Rev. Inst. Med. trop. São Paulo
13(1):51-56, janeiro-fevereiro, 1971

UDC 616.995.122

TISSUE REACTIONS TO *SCHISTOSOMA MANSONI* OVA. III — MICRO-CIRCULATION IN THE INTESTINE OF INFECTED MICE, AS STUDIED BY INJECTION OF CONTRAST MATERIAL

KURT KLOETZEL

SUMMARY

Venous circulation in the bowel wall of white mice has been investigated with the aid of India Ink injection into the portal system, at variable intervals after infection by *S. mansoni.* As a result of the deposition of schistosome ova and granuloma formation the normal architecture of the venous system is severely disorganized, leading to the establishment of a network of fine collateral vessels. These probably function as by-passes to the heavily infiltrated areas of the intestine.

The Author believes that this phenomenon may be held responsible for the shift of granuloma formation from the submucosa to the subserosa, a process which takes place around the 80 th day after infection.

INTRODUCTION

Impressed by the variable course of egg elimination in humans infected by *S. mansoni,* in some species of monkeys as well as in mice (though Cheever-personal communication, could not observe this phenomenon in his group of animals), I have for some time, endeavoured to investigate the mechanisms of egg extrusion through the bowel wall, and been particularly atracted by the hypothesis attributing a specific role to the proteolytic enzyme contained within schistosome ova and released into surrounding tissues [1, 2].

Part I of this series postulated that eggs are extruded into the lumen of the intestine as long as this enzyme and antigen is not neutralized by it's antibody. In effect, the decrease in fecal egg counts was found to be simultaneous to an increased titer of the anti-enzyme antibody [3].

This view had to be modified by the time Part II of this study was reached. While it is true that passage of ova through the bowel wall represents an active phenomenon, only viable eggs ever succeeding in broaching this barrier, few ova accomplish this venture at late stages of infection, simply because they are prevented from reaching the submucosa or *lamina propria* [4].

The period around the 80th to the 90th day is a critical one for schistosome infection in the white mouse. Forstly the rate of formation of granulomata is markedly increased, effectively immobilizing ova within the submucosa and blocking their exit by a florid field of fibrosis, even though the proteolytic enzyme (to which no teleological role can be attached from this stage onwards) goes on destroying the reticulum formed in the center of the granulomata. Moreover, the bulk of egg lesions will now occupy the subserosal layers, fresh supplies of schistosome ova being diverted into the outer le-

Once again I must gratefully acknowledge financial aid from "Fundação de Amparo à Pesquisa do Estado de São Paulo"

Departamento de Medicina Preventiva. Faculdade de Medicina de Mogi das Cruzes. Mogi das Cruzes, São Paulo, Brasil

KLOETZEL, K. — Tissue reactions to *Schistosoma mansoni* ova. III — Micro-circulation in the intestine of infected mice, as studied by injection of contrast material. *Rev. Inst. Med. trop. São Paulo* 13:51-56, 1971.

vels of the wall, an additional obstacle to their ever reaching the villi of the intestinal mucosa.

To explain these events I have postulated [1] that at this stage of infection the venous circulation runs through abnormal routes, efficiently by-passing those areas more heavily infiltrated by ova and granulomata, and sweeping fresh schistosome eggs into the outer layers of the wall.

This is the hypothesis under examination in this final paper.

MATERIAL AND METHODS

This study involved 14 white mice sacrificed at variable intervals (42nd to 107th day) after light infection by *S. mansoni*.

After anaesthesia with ether the animals were opened and bled from the heart. The spleen was grasped between two pieces of blotting paper, and injected through it's lower pole with undiluted India Ink, using a common intradermal needle. Injection was stopped when the finer vessels in the bowel wall appeared to be outlined by the contrast material. (This technique was not uniformly successful; better filling of the mesenteric system can be accomplished by clamping the liver pedicle).

The viscera were then removed in block, and left for one hour in 4% formol. Short lengths of intestine were removed after this interval, cut along the insertion of the mesentery, spread on top of glass slides and left to dry for a few minutes. After additional flatening, a second slide was placed on top of the tissue, the mount being firmly held together by rubber bands and again placed in formol solution.

After a few days the tissues were processed by ordinary histologic procedures, staining being omitted. Normal sections of liver and intestine, stained or unstained, were likewise studied.

