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PULMONARY INVOLVEMENT IN SCHISTOSOMIASIS MANSONI

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The post-treatment pulmonary alterations were evaluated in patients (Study 1) and in mice (Study 2) infected with Schistosoma mansoni.

Study 1: *the patients were examined pre and post-treatment (with ora oxamniquine) and the following exams were performed: sputum for eosinophils and chest x-ray.*

Study 2: *four groups of mice (total = 64) were studied: Group I (infected and treated with oxamniquine); II (infected and not treated); III (not infected and treated) and IV (not infected and not treated). All were x-rayed to check for pulmonary abnormalities pre and post-treatment and lung specimens were studied by optical microscopy and immunofluorescence.*

We have found abnormalities in the parameters checked in both studies and the results suggest an immunological reaction, probably due to deposition of immune complexes in the lungs, with subsequent activation of the complement system. The experimental study showed that the alterations are not dependent of the presence of eggs and/or worms of S. mansoni *in the lungs, thus corroborating the hypothesis of deposition of circulating material.*

There are clinical, radiological, histological and laboratory evidence of pulmonary and renal involvement in various immune complex diseases (Cochrane, 1971). The formation and tissue deposition of these immune complexes in schistosomiasis mansoni may be important in the pathophysiology of this disease (Santoro et al., 1977). The clinical manifestations of the acute phase, the renal and pulmonary involvement are compatible with the formation and deposition of these complexes (Gelfand, 1966; Mendes, 1981; Philips & Fox, 1982).

It is well known that up to three weeks post-treatment a number of patients with schistosomiasis mansoni develop clinical and laboratorial manifestations. These may be mild (urticaria, increases in blood eosinophilia, cough) or severe (dead worm pneumonitis, deterioration of liver function, acute cor pulmonale) (McKensie, 1932; Lambertucci et al., 1982). Serum sickness and even anaphylaxis have been reported post-treatment with antimonials, hycanthone and oxamniquine, and hypersensitivity reactions (type I/III, Gell & Coombs) (Gell et al., 1974) and/or killing and dislodging of the worms have been implicated (Coutinho & Coelho, 1940; Higashi & Farid, 1979; Prata & Machado, 1960).

In this work we evaluated the possible pulmonary alterations post-treatment with oxamniquine in patients (Study 1) and mice (Study 2) infected with *Schistosoma mansoni.*

MATERIAL, PATIENTS AND METHODS

STUDY 1

We have examined 40 patients with hepatosplenic and six with intestinal schistosomiasis (ages 4 to 44). They were treated with a single oral dose of oxamniquine (20 mg/kg) and clinical and laboratory evaluation were performed in all patients.

Clinical evaluation: thorough clinical examination was performed to determine the clinical phase of the disease. The criteria to define the hepatosplenic form was both clinical (palpable liver and spleen) and radiological (presence of esophageal varices).

Post-treatment the patients were examined daily and we looked for alterations especially in the lungs and abdomen (liver and spleen size); axillar temperature was checked regularly.

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Laboratory evaluation: chest x-ray was performed pre-treatment and on days 1, 3, 5 and 10 post-treatment. The patients with any pulmonary changes were x-rayed also on days 15 and 30. The chest films were evaluated on a single-blind manner by three investigators.

STUDY 2

Sixty-four male outbred albino mice, weighing from 18 to 26 g, one month old were studied. They were randomized in 4 groups of 16 animals each:
Group I: infected and treated (IT);
Group II: infected and not treated (INT);
Group III: not infected and treated (NIT);
Group IV: not infected and not treated (NINT), general control of the experiment.

Group I and II mice were infected on their 30th day of life (day 0) through a transcutaneous abdominal injection of 80 cercariae of *S. mansoni* (LE strain, Belo Horizonte); Group I and III mice were treated on day 59 of the experiment with a single intramuscular doses of oxamniquine (200 mg/kg body weight). All animals were killed on day 65.

Laboratory evaluation

a) *Chest x-ray:* all mice were x-rayed on days 59 and 65. A Philips Super 100 with 45 KV and 200 MA at a distance of 1.5 meter was used. Kodak film PE 4006 was employed and the animals were kept still on a special rack. The films were read by three observers, single-blinded: alterations were considered when at least two of the observers agreed.

b) *Light microscopy:* the mice were killed by medullary mechanical fracture on day 65 and necropsied. Sections of the lungs were fixed in 10% formalin and 5 micra specimens stained with hematoxilin-eosin (HE) and Schiff's periodic acid (SPA).

c) *Immunofluorescence:* a 0.05% solution of Tissue Teck Compound (OCT-Miles Laboratories, Inc.) was injected through the trachea to facilitate the slicing of the lungs. Liquid nitrogen (– 30 °C) was used and the 4 micra specimens obtained in a Clay-Adams microtome (Bigazzo & Tilton, 1980).

