OZONE THERAPY

THE HISTO – PATHOLOGICAL CHANGES IN THE LIVERS OF MICE INFECTED WITH SCHISTOSOMA MANSONI

F.M. GAMAL – EDDIN , M.A. EL QADY ;
,M.A. ABDEL – RAHIEM AND A.S. RASMY

A.ALEXANDER

MATERIALS AND METHODS

- A group of mice
- Injected intraperitoneal by cercariae of S.mansoni
- Infected mice were divided into 2 main groups

The 1st Main group

- Intraperitoneal injection by oxygen ozone therapy (O_2 : O_3)
- Ozone concentration 70 ug / ml as a gas mixture.
- Injection was repeated every fourth days for the first main group

The 1st Main group

- O_2 / O_3 b70 ug/ml 1cc each injection , each one cc contains 70 ug/, O_3 , the volume increases as it is shown in the table.

Settings	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Fixed intervals of 3 days between each setting O_3/ O_2 therapy in mice by intraperitoneal gas injection									
First subgroup 3 Doses	1cc	1cc	1cc						
Second subgroup 5 Doses	1cc	1cc	1cc	1.5cc	1.5cc				
Third subgroup 7 Doses	1.5cc	1.5cc	1.5cc	2cc	2cc	2cc	2cc		
Fourth subgroup 9 Doses	2cc	2cc	2cc	2.5cc	2.5cc	2.5cc	3cc	3cc	3cc

The 1st subgroup

- **Injected 1cc of ozone for 3 consecutive doses.**

Settings	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Fixed intervals of 3 days between each setting O_3/ O_2 therapy in mice by intraperitoneal gas injection									
First subgroup 3 Doses	1cc	1cc	1cc						
Second subgroup 5 Doses	1cc	1cc	1cc	1.5cc	1.5cc				
Third subgroup 7 Doses	1.5cc	1.5cc	1.5cc	2cc	2cc	2cc	2cc		
Fourth subgroup 9 Doses	2cc	2cc	2cc	2.5cc	2.5cc	2.5cc	3cc	3cc	3cc

The 2nd subgroup

- Injected 5 times by the same maxiture and concentration
- In the first 3 injections 1cc of the gas
- In the last 2 sessions 1.5cc of the gas

Settings	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Fixed intervals of 3 days between each setting O_3/ O_2 therapy in mice by intraperitoneal gas injection									
First subgroup 3 Doses	1cc	1cc	1cc						
Second subgroup 5 Doses	1cc	1cc	1cc	1.5cc	1.5cc				
Third subgroup 7 Doses	1.5cc	1.5cc	1.5cc	2cc	2cc	2cc	2cc		
Fourth subgroup 9 Doses	2cc	2cc	2cc	2.5cc	2.5cc	2.5cc	3cc	3cc	3cc

The 3rd subgroup

Injected 7 times by the same maxiture and concentration with the same interval between each injection sessions.

In the first 3 injections 1.5cc of the gas.

In the last 4 sessions 2cc of the gas.

Settings	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Fixed intervals of 3 days between each setting O_3/ O_2 therapy in mice by intraperitoneal gas injection									
First subgroup 3 Doses	1cc	1cc	1cc						
Second subgroup 5 Doses	1cc	1cc	1cc	1.5cc	1.5cc				
Third subgroup 7 Doses	1.5cc	1.5cc	1.5cc	2cc	2cc	2cc	2cc		
Fourth subgroup 9 Doses	2cc	2cc	2cc	2.5cc	2.5cc	2.5cc	3cc	3cc	3cc

The 4th subgroup

Injected 9 times by the same maxiture and The fourth concentration with the same interval between each injection sessions.

In the first 3 injections 2cc of the gas.

In the next 3 sessions 2.5cc of the gas.

In the next 3 sessions 3cc of the gas were used.

Settings	1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Fixed intervals of 3 days between each setting O_3/ O_2 therapy in mice by intraperitoneal gas injection									
First subgroup 3 Doses	1cc	1cc	1cc						
Second subgroup 5 Doses	1cc	1cc	1cc	1.5cc	1.5cc				
Third subgroup 7 Doses	1.5cc	1.5cc	1.5cc	2cc	2cc	2cc	2cc		
Fourth subgroup 9 Doses	2cc	2cc	2cc	2.5cc	2.5cc	2.5cc	3cc	3cc	3cc

The 2nd main group

Used as control subject.

