst others, although containing few ovum granulomata, had severe secondary lesions, for instance, haemorrhage, fibrosis or cell infiltration. The mean severity was classified for the various groups as follows:

2,7 in Group I (single, mild infestation);

0,0 in Group II (single, heavy infestation);

3,0 and 3,2 in reinfested Groups III and IV respectively;

0,2 in lightly infested Group V;

3,4 in untreated field Group VI;

1,4 in treated field Group VII.

As in the case of the macroscopic lesion severity, there were highly significant rank correlations between the microscopic severity and the type of granuloma ($r=0,824\,7$; $P<0,001$) and the numbers of ova per mm^2 ($r=0,432\,2$; $P<0,001$) and a similar negative rank correlation with the percentage of live ova

($r=-0,587\,0$; $P<0,001$). In addition there was a highly significant correlation with the grade of lesion ($r=0,353\,8$; $P<0,01$). There was no significant rank correlation with the percentage of ova showing the Hoeppli phenomenon.

3. *The stage of lesion*

This was classified according to the degree of chronicity of the bladder lesions (Table 4).

(a) *Subacute stage* (Stage I). This was characterized by predominantly exudative inflammation with no significant fibrosis. The most common infiltrating reactive cells in this stage were eosinophils, histiocytic or epithelioid cells and, to a lesser extent, plasmacytes and lymphocytes.

(b) In the *chronic active stage* (Stage II) exudative inflammation, combined with some degree of fibroplasia, occurred in the ovum granulomata. The infiltrating cells of this stage also consisted of eosinophils and histiocytes but with a high proportion of plasma cells and lymphocytes.

(c) The *chronic stage* (Stage III) consisted of massive numbers of apparently necrotic or calcified ova with little exudative response and a marked submucosal fibrosis and thickening. Eosinophils at this stage were uncommon.

The lesions in the bladders of 2 animals (E21 and N38) were difficult to classify because of extensive submucosal inflammation. In each case an oedematous, haemorrhagic submucosal stroma was heavily infiltrated by eosinophils and few plasma cells. Number E21, however, was placed in the chronic active stage because of the presence of fibroplasia in the submucosa, whereas, since no fibrosis was evident in the bladder of N38, it was placed in the subacute stage.

The relation between the stage of bladder lesion and the length of infestation is illustrated in Fig. 11. The difference in length of infestation between animals not containing bladder lesions and those with subacute lesions was highly significant ($P<0,001$). Owing to the arbitrary nature of the slaughter schedule, statistical analysis for differences between stages of lesions could not be assessed.

The relation between the stage of bladder lesion and the W–D index is illustrated in Fig. 12. A significant difference ($P<0,02$) was obtained when the W–D indices of the stage without any lesions and the subacute stage were compared. Significant differences were also observed between the W–D indices of Stages I and II ($P<0,02$), but not between Stages II and III ($P<0,1$).

The stage of lesion showed a highly significant correlation with the worm burden ($r=0,464\,3$; $P<0,001$).

Data are presented in Tables 2 and 4 comparing the stages of bladder lesion, percentages of live ova and Hoeppli phenomena, and the number of ova per mm^2 bladder section. When 3 or fewer ova were encountered in a section, the animal was excluded from these comparisons.

The mean percentage of live ova decreased highly significantly ($r=-0,607\,1$; $P<0,001$) with increasing chronicity of the bladder lesion: subacute 44,2%, chronic active 32,4% and chronic 11,4%. A similar negative rank correlation was found between the stage of bladder lesion and ova showing the Hoeppli phenomenon ($r=-0,507\,3$; $P<0,001$).

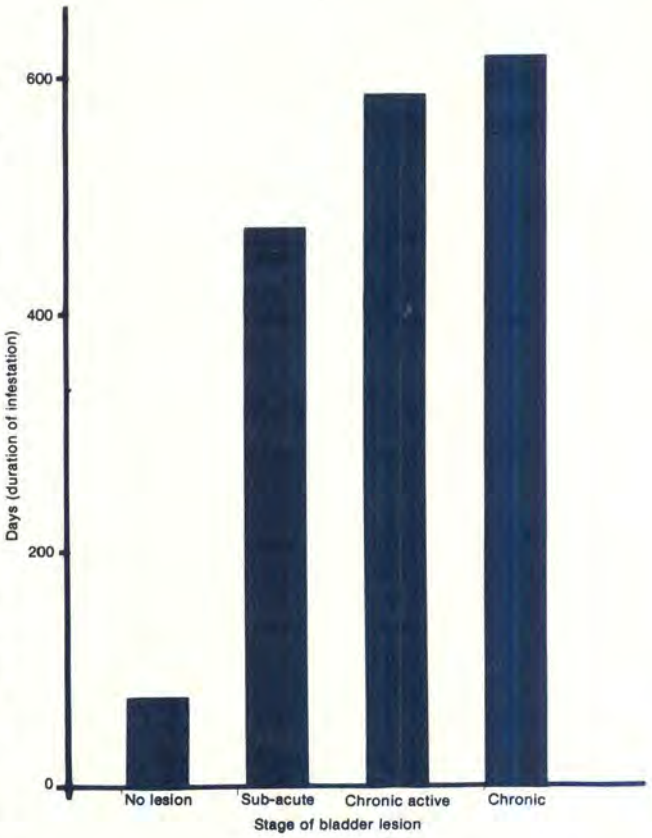


FIG. 11 Relationship between the mean length of infestation and the stage of bladder lesion

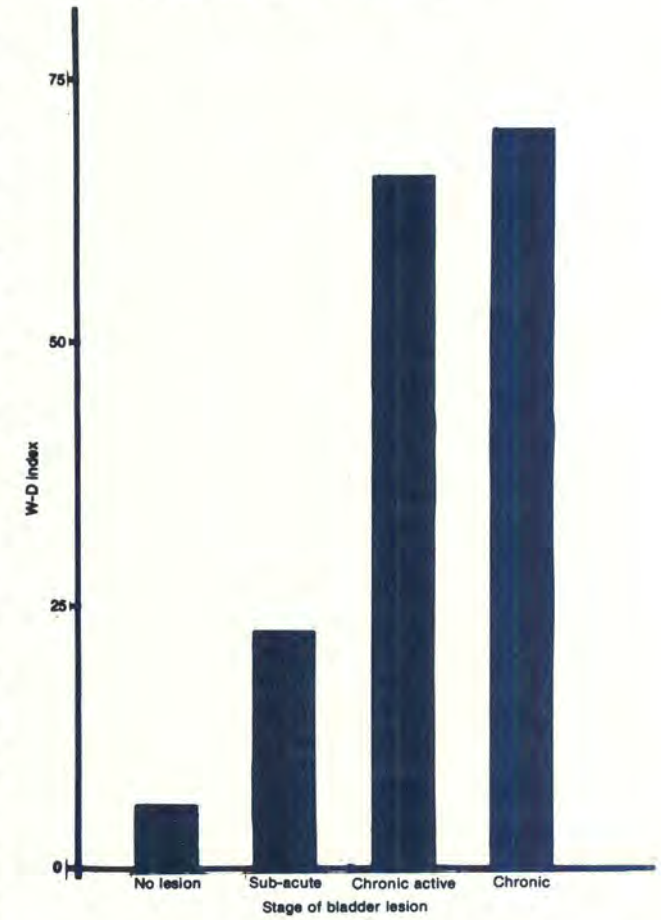


FIG. 12 Relationship between the mean W–D index (Worm-Day index; see p. 84 of the text) and the stage of bladder lesion

4. *Lesion grade*

The bladder lesions were graded microscopically according to the laminar distribution of ovum granulomata (Tables 2 and 5).