- Direct immunofluorescence: the following sera were employed: Fluorescein conjugated goat anti-mice immunoglobulin (Cappel Laboratories, Inc.) and fluorescein conjugated goat anti-mice C3 (Cappel Laboratories, Inc.).

- Indirect immunofluorescence: rabbit anti-*Schistosoma mansoni* antiserum (anti-adult worm); sheep anti-rabbit immunoglobulin fluorescein conjugated was added and sheep anti-rabbit immunoglobulin served as control.

d) *Parasitological evaluation:* the infection was confirmed by coprological examinations. The infected animals were perfusioned according to Pellegrino and Siqueira's technique (Pellegrino & Siqueira, 1956) to collect *Schistosoma* worms from the mesenteric and portal veins; the lungs and liver were also crushed in Petri dishes and examined under a stereoscope to look for trapped worms.

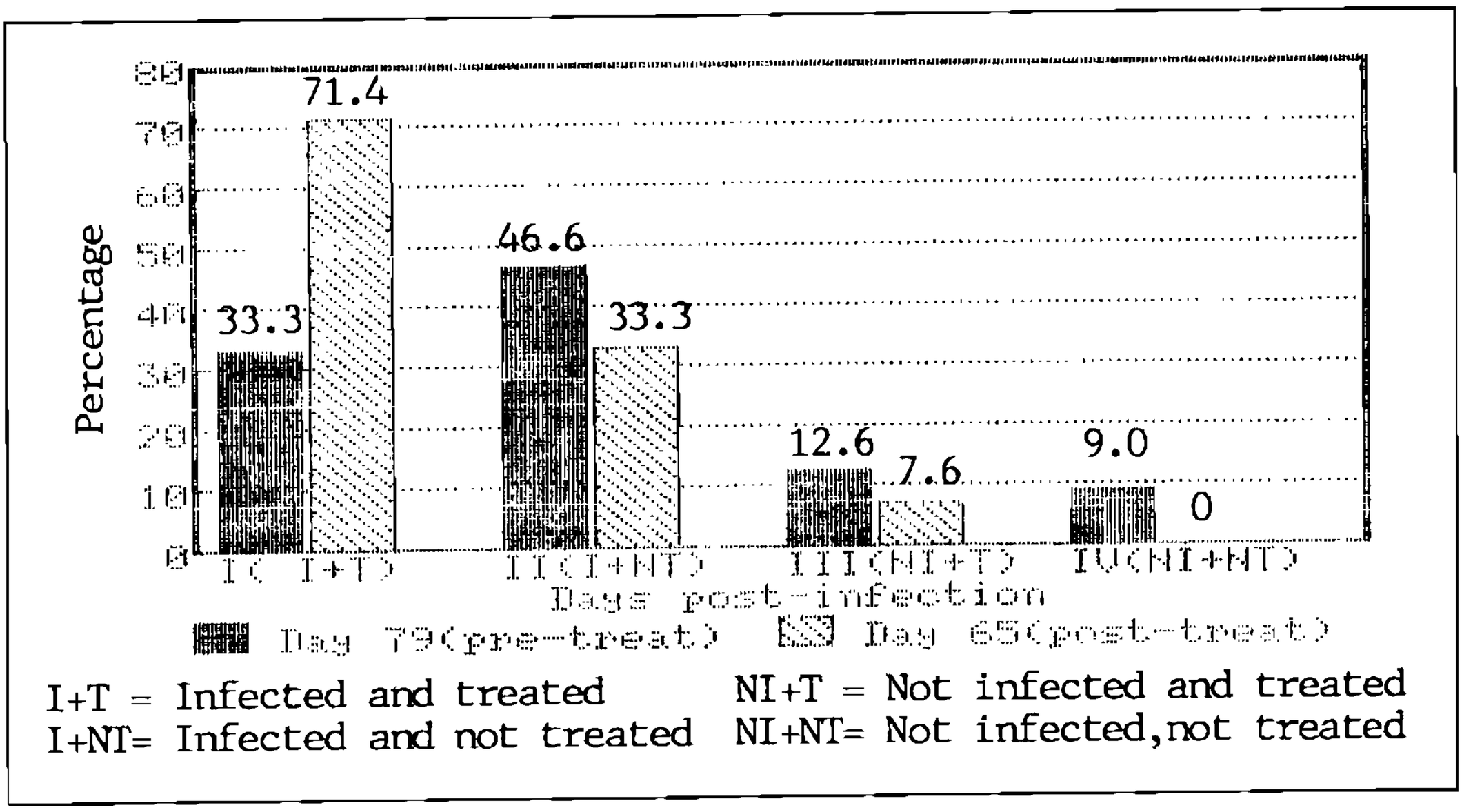
RESULTS

STUDY 1

Thirteen patients (28.8%) had pulmonary alterations on the radiological study: bronchopneumonitis (6 = 46.15%), pneumonitis (4 = 30.77%), prominent medium arc (2 = 15.38%) and pulmonary congestion (1 = 7.69%). These alterations were transitory starting within 3 days post-treatment, with spontaneous recovery in less than 30 days. The presence of sputum eosinophils up to the 12th day post-treatment had a direct correlation with the appearance of x-ray abnormalities and did not depend on the phase of the disease; there was no statistical correlation between the presence of esophageal varices and the pulmonary alterations.

