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Experimental Studies on the Therapeutic Effect of CIBA 32644-Ba against *Schistosoma japonicum* in Mice

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Introduction

It has been reported by LAMBERT (1964) that CIBA 32644-Ba, a nitrothiazole derivative, has a strong schistosomicidal effect with less toxicity than antimonial preparations.

The authors plan to use this drug for the treatment of human schistosomiasis, but before starting a clinical trial, it was felt desirable to confirm the toxicity and the therapeutic effect of the compound in animal experiments. This work is preliminary and incomplete, but, as interesting results were obtained, they will be briefly presented.

Materials and Methods

Albino mice (dd-y strain) of about 25 g were used for the toxicity tests. Thirty-six mice consisting of 24 males and 12 females were divided into 2 groups, A and B; 25 mg/kg and 50 mg/kg of CIBA 32644-Ba were administered in a single dose daily for 10 days to groups A and B respectively. Each group was further divided into 3 sub-groups of 6 mice (4 males and 2 females) and each sub-group was autopsied respectively on the 1st day, 11th day and 21st day after the completion of the administration. Gross and histological changes in the main organs of the mice were noted.

For the therapeutic experiment, 42 albino mice (dd-y strain) of about 25 g were infected with 20-26 cercariae of *Schistosoma japonicum* per mouse by subcutaneous injection. The cercariae

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used for infection were those obtained from *Oncomelania nosophora* experimentally infected with miracidia of *Schistosoma japonicum*. The cercariae obtained from 7 infected snails were mixed and diluted with water, to contain 10 to 13 cercariae in 0.1 ml, and 0.2 ml of the mixture was injected subcutaneously in each mouse. The infected mice were divided into 4 groups A, B, C and D, the first three groups of 10 mice each and the last of 12 mice. The mice of group A received 50 mg/kg of CIBA 32644-Ba in a single dose daily for 10 days, starting 2 weeks after the infection. Groups B and C received respectively 50 mg/kg and 100 mg/kg daily for 10 days, starting 4 weeks after the infection. Group D was used as an untreated control. The mice were all autopsied at 7 to 8 weeks after infection.

Results and Discussion

Toxicity test with CIBA 32644-Ba on mice

No significant abnormal findings were noted histologically in any organs except the testes. In both groups A and B (given 25 mg/kg and 50 mg/kg respectively) inhibition of spermatogenesis was noted in the testes of some animals as shown in Table 1 and Figs. 1-4. When examined at 11 days the changes noted were more severe in the high dosage group (B-2) than in the low dosage group (A-2). That is, in group A-2 there was a moderate decrease in the number of mature sperms in the seminiferous tubules and slight disintegration of the spermatogonia in the parenchyma. In group B-2 there was a significant inhibition of spermatogenesis resulting in a marked decrease of sperms in the tubules, and degenerative changes such as pyknosis and rarely nucleolysis were observed in the parenchyma. However, there was a clear picture of recovery in spermatogenesis in the high-dosage group (B-3) on the 21st day after treatment.

Body weight and appetite were recorded but no significant changes occurred as a result of the administration of the drug.

Therapeutic effect of CIBA 32644-Ba

A total of 30 mice (groups A, B and C) were treated with CIBA 32644-Ba and none of them died during the experiment. However, the results of the autopsies showed that 5 mice in the experimental groups and 3 of 12 control mice were infected only with male worms (unisexual infection). The mice with unisexual infection were excluded from the experimental results, leaving 9, 7, 9 and

TABLE 1

Changes in the testes of mice treated with CIBA 32644-Ba

Groups	Doses	Days after the end of treatment to autopsy	No. of male mice examined	Histological findings
A-1	25 mg/kg × 10	1	4	Slight inhibition of spermatogenesis was found in one of four mice. No necrotic foci were observed.
A-2	25 mg/kg × 10	11	4	Inhibition of spermatogenesis was found in two of four mice.
A-3	25 mg/kg × 10	21	4	Sperms were present in certain seminiferous tubules. Slight atrophy of the parenchyma of the testes still observed.
B-1	50 mg/kg × 10	1	4	Inhibition of spermatogenesis in two of four mice. Marked reduction or disappearance of sperms in the seminiferous tubules. Atrophy of the germinal epithelium or spermatogonia was found.
B-2	50 mg/kg × 10	11	4	Disappearance or marked reduction of sperms in the seminiferous tubules was seen in two of four mice. Pyknosis, karyolysis and degenerative processes of the spermatogonia were found.
B-3	50 mg/kg × 10	21	4	Marked inhibition had subsided. Slight degeneration of the tissues of the testes still observed in two of four mice.
Control			4	No pathological findings.