In Table 2 it can be seen that the mean grading per group (mean laminar distribution) varied very little between Groups I, III, IV and VI in which the cattle, when killed, were untreated and infested for longer than approximately 200 days. The laminar distribution of ovum granulomata (grade) was not related to the number of exposures ($P>0,4$) or to the number of worms present in the cattle.

TABLE 5 Macroscopic grade of the bladder lesions and the severity of the submucosal lesions

Grade of bladder lesion*	Animal number	Microscopic severity of lesion
0.....	N40	3
1.....	E 5	1
	E 9	2
	E20	2
	N32	4
	E21	4
	MEAN	**2,4**
2.....	E 3	2
	E 4	1
	E14	1
	E15	2
	E19	4
	N34	2
	MEAN	**2,0**
3.....	E 1	4
	E 2	4
	E 6	4
	E 7	2
	E 8	3
	E10	4
	E16	3
	E17	4
	E18	4
	E22	4
	E23	3
	E24	3
	E25	3
	N33	4
	N31	4
	N35	4
	N38	4
	MEAN	**3,6**

* See Table 2 for classification

In Table 5 the lesion grade is compared with the severity of the microscopic lesion in the submucosa for each case. Whereas 40% of affected bladders contained ovum granulomata in the superficial layers (mucosa and submucosa), 56,7% had lesions throughout the wall. It is interesting to note that bladders with ovum granulomata only in the superficial layers (Grades 1 and 2) had milder submucosal lesions than those with ovum granulomata throughout the wall ($r=0,353\,8$; $P<0,01$). The mean severity for Grade 1 plus Grade 2 was 2,2 compared with a mean of 3,7 for Grade 3. Notable exceptions were cases E21 and N32 which were undergoing lesion exacerbations at the time of necropsy. Also, the bladder sections of Animal N40 contained no ovum granulomata (Grade 0) but the bladder, markedly fibrotic, was thought to represent an "end-stage" lesion, and was placed in the chronic stage, severity 3.

The grade and stage of bladder lesion were not related ($P>0,05$).

II. Pathology of the Ureters

1. *Macroscopic pathology*

One or both ureters of 23 of the 40 animals (57,5%) contained the characteristically grey-white granulomata which usually extended linearly for a few centimetre, were 1–2 mm wide, raised slightly and associated with haemorrhages (Fig. 5). These granulomata were similar to those of the renal pelves (see below).

Other granulomata which occurred in 14 of the 40 head of cattle (35%), were confluent and focal, forming a granular patch up to 2 cm long and involving the entire circumference of the ureter (Fig. 6). Most ureters were thickened and dilated in these areas and, although no cases of hydronephrosis were observed, 2 affected ureters were dilated up to 4 cm. Since the blood vessels of the urinary tract were not perfused, adult worm pairs were often encountered in small ureteric veins generally near granulomata, and especially the granular patches (Fig. 5).

Ureteric lesions were always associated with lesions in the urinary bladder, and most ureteric granulomata (15 cases) were diffuse and bilateral. In addition, 10 of these 15 cases also contained focal or granular patches associated with some degree of ureteric dilatation. One millimetre diffuse foci were observed in the ureters of 1 animal. Ureters from 2 other cases contained only bilateral granular patches which were located at the proximal end of the ureters in one animal and the distal ends in the other. Left and right unilateral granular patches occurred ln the ureters of 2 and 3 further cases, respectively.

Gross ureteric lesions occurred more commonly in the reinfested animals. All 12 (100%) of the reinfested groups (III and IV) compared to only 5 (27,7%) of the singly-infested groups (I, II and V) had gross ureteric lesions. However, no gross ureteric lesions were observed in the 6 singly-infested animals with infestation periods of less than 185 days. Therefore, disregarding these cases of short duration, there were 6 of the 12 (50%) singly-infested animals with ureteric lesions, while 6 of the 10 (60%) naturally-infested (field) cattle showed gross lesions of the ureters.

2. *Microscopic pathology*

The occurrence of microscopic lesions corresponded to the gross changes in all but 1 animal (E7). In this animal, diffusely scattered foci 1 mm in diameter were observed macroscopically, but repeated sectioning disclosed no microscopic lesions.

Ovum granulomatous lesions were found throughout the wall of the ureter in 19 of the 22 cases (86,4%).

The mucosa

Mucosal hyperplasia denoted by epithelial thickening and corrugation of the mucosa was striking in cases displaying moderate or severe ovum granulomas. In the severely affected cases, the mucosae were up to 15 cell layers thick compared with the 4 or 5 cell layers observed in unaffected ureters. In all cases with hyperplastic epithelium of the ureters the submucosa was also thickened.

The submucosa

The submucosa of the ureters was thickened to various degrees in 18 of the 22 animals (81,2%), the thickening being directly due to a granulomatous response to ova and/or fibrosis.

TABLE 6 A comparison of gross and microscopic lesions between singly and reinfested groups. The severity and grade of gross and microscopic lesions are presented as means of each group

		Number and percentage affected	Severity of gross fundic lesions	Severity of micro. lesion	Grade of lesion*
Singly infested.....	Urinary bladder	11/15 (73%)	2,4	2,5	2,3
	Ureter.....	5/15 (33%)	—	2,0	2,0
Reinfested.....	Urinary bladder	12/12 (100%)	2,8	3,0	2,9
	Ureter.....	12/12 (100%)	—	2,8	2,9

* See Table 2 for classification

Submucosal infiltration of inflammatory cells was evident in the ureters of 28 of the 40 animals (70,0%). In 7 (25,0%) of these cases the submucosa did not contain ovum granulomata. The inflammatory cells were most commonly plasma cells, lymphocytes and rarely eosinophils, except in 6 cases (21,4%) where eosinophils predominated.

The lamina muscularis

Ovum granulomata found in the muscular lamina of the ureters in 19 of the 22 cases (86,4%) were commonly observed between smooth muscle bundles which were markedly separated by associated inflammatory or proliferative responses (Fig. 9). Often the inflammatory reaction appeared disproportionately severe when few ova were present.

Vascular lesions

Vascular lesions of the ureter primarily consisted of perivascular reactive cells of the same types observed in the submucosa. Furthermore, a basophilic, oedematous adventitia of arteries, similar to that seen in the urinary bladder, was observed in the ureters of 3 of the 22 animals (13,6%). This also occurred in vessels located near ovum granulomata. Early mild villous subendothelial proliferation was found in 2 of the 22 cases (9,1%). Prominent submucosal neo-vascularization occurred in 5 of the 22 cases (22,7%).

Relation between lesions of the bladder and ureters

The relation between the mean macroscopic and the mean microscopic lesion severity and that between grades of singly-infested and reinfested groups is given in Table 6. The mean severity of gross and microscopic lesions and the grade of lesions corresponded closely in the ureters and bladder in both singly- and reinfested animals.

III. Kidney Pathology

1. *The renal pelvis*

Of the granulomatous lesions found in the submucosa of the renal pelvis in 8 of the experimental cases, 7 occurred in reinfested animals from Groups IV and V. These grey-white linear granulomata 1–2 mm in width were constantly associated with petechial and ecchymotic haemorrhages and were continuous with those of the ureters radiating in a divergent pattern into the pelvis of the involved kidneys (Fig. 4). The severity of pelvic lesions varied from slight (a few granulomatous, haemorrhagic streaks) to severe (nearly confluent parallel lesions). Renal pelvic granulomata were bilateral in 3 cases. unilateral on the left in 4 cases and right unilateral in 1 case; they were always associated with ureteric lesions.