STUDY 2

Chest x-ray: there were x-ray abnormalities in 33.3% (5/15) of Group I mice pre-treatment and in 71.4% (5/7) post-treatment ($P < 0.05$). The pulmonary alteration in the other three groups were in significant smaller numbers and treatment itself was not responsible for any change in Group III (NIT). These findings are on Graph 1 and illustrated in Plate 1.



Graph 1 : lung x-ray changes in mice pre and post-treatment with oxamniquine.

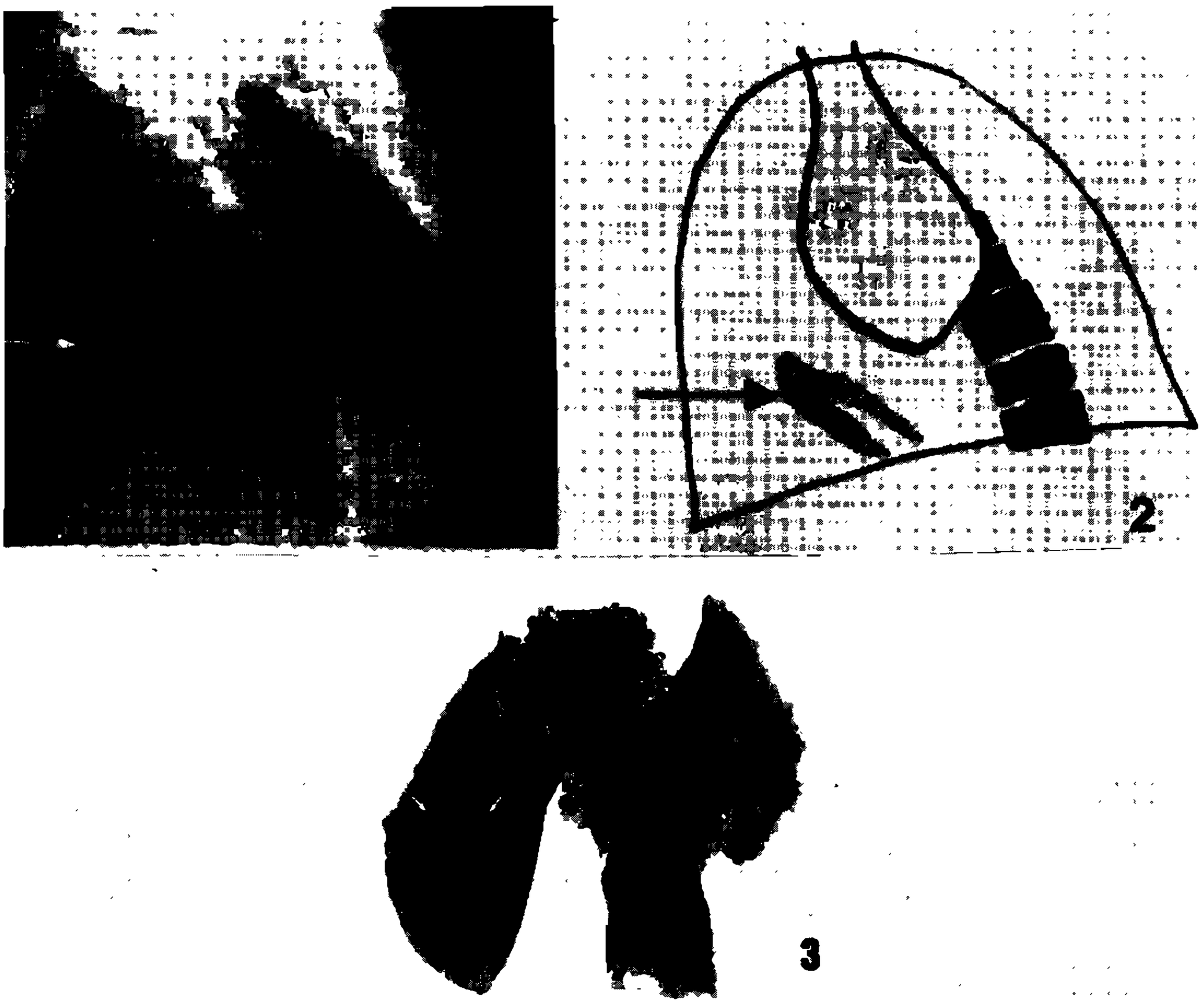


Plate 1 : mouse from Group I (infected with *Schistosoma mansoni* and treated with oxamniquine) – 1. X-ray post-treatment : condensation in the right lung (arrow). 2. Schematic drawing of the x-ray alteration. 3. Necropsy: photomicrography of the same lesion (arrows).