RESULTS

No detailed anatomical studies being carried out, nevertheless I believe that gross examination of these preparations has been rewarding.

The illustrations are self-explanatory. At the earliest stages of egg deposition the normal venous pathways are utilized, ova traveling along the submucosa, then into the mucosa and villi. Submucosal veins are rectilinear and fill evenly with contrast. Venous architecture is well preserved, and filling of villi likewise appears to proceed normally (Figs. 1 and 3).

Whether owing to egg imparction, endophlebitis or compression of venules by granulomata, this situation is soon disturbed, the injection technique not revealing as uniform filling of villi, submucosal veins likewise filling only up to a certain distance from the feeding vessels, the contrast then stopping suddenly. (Would a thorough metabolic investigation at this stage of infection reveal malabsorption in animals with a heavy worm burden?) (Fig. 4).

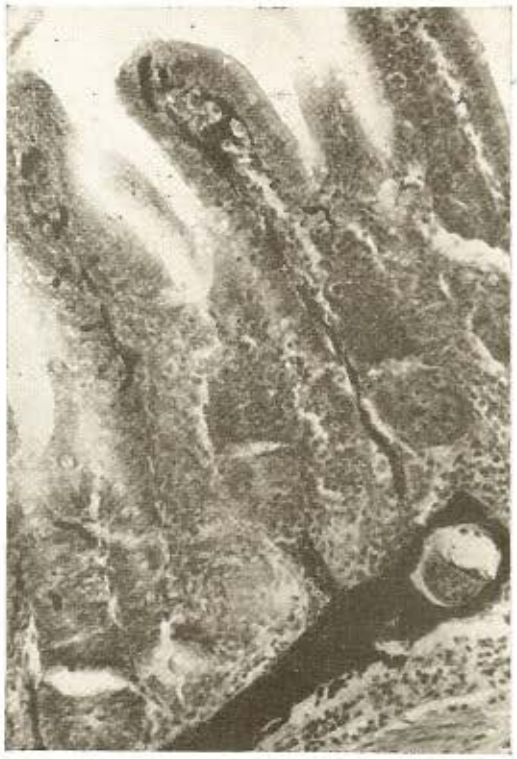

Fig. 1 — 42 days after infection. Large submucosal venule, and partial filling of villi. Contrast stops at schistosome egg within vessel. At no point of this section was contrast visible in the outer layers of the bowel wall. (H. E., 160 X, 20 μ)

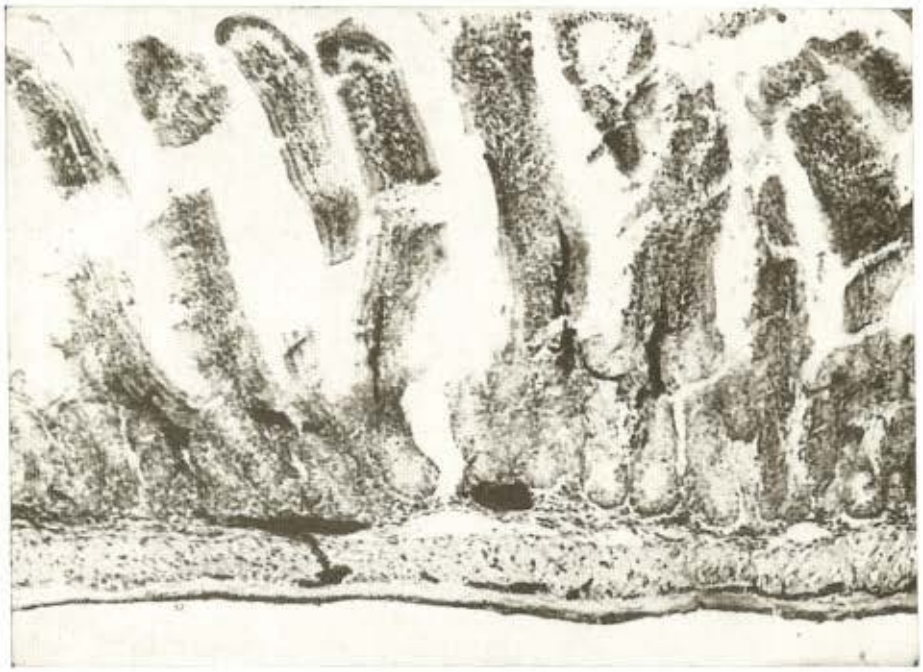

Fig. 2 — 107 days after infection. Though no ova or granulomata are present in this particular section, intestine heavily infiltrated. Patchy filling of submucosal vessels, but contrast present in the boundary between muscle layers, both networks appearing to be connected at one point. (H. E., 80 X, 5 μ)