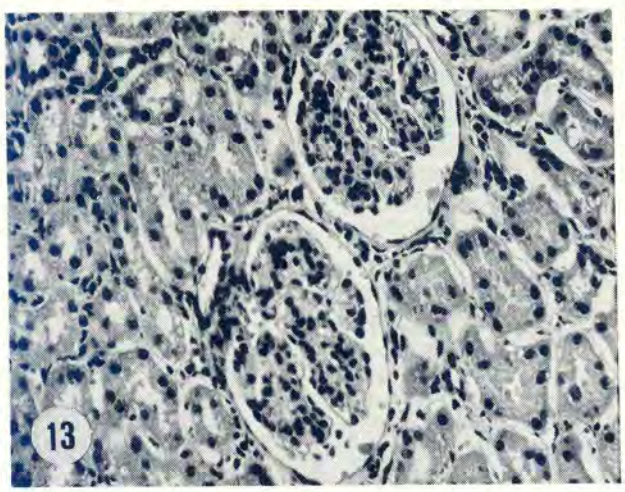
No granulomata were seen in the renal pelvis of the bilharzia field cases.

2. *The renal parenchyma*

While no gross lesions were seen in the renal parenchyma, microscopic lesions were common in each group of animals and were of 2 morphologic types: interstitial nephritis and mesangial proliferation.

Interstitial lymphoid cell infiltration was observed in 27 of the 40 kidneys (67,5%, Fig. 10). Eleven of 18 (61,1%) singly-infested animals, 8 of 12 (67%) reinfested animals, and 8 of 10 (80%) naturally-infested animals had this lesion. In most cases the infiltrate consisted of small lymphocytes, large mononuclear lymphoid cells and plasma cells, while eosinophils comprised part of the cell infiltrate in 1 case (N35). Although usually interstitial, the lymphoid infiltration was occasionally perivascular. Degenerating tubular or glomerular cells with karyorrhectic or pyknotic nuclei were common within these foci in which all stages of tubular and glomerular degeneration were found. Sclerotic thickening of the basement membranes of tubules and glomeruli was associated with loss of tubular epithelium, glomerular adhesions and atrophy. Atrophy of tubules and glomeruli continued until only a syncytium remained, consisting of a giant cell surrounded by a thickened, sclerotic hyalinized basement membrane accompanied by the infiltrating lymphoid cells. These thickened, fibrosed, peri-tubular and peri-glomerular accumulations were only weakly PAS positive and were negative for amyloid using crystal violet staining. The end result appeared to be complete resorption of the degenerate tubules or glomeruli.

Foci of oedematous interstitial fibrous tissue diffusely scattered throughout the kidney cortex were observed in 2 cases (E12 in Group II and N31 in Group VI). Interspersed within the interstitial fibrosis were sparsely scattered lymphocytes, large mononuclear cells and plasma cells. These foci were thought to represent the residuum of interstitial inflammatory reactions.



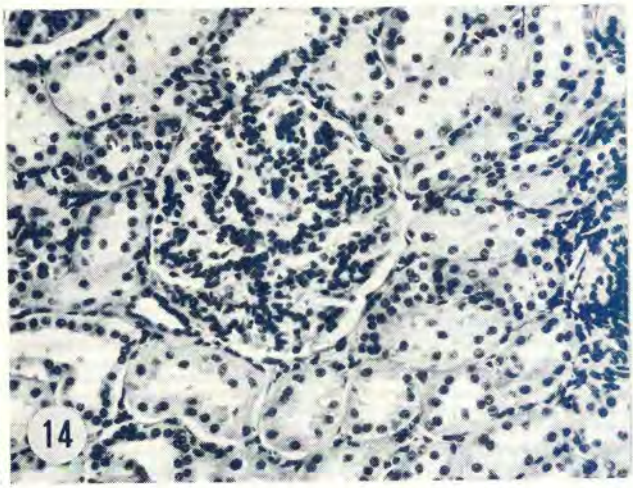


FIG. 13 Photomicrograph of a kidney from a control animal showing normal glomeruli. ×200. H & E
FIG. 14 Photomicrograph of a kidney from an infested animal showing moderate mesangial hyperplasia. ×200. H & E

Hyperplasia of the mesangium was observed in 21 of 39 cases (53,8%) (Fig. 14—compare with Fig. 13), consisting of 12 of 17 (71%) singly infested animals, 6 of 12 (50%) reinfested animals and 5 of 10 (50%) naturally-infested animals. Under microscopic examination, the mesangial cells in affected cases appeared focally or diffusely hyperplastic. Sometimes there was diffuse mesangial proliferation and thickening of the glomerular stalk or of specific focal areas of the glomerulus. Distortion of the normal glomerular shape accompanied focal hyperplasia. Glomerular basement membranes were not thickened and contained no PAS reaction-positive material. With diffuse glomerular hyperplasia, the contents of Bowman's capsule appeared overcrowded. In more severe, diffusely-affected cases, the mesangium developed a tree-like radiation and the mesangial cells often formed columns 2–3 cells thick. Focal adhesions between the glomerulus and Bowman's capsule were frequently seen in more severely affected glomeruli. However, no glomerulosclerosis or glomerular adhesions were observed progressing from the hyperplastic mesangial cells. Mesangial hyperplasia was not observed to progress to atrophic tubules and glomeruli surrounded by interstitial lesions. Renal specimens from the 25 controls examined for the presence of the above lesions showed mesangial proliferation in 6 of 25 cases (24,0%) and focal interstitial nephritis and associated tubular and glomerular degeneration in 8 of 25 cases (32,0%).

IV Urethra and Genitalia

No macroscopic or microscopic urethral or genital lesions were observed in any of the animals.

Discussion

I. The Urinary Bladder

1. *Incidence and importance*

In this study the urinary bladder contained lesions in 75% of the cases, a much higher incidence than that found by other workers, except McCully & Kruger (1969), who found ova in 7 of 9 bovine bladders examined microscopically.

The largest surveys on the frequency of bladder involvement in bovine populations have been done in enzootic areas of Rhodesia. Condy (1960) found *S. mattheei* ova in only 3% of 1 000 bladders examined, although the overall incidence of bilharziasis near Bulawayo was 35%. Later, Lawrence & McKenzie (1972), also working with *S. mattheei*, reported a 10,5% incidence of bladder involvement and an overall incidence of schistosomiasis of 63% near Bulawayo, Rhodesia. Similarly, in a survey in the eastern Transvaal, bladder lesions and ova were found in 3,5% of 346 cattle, while the overall incidence of bilharziasis in the local cattle population was 71% (Pitchford, 1958). Although these authors concluded that bladder lesions were found only in heavily infested animals, their conclusion is not confirmed in the present study, as 7 animals (E3, E5, E7, E8, E9, E10 and E29) had substantial bladder lesions but less than 5 000 worms each.

It must be borne in mind, however, that although there was no correlation between the worm burden and the presence of bladder lesions in the present study (see Results and Discussion below), the mean threshold value for development of these lesions may be below the minimum burdens encountered. Pitchford (1958), Condy (1960) and Lawrence & McKenzie (1972) determined the incidence at abattoirs and, although worm burdens were not determined, these were probably much lower than in the 7 animals having bladder lesions and less than 5 000 worms each. There were, on the other hand, highly significant rank correlations ($P<0,001$) between the gross ($r=0,532\,4$) and microscopic ($r=0,532\,2$) severity of the lesions and the W–D index, indicating that the worm burden did affect the severity of the lesions in older infestations.