9 mice in groups A to D respectively. Even in the mice infected with both sexes, the ratio of female to male was about 1 : 3. The single male worms found in the mice with bisexual infection were, however, noted to be as mature as the male worms paired with female worms.

The results of autopsies are shown in Table 2. In group A, given 50 mg/kg of CIBA 32644-Ba daily for 10 days starting 2 weeks after infection, 9 mice were all infected and the total number of

TABLE 2

Therapeutic effect of CIBA 32644-Ba in mice experimentally infected with Schistosoma japonicum

Groups	32644-Ba daily dose in mg/kg	Mode of application		Resuls					No. of mice positive for eggs in faeces / No. exam.
		No. of daily doses starting 2 weeks after infection	No. of daily doses starting 4 weeks after infection	No. of mice infected / total No. exam.	Worms recovered at necropsy * in each group (by sex)				
					Total No.		No. worms/mouse		
					Female	Male	Female	Male	
A	50 mg/kg	10		9/9	40	91	4.4	10.1	9/9
B	50 mg/kg		10	7/7	24	83	3.4	12.0	7/7
C	100 mg/kg		10	5/9**	4	15	0.4	1.6	0/9
D	Control			9/9	25	87	2.8	9.6	9/9

* autopsied 7–8 weeks after infection.

** all worms were degenerated and only immature worms were found in 2 mice.

TABLE 3

Therapeutic effect of CIBA 32644-Ba in mice experimentally effected with Schistosoma japonicum

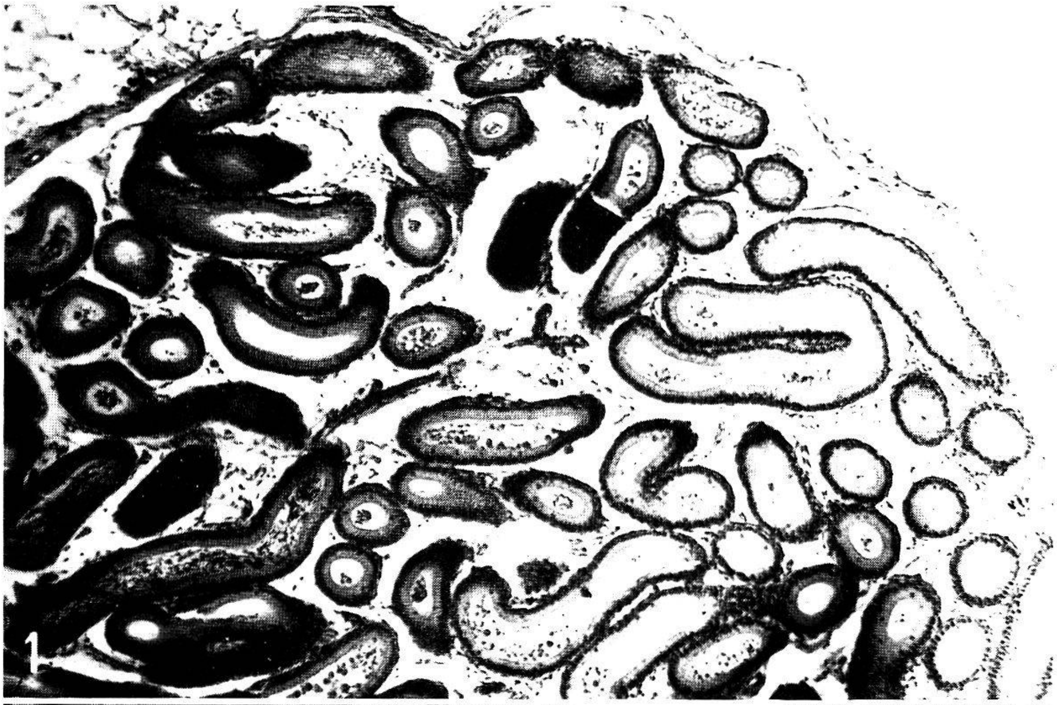
Groups	32644-Ba daily dose in mg/kg	No. mice treated	No. worms recovered from									No. of mice showing egg granuloma in liver	Egg hatching test (Liver)
			Portal vein			Mesenteric vein			Liver				
			Female	Male	Pairs	Female	Male	Pairs	Female	Male	Pairs		
A	50 mg/kg	9	0	27	33	0	2	0	0	23	6	9/9	Positive
B	50 mg/kg	7	0	29	21	0	2	1	1	29	1	7/7	Positive
C	100 mg/kg	9	0	6	3	0	0	0	1	6	0	3/9 *	Negative
D	Control	9	0	33	22	0	4	0	1	26	2	9/9	Positive