Light microscopy: many alterations were found in the lung specimens stained by HE and SPA. In most cases it was not possible to define them as specific for *S. mansoni* infection, because all animals, including Group III and IV were susceptible to other infections (viral, parasitic and/or bacterial), and the lesions were not related to the presence of granuloma or *S. mansoni* worms.

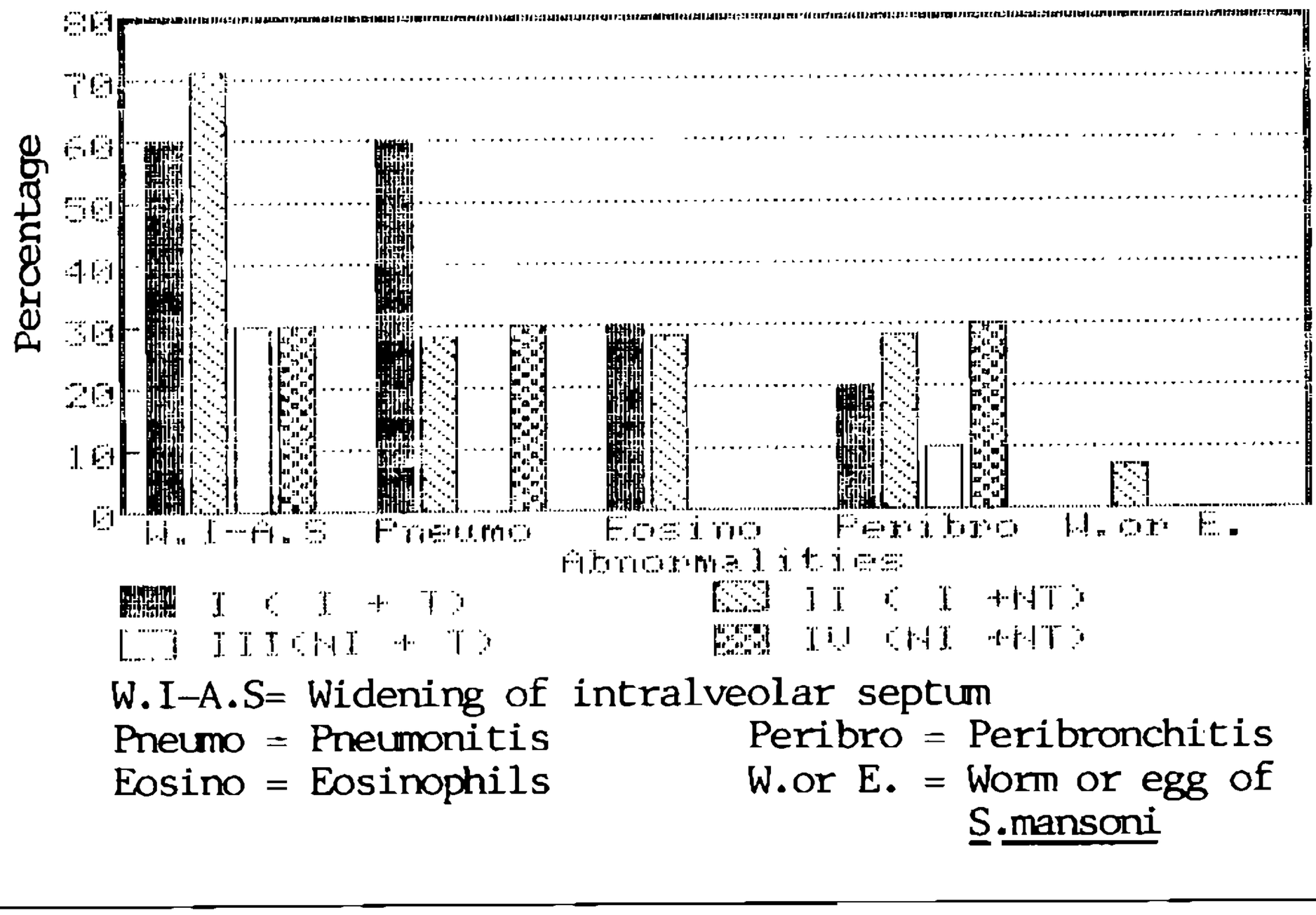
In the infected groups (I and II) the majority of animals showed some abnormality: Group I = 100% (10 out of 10); Group II = 85.8% (12/14). The most frequent alterations were widening of the interalveolar septum (I = 60%, II = 71.3%); interstitial pneumonitis (I = 60%, II = 28.5 %); eosinophil infiltrate (I = 30%, II = 28.5%).