Did not receive any gas mixture (O_2 : O_3)

- Mice were anaesthetized with “ Nun butal ”
- Sacrificed and dissected
- Portal veins were perfused with citrated saline to disimpact any worm.
- Pieces of livers were fixed in 10% formalin solution , dehydrated , cleared , embedded , sectioned and the stain with haemoxylin and eosin.


“ Results ”

In the examined liver speciments

- progressive diminution in the size of the developed granulomas.
- The cellular component of the granulomas become less pronounced
- The amount of the fibrosis got lesser.
- No trace of adult worms appear in the examined sections.
- Replacement of many granulomas by regenerating liver cells.

As shown in the following figers :

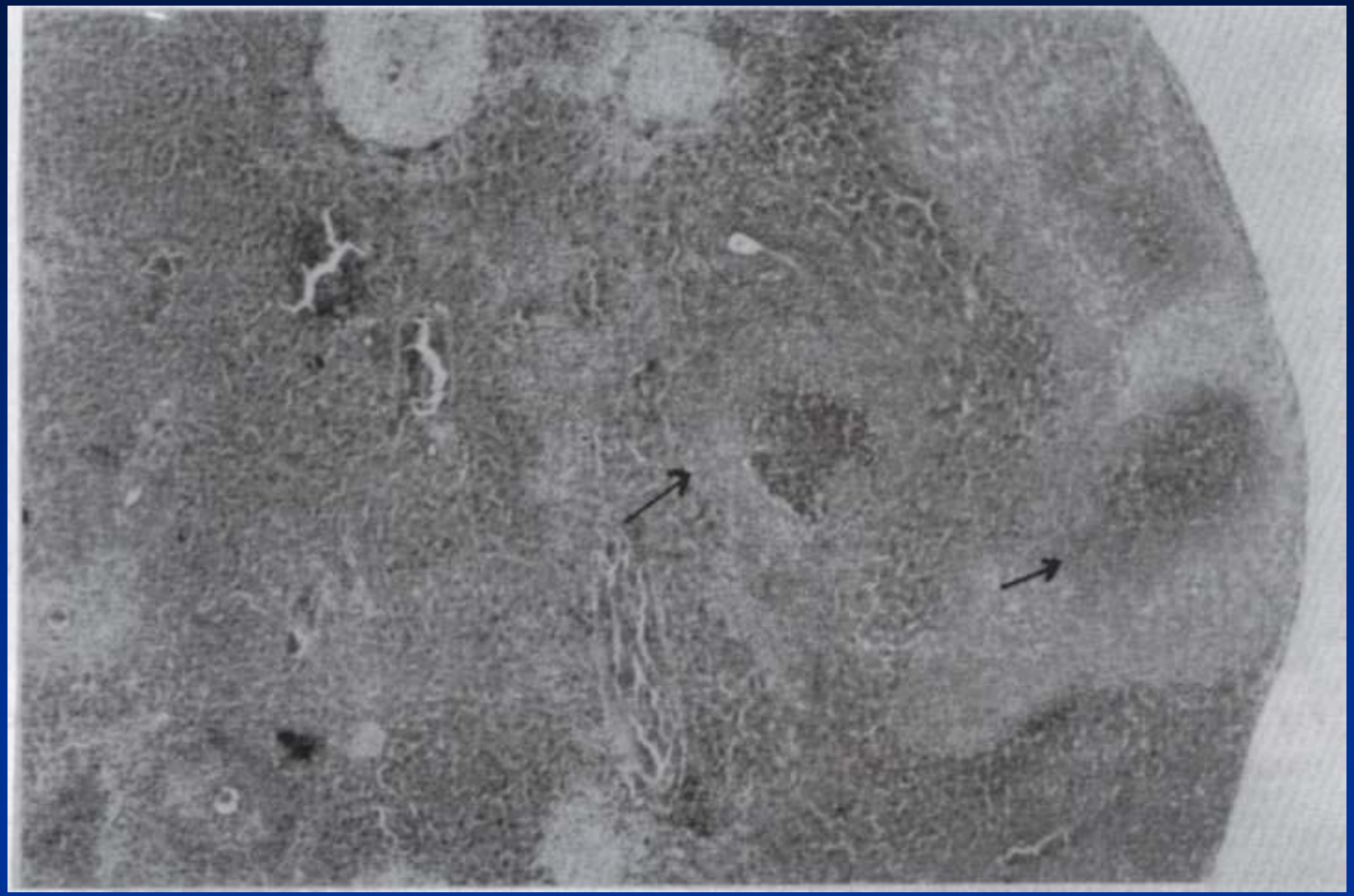

Fig.(1) Section of liver from control animal showing irregular large sized necrotising granulomas with excessive inflammatory reaction (H&E X 100).