* all eggs were degenerated.

mature worms was 40 females and 91 males, which corresponds to 4.4 female and 10.1 male per mouse. The ratio of female to male was 1 : 2.3. In group B, in which 50 mg/kg were administered for 10 days starting 4 weeks after infection, 7 mice were all infected and the total number of worms found was 24 females and 83 males, which corresponds to 3.4 female and 12.0 male per mouse. The ratio of female to male was 1 : 3.5. In the control group D, 9 mice were all infected and 25 female worms and 87 male worms were found, corresponding to 2.8 female and 9.6 male worms per mouse. The ratio of female to male worms was 1 : 3.4. In group C, however, in which 100 mg/kg were administered for 10 days starting 4 weeks after infection, 4 out of 9 mice showed no evidence of infection, and 4 female and 15 male worms were found in the remaining 5 mice, which corresponds to 0.4 female and 1.6 male worms per mouse. The ratio of female to male worms was 1 : 4.2. However, all worms found in group C were markedly atrophied, and their organs, especially the genital organs such as testes, ovary and uterus, were strongly degenerated.

The above-mentioned results are analysed by parasitic sites in Table 3. As can be seen in the table, the largest number of male worms and paired worms was found in the portal veins in groups A and B, treated with 50 mg/kg, and in the control group D. Many living eggs with active miracidia were noted in the livers of all mice in groups A and B. However, in group C, on the higher dosage, only a few degenerated worms were found in the portal veins and livers of 5 of the 9 mice treated, while the eggs were noted in the liver in only 3 mice. Furthermore the eggs found in the liver were all degenerated and no living eggs were found. Histopathologically, in the livers of mice treated with 100 mg/kg of CIBA 32644-Ba the inflammatory responses of the foci around the eggs were distinctly reduced and characterised by an epitheloid-cell reaction around the remnants of egg-shell engulfed with multinucleated giant cells (Figs. 5-8). However, no difference was found in the pathological foci around the eggs in the livers of control mice and those treated with 50 mg/kg of CIBA 32644-Ba.

Usually when the mice are infected with cercariae of *Schistosoma japonicum* by subcutaneous injection most of the worms achieve maturity within 4 weeks after the infection. In this experiment, however, in spite of starting the treatment 4 weeks after infection, no eggs were noted in the liver of 6 mice among 9 in group C. The reason for this is not clear, but it may be that the cercariae used for infection were not mature enough or that females and males were not equally represented. However, from the results mentioned above, it is clear that when 100 mg/kg of CIBA 32644-Ba