Adult worm and egg of *S. mansoni* were found in only one Group II animal. In the non-infected groups (III and IV) there were fewer abnormalities, both qualitative and quantitative: Group III = 50% (5/10), Group IV = 70% (7/10). They comprised mild and isolated widening of the interalveolar septum and peribronchitis. There were no eosinophil infiltrates. Even considering all the lesions in the non-infected (III and IV = 54.5%, 12 out of 20) and comparing with the infected (I and II = 91%, 22 out of 24) this difference is statistically significant ($p < 0.05$). Graph 2 summarizes the results.

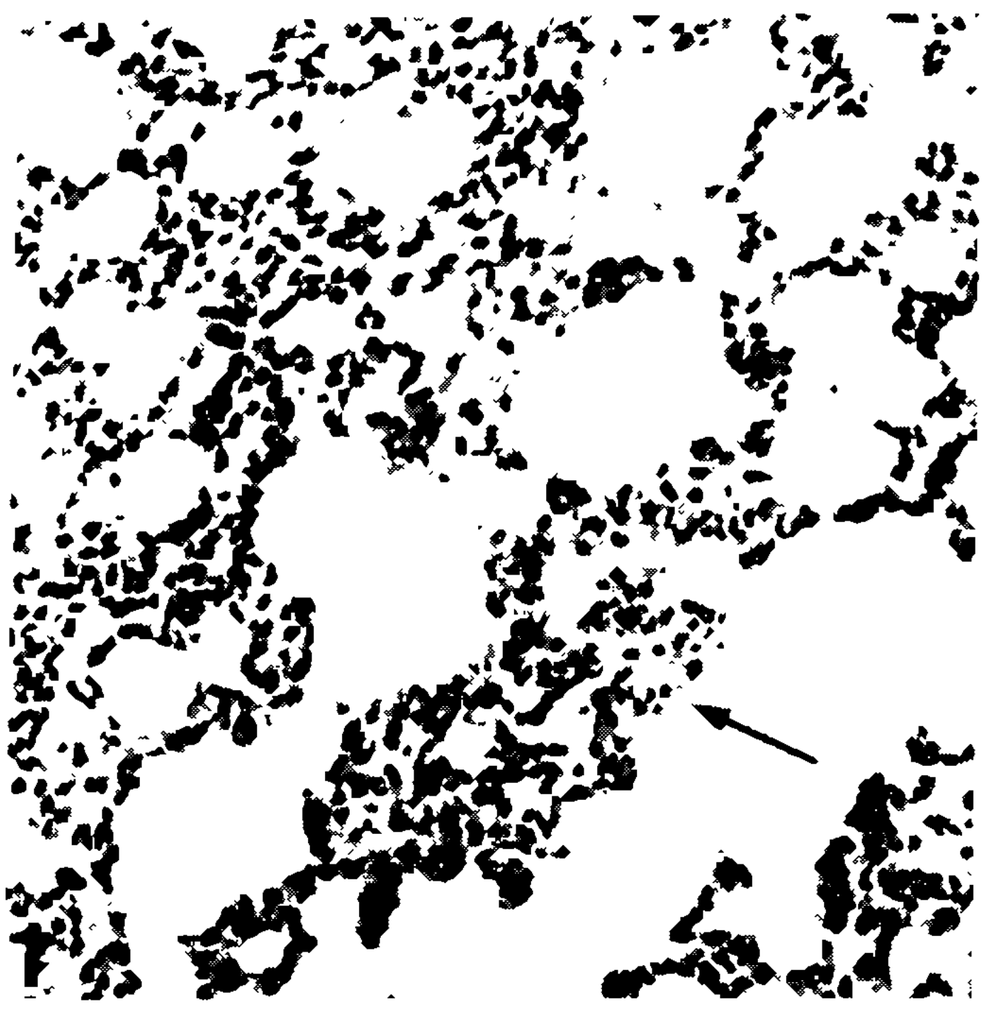
Lung immunofluorescence: the fluorescence (IF) was granular, localizing mainly in the interalveolar septum and in the basal membranes of vessels.