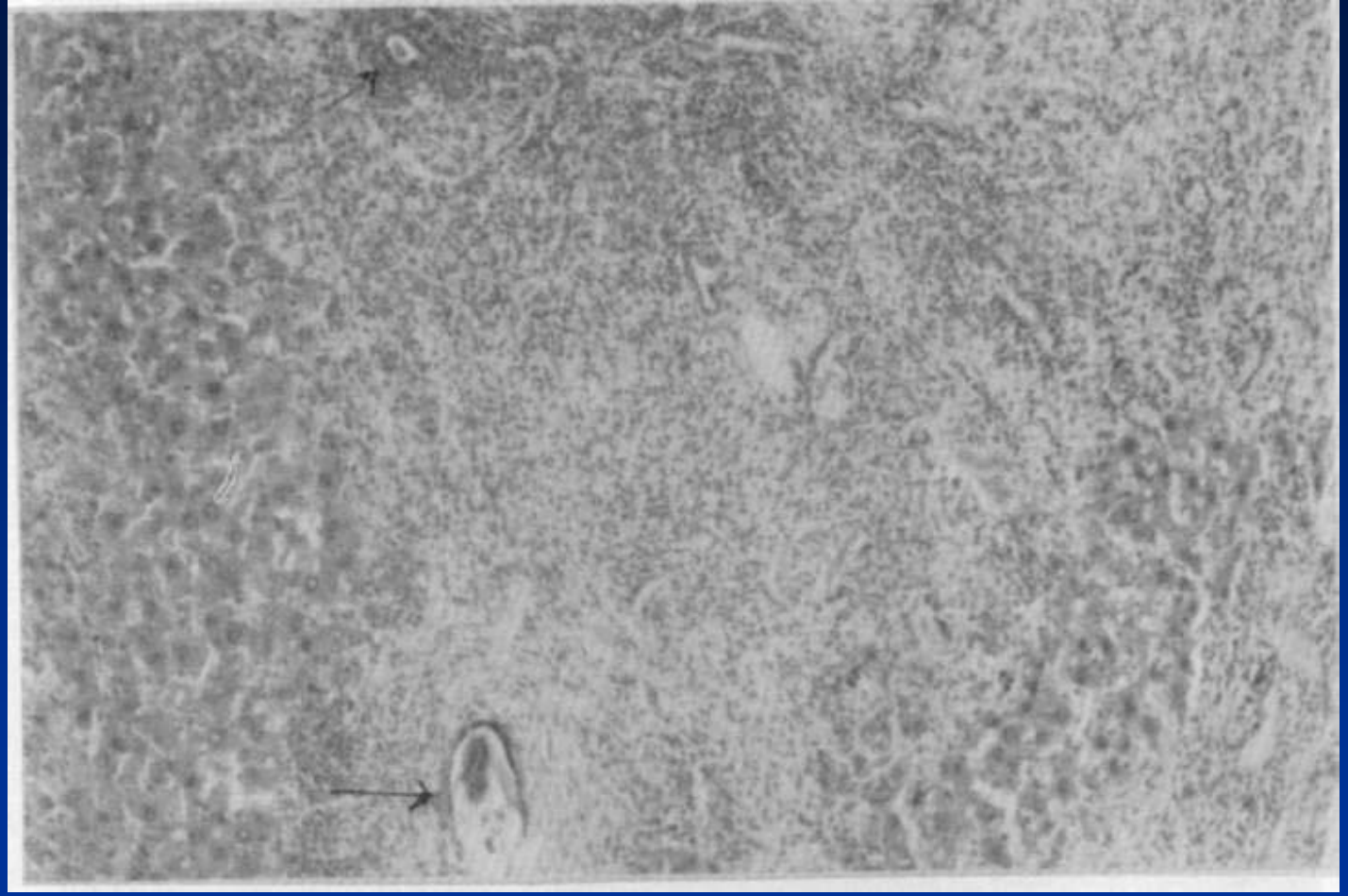

Fig.2 (CONTROL) : Significantly cellular granulomas with scattered ova and little fibrosing reaction (X 300).