In human infestation with *S. haematobium*, the incidence of bladder lesions is often high in endemic regions. Gilles, Lucas, Adeniyi-Jones, Lindner, Anand, Braband, Cockshott, Cowper, Muller, Hire & Wilson (1965) found on radiologic examination that nearly 50% of 9–15-year-old children in Ibadan, Nigeria had abnormalities of the bladder. Later Edington, Von Lichtenberg, Nwabuebo, Taylor & Smith (1970) found lesions in 20% of 673 bladders from unselected necropsies in Ibadan. Expanding on these findings, Young, Farid, Bassily & El-Masry (1973) performed urograms on 353 people with urinary schistosomiasis and discovered lesions of obstructive uropathy in 61%. With intravenous pyelography (IVP), Gelfand (1973) demonstrated

macroscopic bladder lesions in 14 and ureteric involvement in a further 14 of 60 patients passing ova in the urine. The findings of Forsyth & MacDonald (1965) in Tanzania are essentially similar; 27% of 330 children had abnormal IVP's (bladder and ureters). In some endemic areas in South Africa, however, the incidence of bladder involvement in unselected cases appears to be lower than reported elsewhere (Gelfand, 1973, citing Craib, 1968, and Powell, 1967).

From the present investigations it appears, therefore, that *S. mattheei* commonly involves the veins of the ureters and urinary bladders of cattle as in the case of *S. haematobium* infestation of man (Anderson, 1971).

2. *Severity, frequency, types and localities of macroscopic bladder lesions*

The 4 types of macroscopic bladder lesion appear to represent an orderly pathologic progression of bilharzial tissue response. This supposition is supported by the highly significant rank correlation found between the type of lesion and both the macroscopic ($r=0,656\,5$) and microscopic ($r=0,824\,7$) severity of the lesion ($P<0,001$). The 1–2 mm granulomatous foci represent the smallest macroscopic lesion and consist of individual or small aggregates of ovum granulomata. Aggregation of many such granulomata and concomitant inflammation, fibrosis and calcification account for the development of the more severe granular and polypoid patches. Since no quantitative measurements were made to ascertain the parasite load of the urinary tract, any relation between lesion severity and infestation rate is purely speculative. The severity and distribution of lesions probably depend on the number of parasites involved and their intravascular movement, length of infestation (Von Lichtenberg, Edington, Nwabuebo, Taylor & Smith, 1971) and host immunity. Therefore, development of the polypoid patches and the polyp-type of lesion may be related to a number of closely located and relatively stationary worm pairs. It has been observed in rodents infested with single schistosome pairs that the worms (*S. mansoni* and *S. mattheei*) migrate almost continuously (Pellegrino, personal communication, 1974; Van Rensburg, personal communication, 1974). Nevertheless, worms sometimes appear trapped in areas where prominent granulomas have developed, e.g. on the small intestines of reinfested cattle (Van Wyk, unpublished data, 1971) and possibly this may occur in the bladder as well.

The bladder lesions described in cattle do not correspond to those commonly observed at necropsy in man or chimpanzees, but rather to lesions found earlier in the pathogenesis of infestation in these hosts (Edington *et al.*, 1970). The polypoid patches observed in cattle possibly require a longer period of infestation to develop into fibrous and later sandy patches than in man (Warren, 1973). The infestation period was artificial as most of these animals did not die from the effects of bilharziasis but were euthanized at predetermined times. Moreover, the infestation in man is measured in years and decades as compared to weeks and months in the animals of the present study. Sadun, Von Lichtenberg, Cheever, Erickson & Hickman (1970), however, found sandy patch-type lesions in chimpanzees infested 11 months or less, but extrapolation from this could be misleading because the chimpanzee may be an aberrant host for *S. haematobium*. In the present study several urinary bladders contained microscopic lesions which appeared similar to those described as fibrous patches in man. In several cases prolific fibrosis and extensive ovum calcification were observed microscopically, but the macroscopic characteristics of whorled fibrous structures or a sandy surface appearance were absent. These bovine lesions may represent an early stage of a lesion similar to the fibrous patches observed in man.

Lesions in the bladder of cattle have not been adequately described previously. Condy (1960) listed 3 types of bladder lesion: a very early lesion consisting of petechiae and ecchymoses, an acute allergic type characterized by oedema and haemorrhage, and chronic lesions composed of various sized nodules. Examples of the latter type were prevalent (identified as granular and polypoid patches) in this study and were also seen by McCully & Kruger (1969), but the very early type of lesion was not observed. In addition, two bladders (cases E21 and N38) contained lesions characterized by oedema, haemorrhage and excessive eosinophil infiltration. These lesions may have represented hypersensitive reactions but the aetiological stimulus was not determined.

As in man and in the chimpanzee, the fundic portion of the bovine bladder contained the most common and prominent lesions (Gilles *et al.*, 1965; Sadun *et al.*, 1970; Edington *et al.*, 1970). In fact in this study bladder lesions invariably involved the fundus. Lesions in the neck and trigone were less common and associated with severely affected bladders. In contrast Edington *et al.* (1970) found a frequency of 66% trigone involvement in human cases with bladder lesions.

Macroscopic lesions of the bovine bladder may be more obvious than in the human bladder. Edington *et al.*, (1970) warned that even the most heavily affected bladders of man appeared unremarkable on gross examination and may be overlooked if microscopic examination is not undertaken. In this study microscopic lesions were not found where gross examination was negative. The necropsy procedure involved mechanical distension of the bladder and examination in strong light.

3. *Microscopic evaluation of bladder lesions*

The Hoeppli reaction: The occurrence of this lesion within the ovum granuloma appears to be a more prominent feature in cattle than in man or most experimental animals. Von Lichtenberg *et al.*, (1966) concluded that the Hoeppli reaction occurred most frequently in acute cases (9–15 weeks post exposure, when the host antibody titre was at peak levels) in organs with heavy egg deposition and involved not more than 10% of the eggs concurrently. In cattle, the Hoeppli reaction most frequently accompanied the subacute or exudative phase of lesion and involved up to 12% of ova counted. The Hoeppli reaction has been identified as schistosome antigen-host antibody complexes by immunofluorescence (Von Lichtenberg *et al.*, 1966).

In these cattle the Hoeppli reaction invariably appeared around necrotic or necrosing ova (when the soluble egg antigens were being rapidly released). Its appearance also corresponded with the presence of a large number of eosinophils many of which undergo necrosis themselves. It can be concluded that in cattle the Hoeppli reaction is a host response

against the rapid release of large amounts of antigenic ovum material at a time when large numbers of ova are undergoing necrosis.

Vascular lesions: As in the work of McCully & Kruger (1969), a variety of vascular lesions was observed in the bladders and ureters. Thus perivascular cuffing and inflammation and arteriolar medial hypertrophy were associated with contiguous areas of diffuse ovum inflammatory response. Eosinophilic vasculitis and endothelial hyperplasia were probably precipitated by the passage of ova through the vessel wall or possibly by irritation caused by the adult worms. Likewise, thrombosis of venules was due to emboli formed by aggregates of ova and dead worms, probably predisposed, by the tortuosity of the venous complex of the bladder, as Sadun *et al.*, (1970) suggested.

The grade of bladder lesion: The grade of microscopic lesion (laminar distribution of granulomata) was related to the microscopic severity of the lesion ($P<0,01$); bladders with ova only in the superficial layers usually had less severe lesions than those with ova throughout the bladder wall. On the other hand, there was no significant correlation between the grade of bladder lesion and the worm burden or the duration of infestation.

The stage of bladder lesion: Statistically, the subacute stage of bladder lesion was directly related to the duration of infestation ($P<0,001$) and the W–D index compared with cases lacking lesions. The chronic active ($P<0,01$) stages were also directly related to the W–D index when compared with cases not revealing lesions. Furthermore there was a highly significant rank correlation between the stage of bladder lesion and the worm burden ($P<0,001$). Similarly, the stage of bladder lesion in human schistosomiasis has been related to time (i.e. the age of the patient) and to the intensity of infestation indicated by the number of ova per gramme of bladder tissue (Von Lichtenberg, Edington, Nwabuebo, Taylor & Smith, 1971).