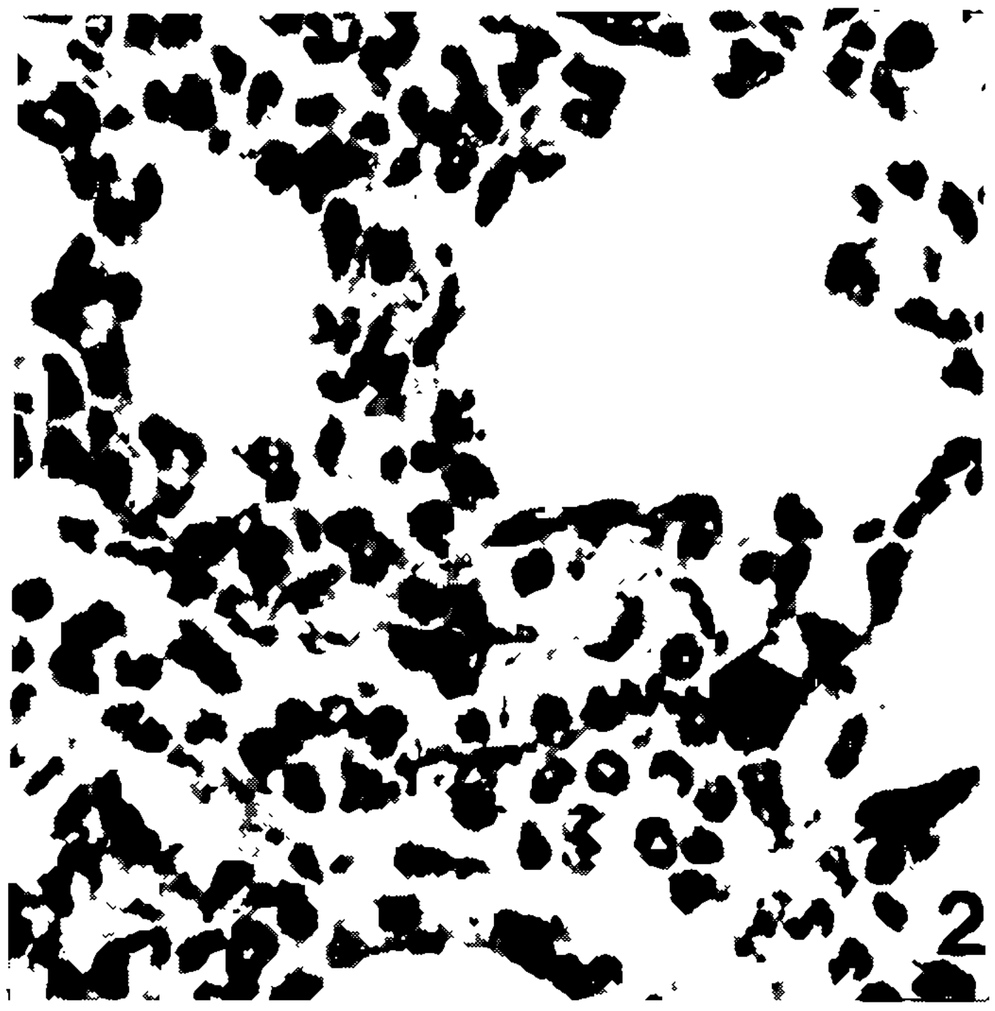
- Deposition of immunoglobulins: in the infected groups, the IF for immunoglobulin was positive in 18 mice (81.8%): Group I = 62.5% (5/8), Group II = 93% (13/14). In the non-infected Groups the IF was positive in only 4 animals (17.4%): Group III = 25% (2/8), Group IV = 20% (3/15). The difference between the infected and non-infected was significant ($p < 0.05$).
- Deposition of C3: the infected animals, 5 (all from Group I) had positive IF for C3 (22.7%, 5 out of 22): Group I = 62.5%, 1, 5 out of 8). Groups III and IV were all negative.
- Deposition of *S. mansoni* antigens: in Group I and II there were 2 positive cases (9.1%, 2 out of 22), both in Group I = 25% (2/8). Groups III and IV were negative.

These results are condensed in Graph 3 and illustrated in Plate 2.



Graph 2: light microscopy in the lungs of mice post-treatment with oxamniquine.





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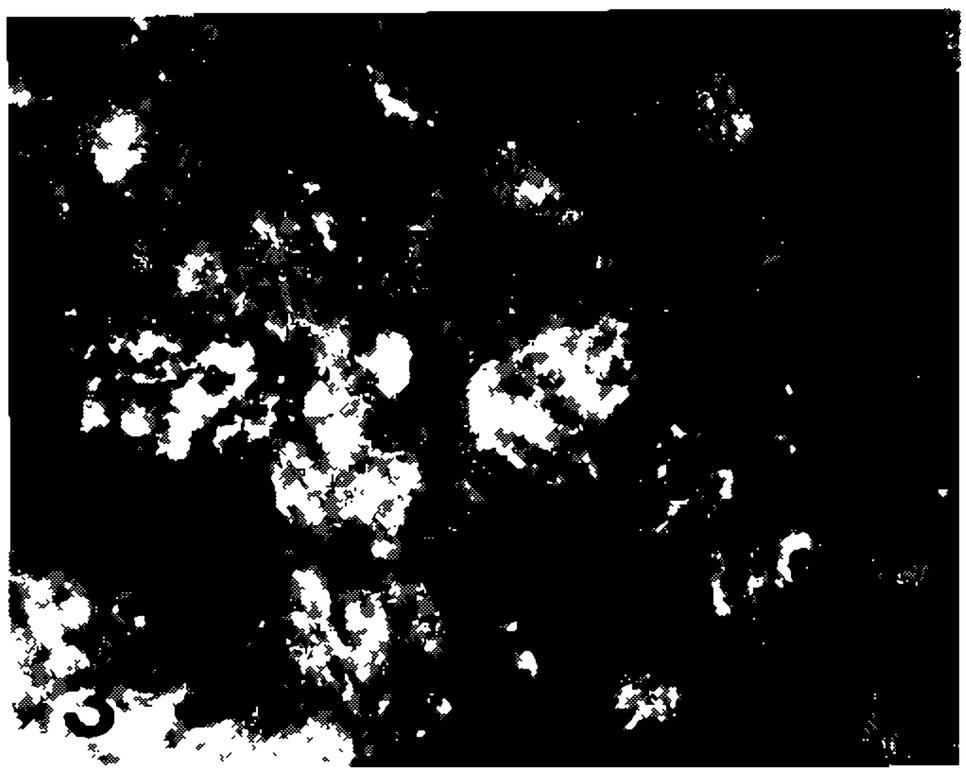