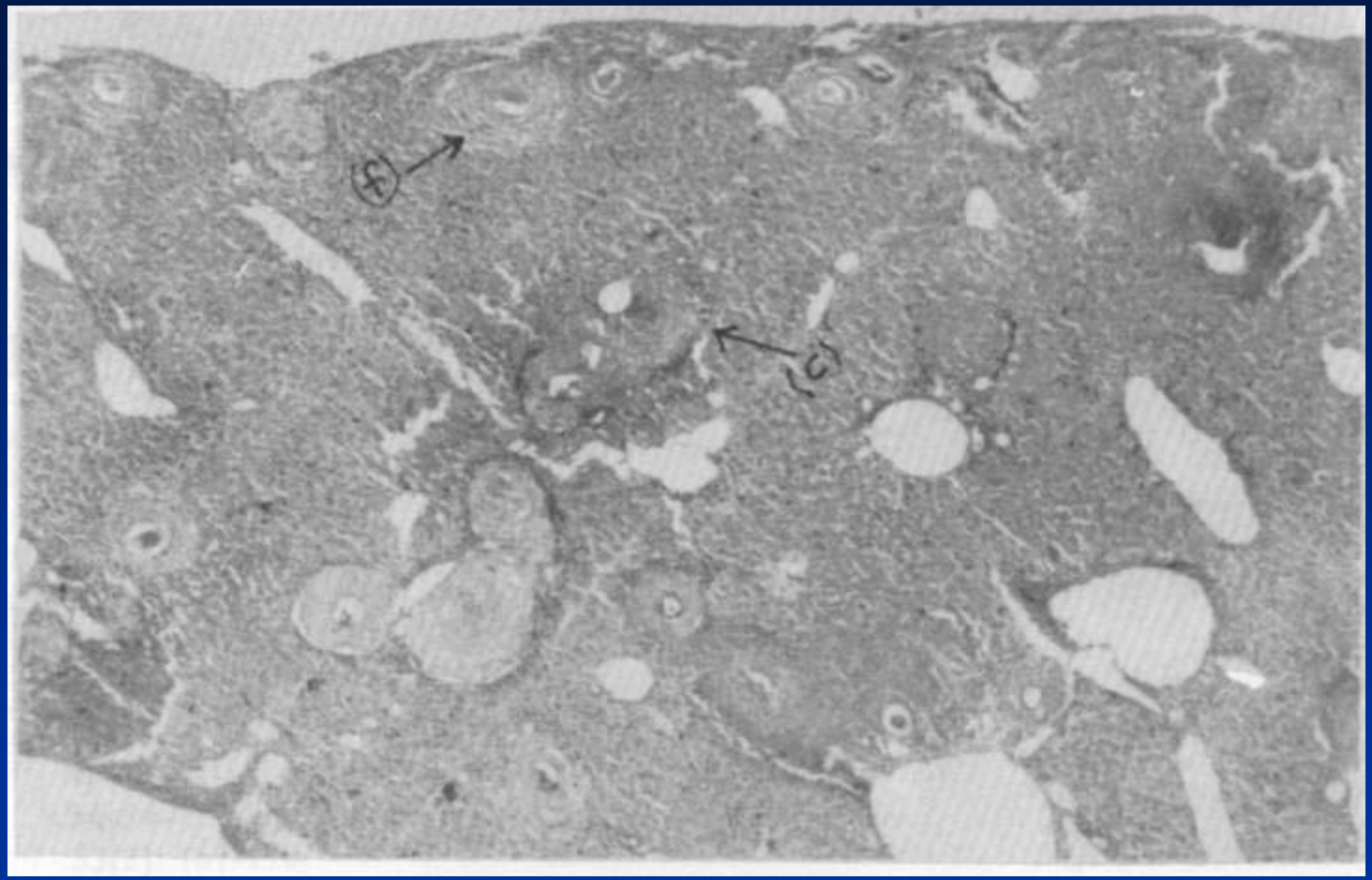

Fig.3 (mouse liver after 3 doses of O_2 : O_3 mixture : Many granulomas of large and moderate size. Some are cellular (c) while others are fibrotic (f).