Fig. 3 — 42 days. Eggs still confined within submucosal vessels. Note orderly architecture, good filling, and the absence of any recognizable collateral venule or capillary (Whole mount, 25 X)

Fig. 4 — 80 days. Ova imparcted in submucosal veins. No filling of villi tributary to these vessels. No collaterals to be seen (Whole mount, 25 X)

At a still later stage nature tries to overcome the obstacles, a network of very fine vessels becoming outlined by India Ink. These capillaries do not lead directly to villi, and appear to run through the outer layers of the bowel wall. The submucosal venules fill in a patchy fashion, but filling of villi is again normal; everything leads to the view that these short stretches of patent submucosal venules are interconnected, and drain into, the network of collateral circulation (Figs. 2, 5, 6 and 7).

DISCUSSION

Obstructed areas of the intestinal wall being by-passed by a system of hitherto inapparent pathways, and these coursing mainly along the boundary between the two muscle layers or in the submucosa, as seems very likely, ova traveling counter-current to the blood flow in the portal system will invariably have to course through the outer wall of the bowel, there being retained within the finer vessels. This will finally lead to a marked thickening of the subserosal layers, specially in the vicinity of the perforating mesenteric venules (See Fig. 4 in Part II of this series-4).

On the sole basis of these few and rough observations it would be rash to venture a

Fig. 5 — 107 days after infection. A number of *S. mansoni* ova in area devoid of larger vessels, but permeated by a fine network of capillaries, which do not appear to lead to villi. Areas peripheral to the figure show partial filling of villi, disorganized structure of the venules which do not fill with contrast throughout their length (Whole mount, 25 X)

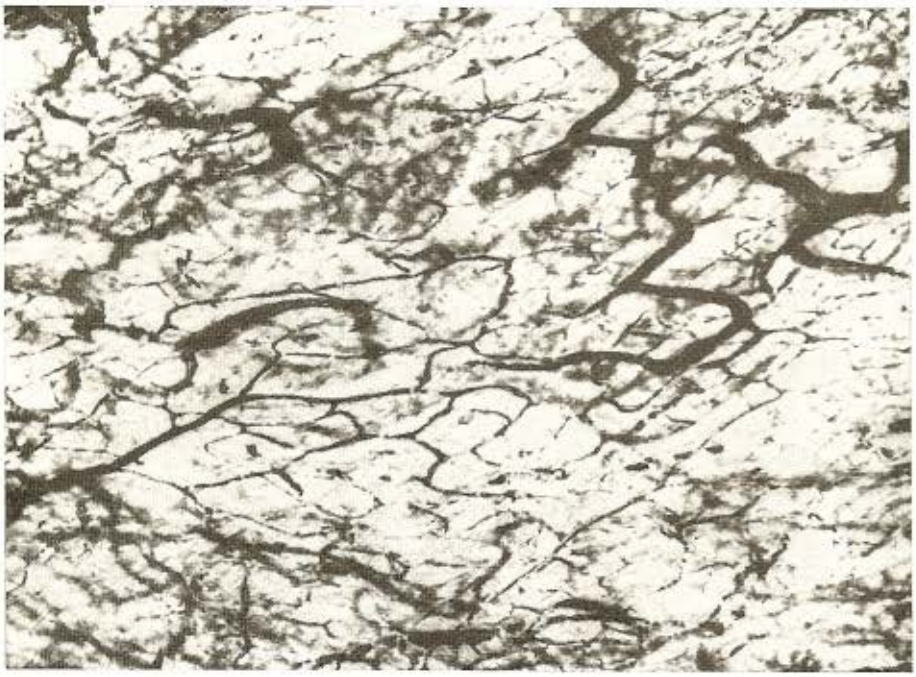

Fig. 6 — 107 days. Enlarged area from same slide. A fine network of venous channels is seen connecting short stretches of larger vessels, probably submucosal. Villi not in focus (Whole mount, 63 X)