The stage of bovine bladder lesion could also be related to the percentage of live ova counted microscopically ($P<0,001$), which decreased with increasing chronicity of the lesion and the duration of infestation. These results are again comparable with the findings of Von Lichtenberg, *et al.*, (1971) in man. Furthermore, these workers found an increase in the number of ova per gramme of bladder tissue throughout the early active, chronic active and residual lesion stages and a decrease only in the inactive stage of lesion. This work was corroborated by Smith, Torky & Mansour (1972) who found that the 24 h rate of egg excretion as a function of tissue egg burden is more than 10 times greater in the active stage of disease than in the inactive disease. These workers also found a good correlation between the tissue egg burden and the urine egg excretion which is not the case in cattle where no ova were found in the urine of animals with bladder lesions (Condy, 1960).

The decrease in percentage live ova as the infestation ages has important implications which are not immediately obvious. Cheever & Powers (1971) and Cheever & Anderson (1971) have shown that the initial "half life" (between egg deposition and either excretion or destruction in the body to an unrecognizable morphological form) of *S. mansoni* ova varies from 8 days in rhesus monkeys to 30 days in mice. Furthermore, in the latter, many ova accumulate with time, because the eggs which remain after 30 days have subsequently a markedly lengthened half-life. In the monkey this accumulation did not occur. It is also known that *S. mansoni* ova remain alive for a maximum of only about 28–32 days in the tissues of mice (Gönnert, 1955; Maldonado, 1959). Because of similarities in the life cycles, this maximum survival probably applies also to *S. mattheei.*

In view of the duration of the infestation (in months) in these cattle, it is unlikely that the inverse ratio between percentage live ova and the chronicity of the lesion was due to death of individual ova in a pool of live ova. This finding probably reflects a decreased rate of egg production coupled with retention of dead eggs in granulomata (Lawrence, 1973). Furthermore, it indicates that schistosome ova are probably not as efficiently destroyed in cattle as in rhesus monkeys (Cheever & Powers, 1971), as some accumulate in the tissues in spite of reduced egg production as the worms age (Lawrence, 1973).

II. The Ureters

1. *Incidence*

This appears to be the first report of bilharzial lesions of the ureters in cattle. Such lesions occurred in 57,5% of the animals in this study.

In the human and some other primates the incidence of ureteric involvement in urinary bilharziasis at necropsy is also high. Thus, Bhagwandeen (1967) and Von Lichtenberg, *et al.*, (1971) reported an incidence of 73% and 75% respectively in man, while Obuyu (1970) found lesions in the ureters of 3 of 4 experimentally infested vervet monkeys and Sadun *et al.* (1970) in 6 of 8 chimpanzees in another study.

Dilatation of the ureter or hydro-ureter occurs in 18,5% to 37% of cases in human urinary bilharziasis (Bhagwandeen, 1967; Von Lichtenberg, *et al.*, 1971; Gelfand, 1973) and 75% in vervet monkeys (Obuyu, 1970) and chimpanzees (Sadun, *et al.*, 1970). Similarly hydronephrosis has been reported in 7,3%–20% and in 1 instance in 58% of humans with urinary bilharziasis (Gelfand, 1948; Kisner, 1952; Honey & Gelfand, 1960; Bhagwandeen, 1967; Gelfand, 1973).

2. *Importance*

Although 57,5% of the cattle in the present study had gross lesions similar to those leading to hydroureter in man, their clinical and pathological significance in cattle is unknown. While granulomatous lesions of the ureters of man tend to lead to hydroureter and hydronephrosis which may in turn lead to pyelonephritis and uraemia (Sayegh, 1950; Makar, 1968; Da Silva, De Brito, Camargo, De Boni, López & Gunji, 1970), none of these sequelae of hydroureter were observed in the present study. Possibly the infestations were not of sufficient duration for their development.

3. *Types and localities of macroscopic ureteric lesions*

As in human *S. haematobium* infestations (Bhagwandeen, 1967; Von Lichtenberg, *et al.*, 1971), ureteric lesions in cattle are found only in the presence of bladder lesions. The most frequent (65%) ureteric lesions in cattle were extensive bilateral linear granulomata. Granular patches accompanied the linear granulomata in 67% of the cases but occurred alone in 4 other cases. The granular patches occurred more frequently in the distal half of the ureter but did not appear to favour a specific site.

In man (Gelfand, 1948), the granular patches are the most common ureteric lesion and, according to most workers, have a definite predilection for the lower third of the ureter, especially the intrapelvic ureter (Sayegh, 1950). This was confirmed by Von Lichtenberg, *et al.*, (1971) who noted an incidence of 14,0% of lower ureteric lesions compared with an incidence of only 1,6% in the upper ureter. Bhagwandeen (1967), however, in a study of 60 ureters from cases with urinary bilharziasis, reported an incidence of 23% ureteric involvement in the lower third, 12% in the lower two-thirds and 27% along the whole ureteric length. He concluded that the entire ureter is more frequently involved than was previously reported.

Dilatation of the ureter has been reported as occurring both throughout its length and segmentally in man (Gelfand, 1948; Von Lichtenberg *et al.*, 1971) whereas the bovine lesion was only observed to be segmental, in association with granular patches.

4. *Pathogenesis of hydro-ureter*

Dilatation and tortuosity of the entire ureter in man is usually thought to be due to stenosis of the the lower third or intramural ureter (Sayegh, 1950). The stenosis may be due to a ureterolith (Young *et al.*, 1973), or dense fibrosis of the ureter (Makar, 1968), which usually occurs at the orifice and is caused by the extensive inflammatory response, fibrosis and calcification.

Nevertheless, this theory, that stenosis is the usual cause of hydro-ureter in bilharziasis, has been challenged by Gelfand (1948), Bhagwandeen (1967) and Von Lichtenberg, *et al.*, (1971), who found the incidence of hydro-ureter to be much higher than that of stenosis; for instance 22,7% compared with 2,7% respectively (Gelfand, 1948). These authors concluded that stenosis is a late and uncommon complication of ureteric involvement, whereas segmental dilatation was found early in the disease in young patients and was related to intensity of infestation and severity of the disease in the bladder and ureters (Bhagwandeen, 1967; Von Lichtenberg, *et al.*, 1971).

Edington *et al.*, (1970), Sadun *et al.*, (1970) and Von Lichtenberg, *et al.*, (1971) were of the opinion that polypoid patches in the ureter and dilatation caused functional impairment or aperistalsis, resulting in hydro-ureter.

In the present study the lesions of the ureters were similar to those on which Gelfand (1948) based his proposal for the pathogenesis of ureteric dilatation and hydronephrosis. In addition the incidence of involvement was as high as in man. These lesions may, therefore, be of clinical significance in cattle, although it is an interesting question why no associated hydronephrosis was observed in this study.

5. *Immunity and re-infestation*

Van Wyk & Bartsch (1971) showed that an immunizing dose of cercariae protected cattle, at least partially, against a double lethal dose (for fully susceptible animals) of cercariae.

Lawrence (1973) observed a peak schistosome egg count in cattle 10–11 weeks post infestation, followed by a rapid decline to very low numbers. Reinfestation of these animals produced only a slight or no rise in faecal egg count despite the fact that a large proportion of the challenge cercariae developed to adulthood.