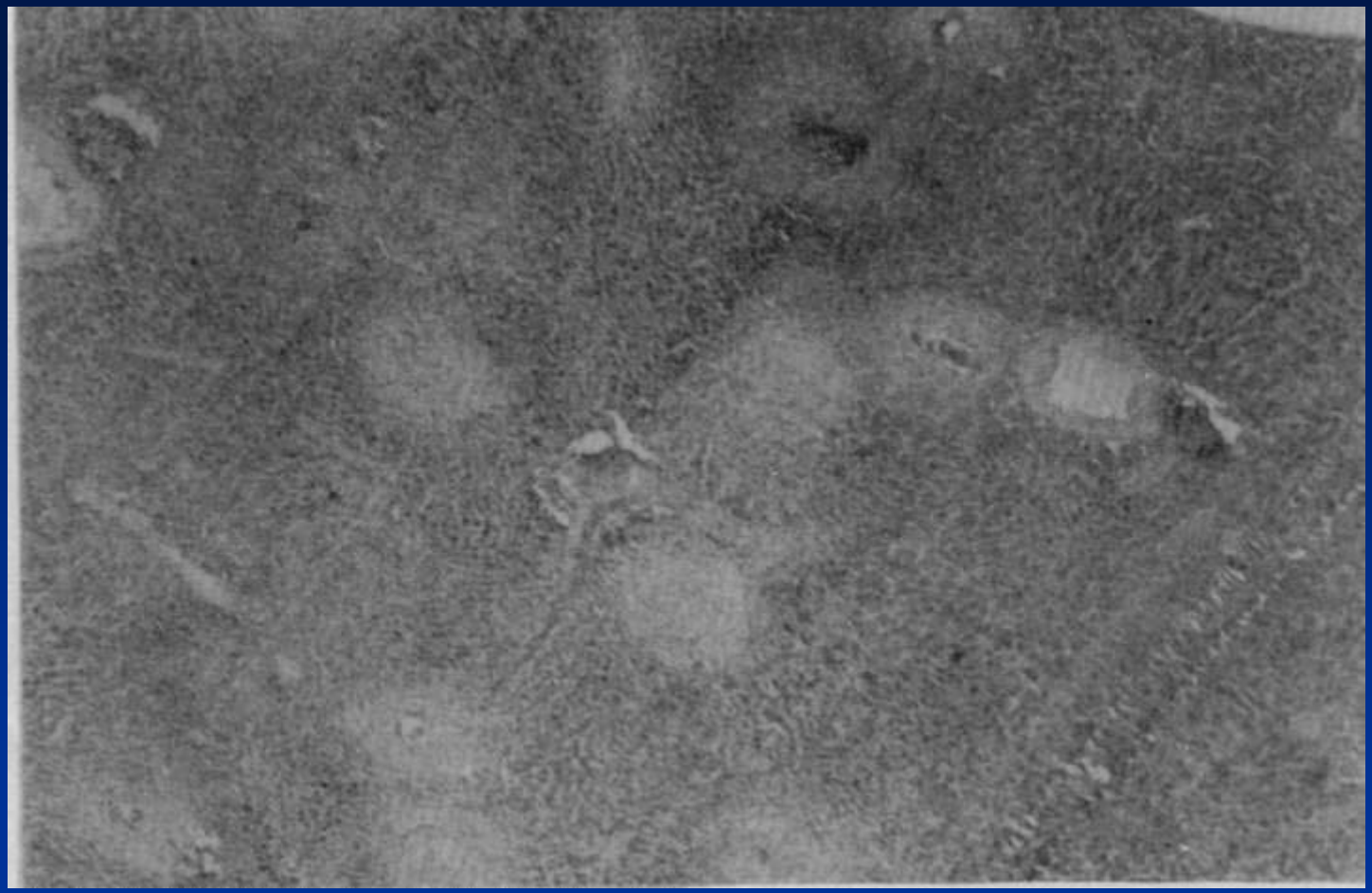

Fig.4 (5 doses) : Moderate-sized granulomas showing moderate cellulairty with more apparent fibrosis (X120).