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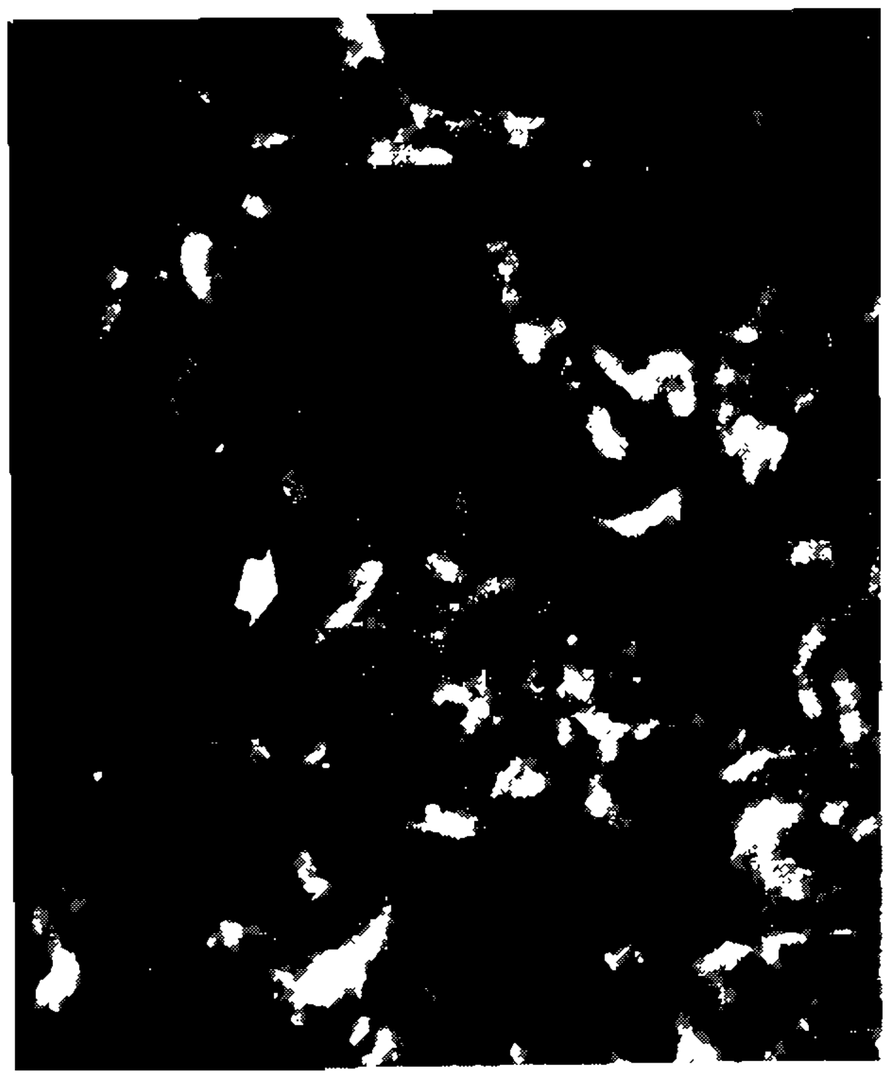
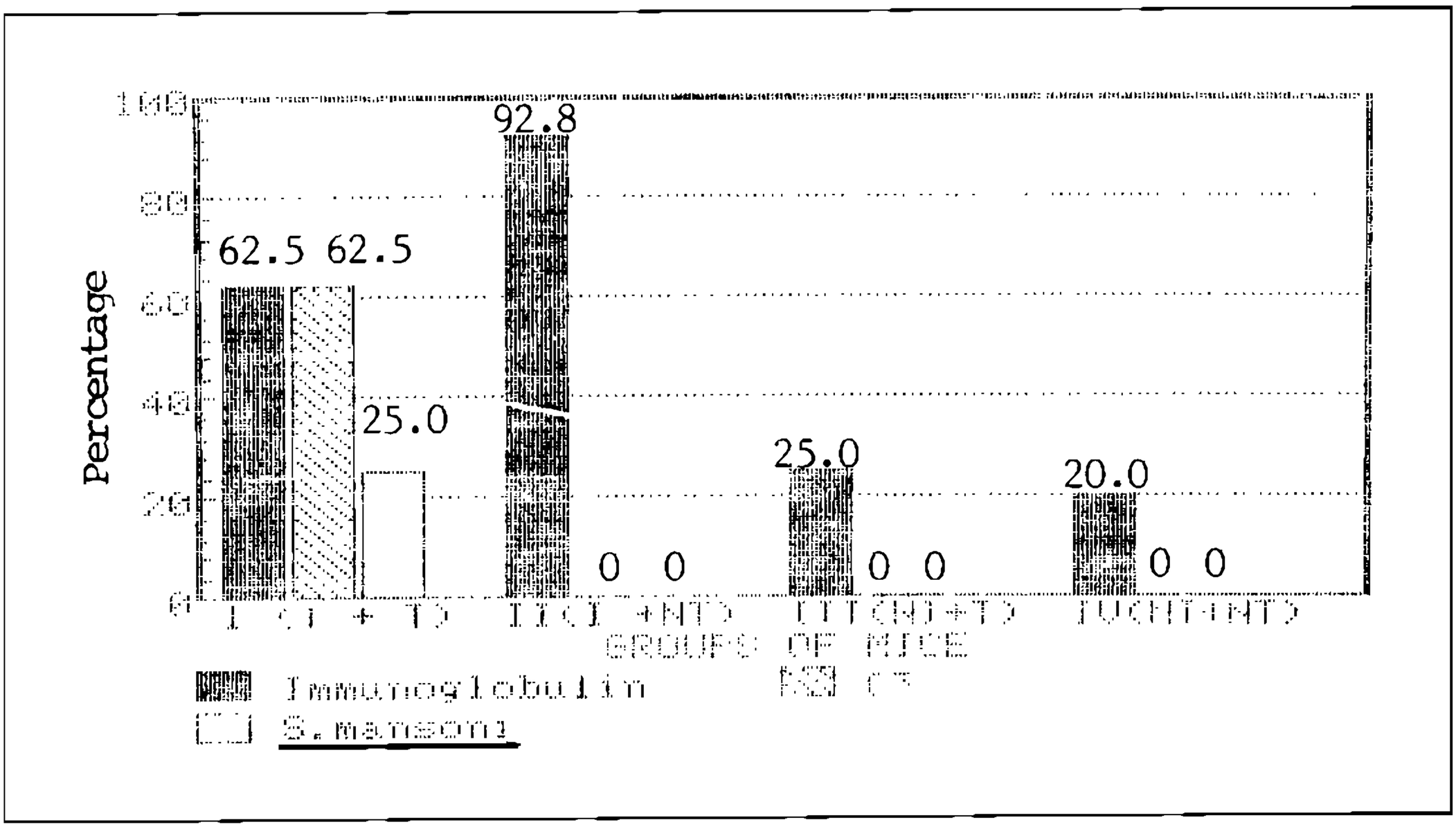