The decrease in faecal egg count may be due to immune responses directed against both the ova and adult flukes or decreased ovum passage through the tissues. The progression from the acute to the chronic stage of *S. mansoni* infestation is accompanied by a decrease in size of granulomata, possibly because of enhanced antigen destruction (Von Lichtenberg, 1967), and a marked increase in connective tissue in the intestines, which may act as a mechanical obstruction to egg passage (Gelfand, 1967; Buchanan, 1971). The latter observation was also supported by our finding a significant correlation between large numbers of ova and chronicity of lesion in conjunction with the increase in fibrous tissue in the chronic lesions.

Reinfestation had a greater effect on the number of lesions of the urinary tract than on the composition of the lesions. Thus, lesions were present in the reinfested animals in a greater percentage of bladders (100% c.f. 92%) and ureters (100% c.f. 50%) but in fewer kidneys (50% c.f. 71%). On the other hand it must be remembered that the reinfested cattle were more heavily infested for longer periods of time than the singly infested cattle.

Although gross and microscopic lesions were basically similar in composition, the lesions in the reinfested animals tended to be more severe, both grossly ($P<0,01$) and microscopically ($P<0,02$). A more interesting difference was the tendency for the bladder lesion in reinfested animals to be in the chronic active or chronic stage whereas those in the singly infested animals were usually in the subacute stage. Lesions of reinfested animals were therefore usually more severe and more chronic than those of singly infested animals. However, owing to the differences in worm burden, it may be misleading to conclude that these results were due solely to reinfestation.

There was no significant correlation between the occurrence of bilharzial lesions in the urinary tract and the total number of parasites collected from the lungs, liver and intestines, or the duration of infestation. These results are somewhat inconclusive because the number of parasites in the urinary tract was not determined and the duration of infestation was arbitrary, since most of the animals were slaughtered according to a fixed schedule, before the terminal stages of the disease. Furthermore, as postulated earlier, the lowest worm burdens in the experimental cattle may have exceeded the mean threshold required to produce lesions of the urinary system.

6. *Parasitic migration to the urinary tract*

Neither gross nor microscopic bladder lesions were observed in the 6 animals that died or were killed *in extremis* 57–70 days after a single infestation. Only minor lesions were found in 1 (E29) of the 2 animals lightly infested for 185 days. These findings imply that the prepatent period of urinary infestation may be considerably longer than the 45–49 days reported for intestinal infestation (Hussein, 1973; Lawrence, 1973; Van Wyk, unpublished data, 1971). In vervet monkeys infested with *S. haematobium*, Obuyu (1970) reported similar differences, namely that ova were found in the faeces of the monkeys 10–16 weeks after infestation, while they appeared in the urine only 16–27 weeks after infestation.

The schistosomes may reach the urinary tract as a result of aberrant migration, or of overcrowding of the intestinal sites or as part of the normal migratory pattern. These results do not support the overcrowding hypothesis since 7 animals with low worm burdens (under 5 000) and infested for long periods had urinary tract lesions, while animals with high worm burdens (above 80 000) and shorter periods of infestation did not have them.

Movement of the parasites to the urinary tract involves migration from the portal venous system to branches of the posterior vena cava. It is unknown to what extent collaterals (anastomoses in the vicinity of the urinary tract) available to the schistosomes in man also occur in cattle.

Portal hypertension, with a consequent increase in the number of collateral vessels, may facilitate aberrant worm migration. The time required for the development of collateral circulation may account for the delay in the arrival of the parasites in the urinary blood vessels. Another possibility is that the migrating shistosomulae may continue to migrate in the posterior vena cava instead of moving into the hepatic vein (Kruger, Heitmann, Van Wyk & McCully, 1969), but in this case the prepatent period of urinary schistosomiasis would not be much longer than that of the intestinal form.

Further studies are required to elucidate the host-parasite relationship of urinary schistosomiasis in cattle and answer the questions raised by the above observations.

III. The Kidneys

1. *Lesions of the renal pelvis*

We can find no reference to the occurrence of bilharzial granulomata of the renal pelvis in man, cattle or experimental animals. Linear granulomatous lesions were observed in 20% of the renal pelves in this study. All the affected animals were in the experimentally infested groups. Lesions at this site may possibly develop only with heavy urinary infestation since they were less commonly observed than ureteric lesions and were always associated with the more severe lesions in the ureters. However, despite heavy infestations, none of the field cases had these lesions. The peculiar linear distribution of the granulomata provide evidence that the worm pairs may migrate as they produce ova. This agrees with observations by Pellegrino (personal communication, 1974) and Van Rensburg (personal communication, 1974) on mice and hamsters infested with single pairs of *S. mansoni* and *S. mattheei*, respectively, where the entire length of the intestine contained granulomata.

The linear granulomata of the pelvis as in the ureters appear to be of little pathological significance.

2. *Interstitial nephritis*

The incidence of multifocal interstitial lymphoid infiltration and associated nephron atrophy was more than double in the bilharzia infested cattle (67,5%) than in the uninfested controls (32,0%). Hydronephrosis was not present to account for the interstitial inflammation, nor was the cause or the pathogenesis of the interstitial lesions evident.

In man, interstitial cellular infiltration with tubular and glomerular atrophy has been reported in urinary schistosomiasis and is considered secondary to pyelonephritis (Sabbour, El-Said & Abou-Gabal, 1972). Kidneys in the cases studied by them also contained glomerular lesions ranging from mesangial proliferation to glomerulosclerosis, and a high percentage had significant proteinuria, hypoalbuminaemia and varying degrees of subcutaneous oedema. Schistosomiasis was responsible for 50,5% of cases in their renal unit and renal failure was occasionally the direct cause of death in these patients.

3. *Mesangial hyperplasia*

Glomerular lesions were observed in 53,8% of the cases studied but these could not be correlated to either single infestation or reinfestation, to the worm burden or to the duration of infestation. The incidence of these lesions in the infested animals was more than double that of the abattoir controls (24,0%). Mesangial proliferation, thickening and glomerular adhesions did not appear to develop into the atrophic and sclerotic lesions that accompanied the interstitial infiltration of lymphoid cells. In human bilharziasis, on the other hand, Andrade, Andrade & Sadigursky (1971) graded glomerular lesions according to severity and speculated that there may be a progression leading to glomerulosclerosis.

Similar renal changes, termed schistosomal nephropathy, have been reported in man infested with *S. haematobium* (Gelfand & Weinberg, 1968) and *S. mansoni* (Da Silva *et al.*, 1970; Andrade *et al.*, 1971) and in experimentally infested chimpanzees (Von Lichtenberg, Sadun, Cheever, Erickson, Johnson & Boyce 1971; Cavallo, Galvanak, Sadun & Von Lichtenberg, 1972), rabbits (Von Lichtenberg, Sadun & Bruce, 1972) and Cebus monkeys (De Brito, Gunji, Camargo, Ceravolo & Da Silva, 1971) harbouring either *S. japonicum* or *S. mansoni*.

Schistosomal renal disease in man, whether related to interstitial nephritis or glomerular lesions, is of clinical importance. However, the importance of the reported glomerulopathy and interstitial nephritis in cattle is unknown. Hypoalbuminaemia, hypergammaglobulinaemia and mild proteinuria were observed in the sole experimental animal tested, but haemodilution, owing to an increased plasma volume, seems to be a constant finding in animals and humans infested with schistosomiasis, and hence the hypoalbuminaemia in this one case is probably of significance (Dargie, MacLean & Preston, 1973).