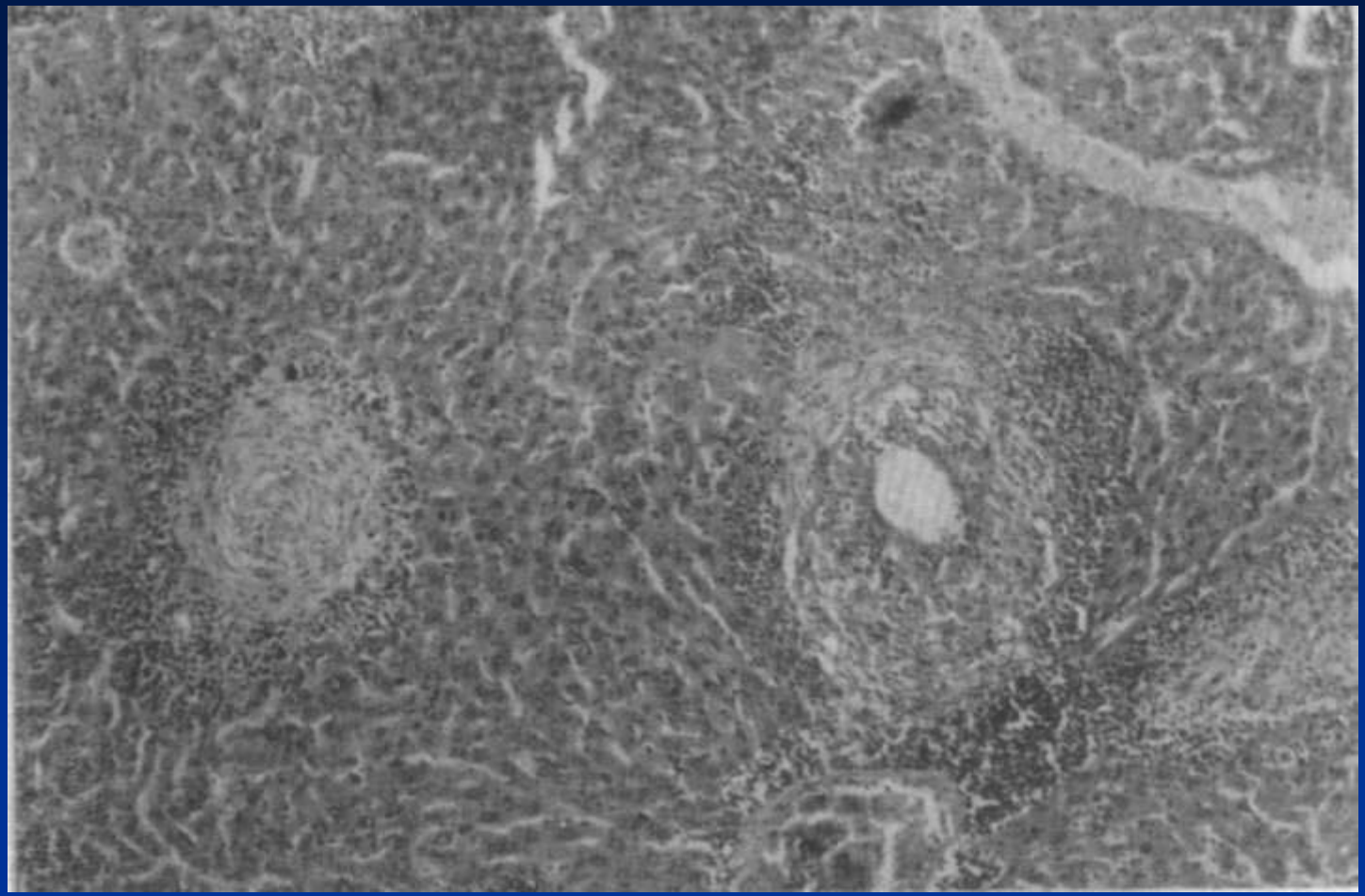

Fig.5 (7 doses) : Moderate and small sized granulomas , showing significant fibrosing reaction with reduction of inflammatory cells (X200).