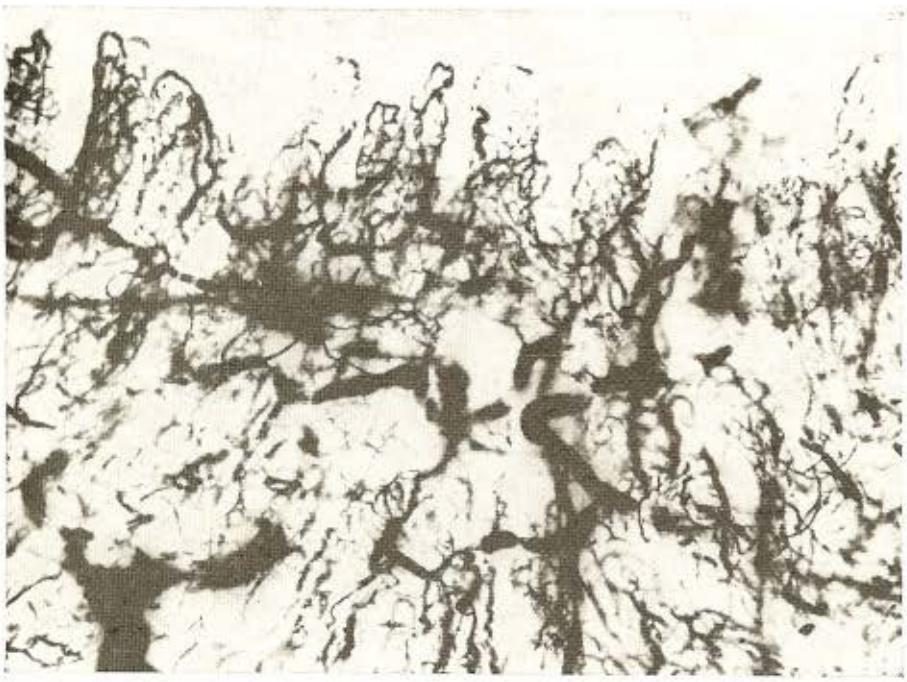

Fig. 7 — 107 days. Good filling of villi but structure of larger veins entirely disorganized, showing patchy filling. A number of fine, vertical channels can be seen on closer inspection (Whole mount, 63 X)

guess whether one is facing a process of neoformation of venous pathways (such as the angiomatoid lesions described in the liver), or whether the described phenomena simply represents an unusual patency of pre-existing venous capillaries, normally of very fine caliber and inapparent at earlyer stages of infection.

RESUMO

Reações do tecido aos ovos de Schistosoma mansoni. — *III Micro-circulação no intestino de camundongos infetados, estudada através da injeção de contraste*

A circulação venosa da parede intestinal de camundongos brancos, a intervalos variados após infecção por *S. mansoni*, foi investigada por intermédio da injeção do sistema porta com tinta Nankim. Constatou-se severa desorganização da arquitetura venosa, conseqüente à deposição de ovos e formação de granulomas. Notou-se formação de uma rêde colateral de finos vasos, possìvelmente funcionando como sistema "By-pass" às áreas mais densamente infiltradas do intestino.

Acreditamos que semelhante fenômeno pode ser responsabilizado pelo desvio de ovos da submucosa para a subserosa, processo que tem lugar em tôrno do 80º dia após a infecção.

REFERENCES

1. KLOETZEL, K. — A collagenase-like enzyme diffusing from eggs of *Schistosoma mansoni*. *Trans. Roy. Soc. Trop. Med. & Hyg.* **61**:608-609, 1967.

2. KLOETZEL, K. — A collagenase-like substance produced by eggs of *Schistosoma mansoni*. *J. Parasit.* **54**:177-178, 1968.

3. KLOETZEL, K. — Tissue reactions to *Schistosoma mansoni* eggs. I — Serological and histological reactions at various intervals after infection. *Trans. Roy. Soc. Trop. Med. & Hyg.* **63**:459-469, 1969.

4. KLOETZEL, K. — Tissue reactions to *Schistosoma mansoni* eggs. II — Distribution of eggs in faeces and at different levels of the intestinal wall, at variable intervals after infection. *Trans. Roy. Soc. Trop. Med. & Hyg.* **64**:116-121, 1970.

Recebido para publicação em 15/6/1970.