Mesangial hypertrophy and hyperplasia have frequently been reported in ultrastructural studies of schistosomal nephropathy (Da Silva *et al.*, 1970; Sabbour *et al.*, 1972; Von Lichtenberg *et al.*, 1972). Focal electron-dense, granular deposits were observed on the endothelial side of the basement membrane near or underlying mesangial cells and within the mesangial and endothelial cells (Da Silva *et al.*, 1970, Sabbour *et al.*, 1972). These ultrastructural findings were augmented by the immunofluorescent studies of Da Silva *et al.*, (1970), who demonstrated immunoglobulins (IgG, and IgM) and the β_{1C} fraction of complement in the glomeruli. They concluded that the glomerular capillary walls were probably the depot for immune complexes rather than a specific, antigen-antibody reaction site.

Hypergammaglobulinaemia is a common finding in bilharzia-infested man and animals. However, schistosome-specific circulating antigens have only recently been reported in infested animals (Berggren & Weller, 1967). Subsequently, Gold, Rosen & Weller (1969) described a specific schistosomal antigen in the circulation and also in the urine and

attributed its occurrence in the urine to its low molecular mass (less than 10 000). It is thought that the reaction between circulating host antibody and foreign antigen forms soluble complexes which may concentrate in the glomeruli resulting in injury (Dixon, Wilson & Marquardt, 1971). Immune-complex glomerular injury has been shown experimentally to stimulate mesangial proliferation and thickening and may initiate a progression toward chronic glomerulonephritis (Mauer, Sutherland, Howard, Fish, Najarian & Michael, 1973). It is possible that this hyperplasia and hypertrophy facilitate the removal of the deposited bilharzia immune-complexes and, if effective, prevent the development of glomerular damage leading to glomerulosclerosis.

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References

ANDERSON, W. A., ed., 1971. Pathology. 6th Edition. Mosby St. Louis.

ANDRADE, Z. A., ANDRADE, S. G. & SADIGURSKY, M., 1971. Renal changes in patients with hepatosplenic schistosomiasis. *The American Journal of Tropical Medicine & Hygiene*, 20, 77–83.

BERGGREN, W. L. & WELLER, T. H., 1967. Immunoelectrophoretic demonstration of specific circulating antigen in animals infected with *Schistosoma mansoni*. *The American Journal of Tropical Medicine & Hygiene*, 16, 606–612.

BHAGWANDEEN, S. B., 1967. The pathology of ureteric bilharziasis. *The South African Medical Journal*, 41, 950–955.

BUCHANAN, W. M., 1971. Cirrhosis and bilharzial fibrosis of the liver in Rhodesia. *Central African Journal of Medicine*, 17, 139–144.

CAVALLO, P. A. W., GALVANAK, E. G., SADUN, E. H. & VON LICHTENBERG, F., 1972. The nephropathy of hepatosplenic schistosomiasis. *The American Journal of Pathology*, 66, 33a.

CHEEVER, A. W. & ANDERSON, LOREN A., 1971. Rate of destruction of *Schistosoma mansoni* eggs in the tissues of mice. *The American Journal of Tropical Medicine & Hygiene*, 20, 62–68.

CHEEVER, A. W. & POWERS, K. G., 1971. Rate of destruction of *Schistosoma mansoni* eggs and adult worms in the tissues of rhesus monkeys. *The American Journal of Tropical Medicine & Hygiene*, 20, 69–76.

CONDY, J. B., 1960. Bovine schistosomiasis in Southern Rhodesia. *The Central African Journal of Medicine*, 6, 381–384.

DARGIE, J. D., MACLEAN, J. M. & PRESTON, J. M., 1973. Pathophysiology of ovine schistosomiasis. III. Study of plasma protein metabolism in experimental *Schistosoma mattheei* infections. *The Journal of Comparative Pathology*, 83, 543–557.

DA SILVA, L. C., DE BRITO, T., CAMARGO, M. E., DE BONI, D. R., LÓPEZ, J. D. & GUNJI, J., 1970. Kidney biopsy in the hepatosplenic form of infection with *Schistosoma mansoni* in man. *Bulletin of the World Health Organization*, 42, 907–910.

DE BRITO, T., GUNJI, J., CAMARGO, M. E., CERAVOLO, A. & DA SILVA, L. C., 1971. Glomerular lesions in experimental infections of *Schistosoma mansoni* in *Cebus apella* monkeys. *Bulletin of the World Health Organization*, 45, 419–422.

DIXON, F. J., WILSON, C. B. & MARQUARDT, H., 1971. Experimental immunologic glomerulonephritis. *In*: Advances in Nephrology. HAMBURGER, J., CROSNIER, J. & MAXWELL, M. H., editors. Year Book Medical Publishers, Inc. Chicago. Illinois.

DOWNIE, N. M. & HEATH, R. W., 1965. Basic statistical methods. Harper & Row: New York.

EDINGTON, G. M., VON LICHTENBERG, F., NWA-BUEBO, I., TAYLOR, J. R. & SMITH, J. H., 1970. Pathologic effects of schistosomiasis in Ibadan, Western State of Nigeria. I. Incidence and intensity of infection; distribution and severity of lesions. *The American Journal of Tropical Medicine & Hygiene*, 19, 982–995.

ELSDON-DEW, R., 1967. Is bilharzia a problem? *The South African Medical Journal*, 41, 969–970.

FORSYTH, D. M. & MACDONALD, G., 1965. Urological complications of endemic schistosomiasis in school-children. Part I. Usagara School. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 59, 171–178.

GELFAND, M., 1948. Bilharzial affection of the ureter. *The British Medical Journal*, 1, 1228–1230.

GELFAND, M., 1967. A clinical study of intestinal bilharziasis (*Schistosoma mansoni*) in Africa. London: Edward Arnold Ltd. 152–156.

GELFAND, M., 1973. The effects of bilharziasis in Rhodesia. *The Central African Journal of Medicine*, 19 (Suppl.), 1–11.

GELFAND, M. & WEINBERG, R., 1968. Early inflammatory changes in the kidney in bilharzia of the lower urinary tract. *The Journal of Tropical Medicine & Hygiene*, 71, 285–287.

GILLES, H. M., LUCAS, A., ADENIYI-JONES, C., LINDNER, R., ANAND, S. V., BRABAND, H., COCKSHOTT, W. P., COWPER, S. G., MULLER, R. L., HIRA, P. R. & WILSON, A. M. M., 1965. *Schistosoma haematobium* infection in Nigeria. II. Infection at a primary school in Ibadan. *Annals of Tropical Medicine & Parasitology*, 59, 441–450.

GOLD, R., ROSEN, F. S. & WELLER, T. H., 1969. A specific circulating antigen in hamsters infected with *Schistosoma mansoni*. *The American Journal of Tropical Medicine & Hygiene*, 18, 545–552.

GÖNNERT, R., 1955. Schistosomiasis-Studien. II. Über die Eibildung bei *Schistosoma mansoni* und das Schicksal der Eier im Wirtsorganismus. *Zeitschrift für Tropenmedizin und Parasitologie*, 6, 33–52.

HARLEY, J., 1864. On the endemic haematuria of the Cape of Good Hope. *Medico-Chirurgical Transactions*. London, 47, 55–72.

HOLLINSHEAD, W. H., 1956. Anatomy for Surgeons: Vol. 2. The Thorax, Abdomen and Pelvis. Hoeber-Harper, New York.

HONEY, R. M., & GELFAND, M., 1960. The urological aspects of bilharziasis in Rhodesia. E. & S. Livingstone, Edinburgh.

HUSSEIN, M. F., 1968. Observations on the pathology of natural and experimental bovine schistosomiasis. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 62, 9.

HUSSEIN, M. F., 1971. The pathology of experimental schistosomiasis in calves. *Research in Veterinary Science*, 12, 246–252.

HUSSEIN, M. F., 1973. Animal schistosomiasis in Africa; a review of *Schistosoma bovis* and *Schistosoma mattheei*. *The Veterinary Bulletin*, 43, 341–347.