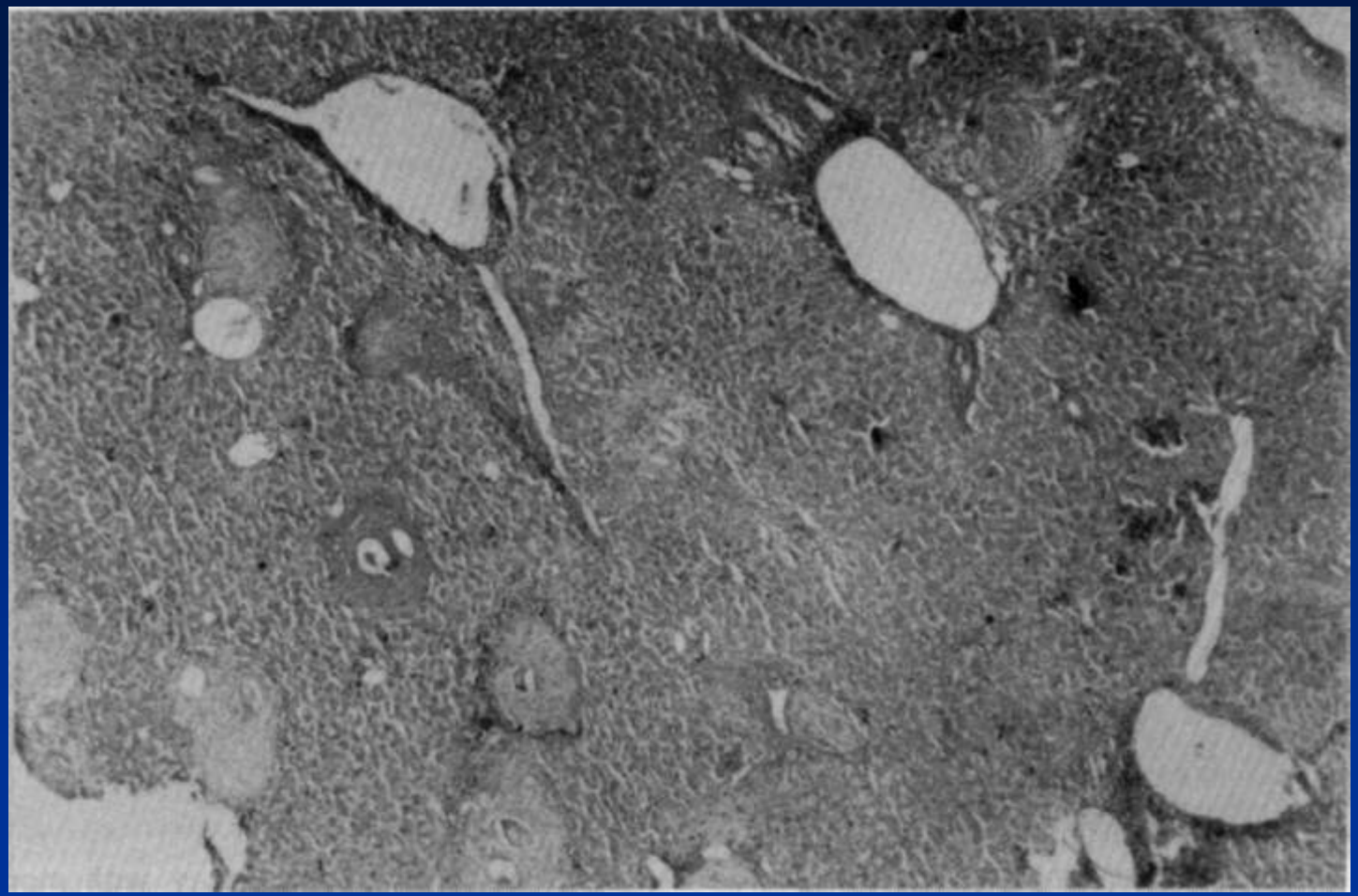

Fig. 6 (9 doses) : Moderate number of widely spaced granulomas, mostly of small size and showing significant fibrosis. The inflammatory cells are few (X100).