Plate 2: mouse from Group I (infected with *Schistosoma mansoni* and treated with oxamniquine) – 1. Optical microscopy (lung): widening of the intralveolar septum (HE x 16). 2. Detail of photo 1 (HE x 40): inflammatory infiltrate with eosinophils (arrow). 3. Fluorescence microscopy (lung): incubated with anti-immunoglobulin serum (40 x). 4. Fluorescence microscopy (lung): incubated with anti-C3 serum (16 x). 5. Fluorescence microscopy (lung): incubated with anti-*S. mansoni* serum (16 x).



Graph 3: lung immunofluorescence in mice pre and post-treatment with oxamniquine.

Parasitological evaluation: all mice from Groups I and II were perfusioned on day 65. The average number of adult *Schistosoma mansoni* worms in the mesenteric and portal veins were: Group I = 11.1; Group II = 37.8. The liver and lungs were crushed in Petri dishes and microscopically examined. Worms were found in the liver: Group I = 11.6, II = 0.7; in the lungs: Group I = 0, Group II = 0. This shows that post-treatment, worms were dislodged to the liver but they were not found in the lungs, except in one occasion on light microscopy in Group II.

CONCLUSION

Clinical and radiological pulmonary manifestations are common in patients infected by *S. mansoni* (Pedroso et al., 1984). These alterarions may be:

a) Secondary to deposition of immune complexes, activation of the complement system and migration of leukocytes, macrophages and may be independent of the local presence of worms and/or eggs, similar to the renal manifestations already reported (Lambert & Houba, 1974; Van Mark, 1983; Wilson & Dixon, 1983);

b) Enhanced by the liberation of more worm antigens, secondary to the treatment.

Our results suggest an immunological reaction, probably due to deposition of immune complexes in the lungs with subsequent activation of the complement system. The experimental study shows that the alterations were not dependent on the presence of eggs and/or worms in the lungs, corroborating the hypothesis of deposition of soluble material.

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