KISNER, C. R., 1952. Vesical bilharziasis in South Africa; an analysis of the cystoscopic appearances of vesical bilharziasis before and after treatment with various anti-schistosomal drugs, and a coincidental study of bilharzial strictures of the ureter. M.D. Thesis. University of the Witwatersrand.

KLOETZEL, K., 1967. Egg and pigment production in *Schistosoma mansoni* infections of the white mouse. *The American Journal of Tropical Medicine & Hygiene*, 16, 293–299.

KRUGER, S. P., HEITMANN, L. P., VAN WYK, J. A. & McCULLY, R. M., 1969. The route of migration of *Schistosoma mattheei* from the lungs to the liver in sheep. *Journal of the South African Veterinary Medical Association*, 40, 39–43.

LAWRENCE, J. A., 1973. *Schistosoma mattheei* in cattle: the host-parasite relationship. *Research in Veterinary Science*, 14, 400–402.

LAWRENCE, J. A. & McKENZIE, R. L., 1972. Schistosomiasis in farm livestock. *The Rhodesia Agricultural Journal*, 69, 79–83.

LE ROUX, P. L., 1929. Remarks on the habits and the pathogenesis of *Schistosoma mattheei*, together with notes on the pathological lesions observed in infested sheep. *15th Report of the Director of Veterinary Services, Union of South Africa*, 347–406.

MAKAR, N., 1968. The bilharzial ureter. *The British Journal of Surgery*, 36, 148–158.

MALDONADO, J. F., 1959. The longevity of the unhatched miracidium of *Schistosoma mansoni* in the tissues of mice. *The American Journal of Tropical Medicine & Hygiene*, 8, 16–19.

MALEK, E. A., 1969. Studies on bovine schistosomiasis in the Sudan. *Annals of Tropical Medicine & Parasitology*, 63, 501–513.

MAUER, S. M., SUTHERLAND, D. E. R., HOWARD, R. J., FISH, A. J., NAJARIAN, J. S. & MICHAEL, A. F., 1973. The glomerular mesangium. III. Acute immune mesangial injury: a new model of glomerulonephritis. *The Journal of Experimental Medicine*, 137, 553–561.

McCULLY, R. M. & KRUGER, S. P., 1969. Observations on bilharziasis of domestic ruminants in South Africa. *The Onderstepoort Journal of Veterinary Research*, 36, 129–162.

MILLER, R. G., 1966. Simultaneous statistical inference. McGraw-Hill Book Co., New York.

OBUYU, C. K. A., 1970. Preliminary communication on gross pathological lesions in the genito-urinary tract of vervet monkeys experimentally infected with *Schistosoma haematobium*. *Annals of Tropical Medicine & Parasitology*, 64, 395–398.

PITCHFORD, R. J., 1958. Animal reservoirs of human bilharziasis in the Eastern Transvaal. *Bulletin of the World Health Organization*, 18, 1080.

PITCHFORD, R. J., 1959. Cattle schistosomiasis in man in the Eastern Transvaal. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 53, 285–290.

PITCHFORD, R. J., 1961. Observations on a possible hybrid between the two schistosomes *S. haematobium* and *S. mattheei*. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 55, 44–51.

SABBOUR, M. S., EL-SAID, W. & ABOU-GABAL, I., 1972. A clinical and pathological study of schistosomal nephritis. *Bulletin of the World Health Organization*, 47, 549–557.

SADUN, E. H., VON LICHTENBERG, F., CHEEVER, A. W., ERICKSON, D. G. & HICKMAN, R. L., 1970. Experimental infection with *Schistosoma haematobium* in chimpanzees. *The American Journal of Tropical Medicine & Hygiene*, 19, 427–458.

SAYEGH, E. S., 1950. Late complications of urinary bilharziasis. *The Journal of Urology*, 63, 353–371.

SIEGEL, S., 1956. Nonparametric statistics for the behavioural sciences. McGraw-Hill Book Co., New York.

SMITH, J. H., TORKEY, H. & MANSOUR, N., 1972. The relationship of egg excretion and tissue egg burden in urinary schistosomiasis. *The American Journal of Pathology*, 66, 33a.

VAN WYK, J. A., 1973. Studies on Schistosomiasis. 4. Differential staining of live and dead cercariae after immobilization with physostigmin. *The Onderstepoort Journal of Veterinary Research*, 40, 23–30.

VAN WYK, J. A. & BARTSCH, R. C., 1971. Stootsiektesindroom by eksperimentele hiperergiese schistosomiase/ *Staggers syndrome in experimental hyperergic schistosomiasis*. *Journal of the South African Veterinary Medical Association*, 42, 274.

VAN WYK, J. A. & GROENEVELD, H. T., 1973. Studies on schistosomiasis. 5. Sampling methods for estimating numbers of cercariae in suspension with special reference to the infestation of experimental animals. *The Onderstepoort Journal of Veterinary Research*, 40, 157–174.

VAN WYK, J. A., BARTSCH, R. C., VAN RENSBURG, L. J., HEITMANN, L. P. & GOOSEN, P. J., 1974. Studies on schistosomiasis. 6. A field outbreak of bilharzia in cattle. *The Onderstepoort Journal of Veterinary Research*, 41, 39–49.

VEGLIA, F. & LE ROUX, P. L., 1929. On the morphology of a schistosome (*Schistosoma mattheei*, sp. nov.) from the sheep in the Cape Province. *15th Annual Report of the Director of Veterinary Research, Union of South Africa*, 335–346.

VON LICHTENBERG, F., 1967. The bilharzial pseudotubercle: a model of the immunopathology of granuloma formation. *In*: Immunologic aspects of parasitic infections. Scientific Publication No. 150, Pan American Health Organization, Washington, D.C.

VON LICHTENBERG, F., SADUN, E. H. & BRUCE, J. I., 1962. Tissue responses and mechanisms of resistance in schistosomiasis mansoni in abnormal hosts. *The American Journal of Tropical Medicine & Hygiene*, 2, 347–356.

VON LICHTENBERG, F., SADUN, E. H. & BRUCE, J. I., 1972. Renal lesions in *Schistosoma japonicum* infected rabbits. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, 66, 505–507.

VON LICHTENBERG, F., SMITH, J. H. & CHEEVER, A. W., 1966. The Hoeppli phenomenon in schistosomiasis. Comparative pathology and immunopathology. *The American Journal of Tropical Medicine & Hygiene*, 15, 886–895.

VON LICHTENBERG, F., EDINGTON, G. M., NWABUEBO, I., TAYLOR, J. R. & SMITH, J. H., 1971. Pathologic effects of schistosomiasis in Ibadan, Western State of Nigeria. II. Pathogenesis of lesions of the bladder and ureters. *The American Journal of Tropical Medicine & Hygiene*, 20, 244–254.

VON LICHTENBERG, F., SADUN, E. H., CHEEVER, A. W., ERICKSON, D. G., JOHNSON, A. J. & BOYCE, H. W., 1971. Experimental infection with *Schistosoma japonicum* in chimpanzees. Parasitologic, clinical, serologic and pathological observations. *The American Journal of Tropical Medicine & Hygiene*, 20, 850–893.

WARREN, K. S., 1973. The pathology of schistosome infections. *Helminthological Abstracts, Series A, Animal & Human Helminthology*, 42, 591–633.

YOUNG, S. W., FARID, Z., BASSILY, S. & EL-MASRY, N. A., 1973. Urinary schistosomiasis in Egypt: Further radiological correlations. *The Transactions of the Royal Society of Tropical Medicine & Hygiene*, 67, 417.