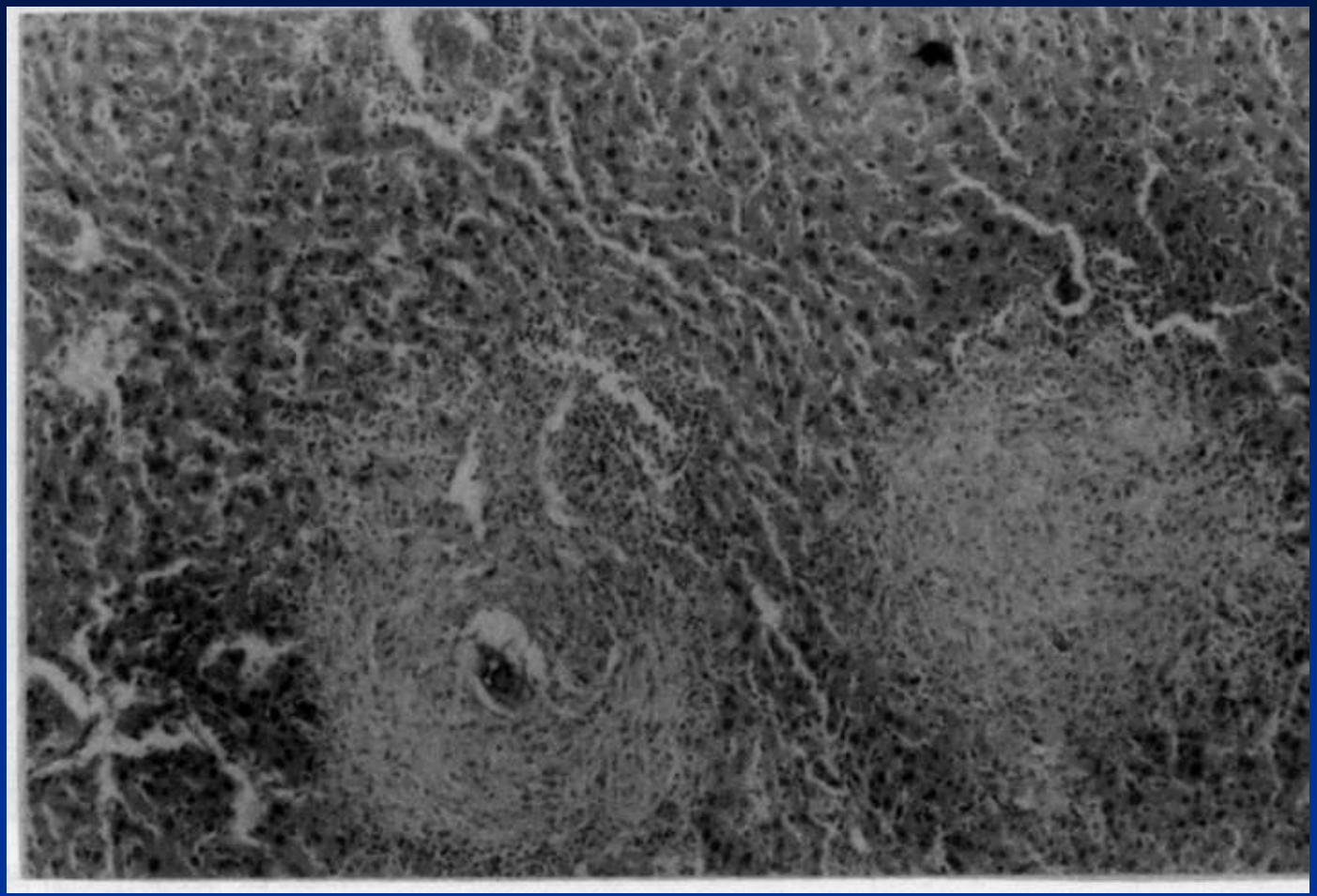

Fig .7 (9 doses) : Hypo cellular granulomas, moderately fibrotic - a step towards healing. (X300).


“ Discussion ”

This chronic disease causes of :

- Hepatic granulomas
- Periportal fibrosis
- Portal hypertension
- Splenomegaly
- Ascites
- Potentially fatal esophageal varices

- liver granulomas develop as a result of hypersensitivity seaction to antigens

derived from

Trapped eggs

Adult worm.

- **Many factors affect the modulation and regulation of granulomas such as :**

  - Age of the host
  - Prior sensitization
  - History of treatment

- CD_4 & CD_8 T cells & cytokinest [IL – 7] produced during penetration of cercariae into the skin.
- IL – 6 produced in acute stage of the disease.
- IFN gamma and TNF- alpha produced later.
- TNT & react to adult antigen rather than to ova antigen.
- IFN gamma effect on the parasite and reduced hepatic fibrosis.

- Many drugs have been used including:

- Corticosteroids
- Non – steroidal.
- Anti inflammatory drugs.
- Colchicine.
- Even praziquantel.

No one could be considered satisfactory.

Observation Of Treated Animals

- Increased Physical activity
- Increased in the appetite
- No acute toxic reaction

- **The metabolic path way of O_2 : O_3 gas mixture which passes through dismutation by super oxioids dismutases (SOD_s) produces reactive oxygen species “ H_2O_2 ”.**

- The absence of any trace of adult worms in the liver sections and the most of mesenteric veins , in contrast to those achieved in the control group raises an important inquiry about their disappearance .

- **The worms succeeded to colonize two anti oxidant enzymes**

Super oxide Dismutase (SOD)

Glutathione peroxidase (GPX)

O_3 Changes inside the body to reactive oxygen species (ROS_s) and succeed to trigger and circumvent the two anti – oxidant enzymes on the worm gut epithelium surface membrane and play an integral part in finding inlets for the host blood carrying ROS_s especially in the gut epithelium of the worms collecting .

Host defense effector elements to cause gradation and fragmentation of the adult worms – in O_2 : O_3 treated mice.

Nowadays , there are advocations to use foreign molecules not of the worm origin on which the worms are acquainted and can recognize , so as to activate and stimulate the immune elements of the host to break down the hostile micro – environment around the worms .

This is of utmost importance in schistosomiass vaccinology.

However , what happens accurately is under investigation to determined the proper worm areas affected and the kind of activated cellular and humeral immunity levels and will be published in due time.


Dr . Adel Alexander
Thank you for your time