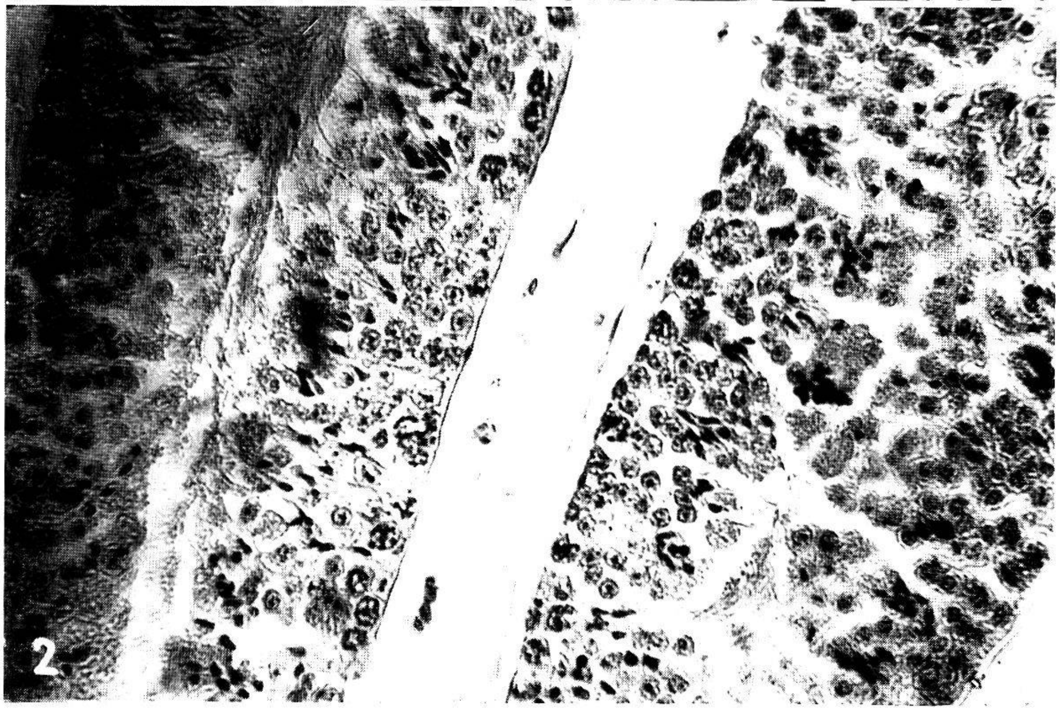
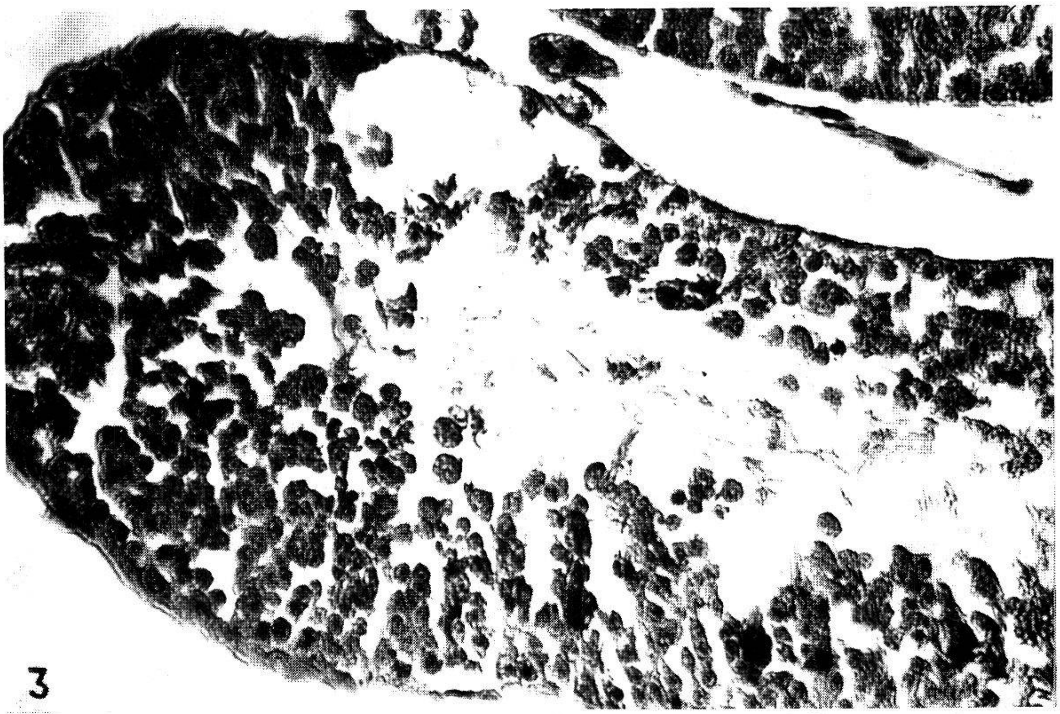


Fig. 1-4. Histological findings in the testes of mice given CIBA 32644-Ba for 10 days. (Autopsied on the 11th day after the last administration.)

Fig. 1. A slight reduction in the number of mature sperms in seminiferous tubules as seen in mice given 25 mg/kg.

Fig. 2. A moderate reduction in the number of mature sperms and disintegration of spermatogonia with pyknosis, vacuolisation and nucleolysis, as seen in mice given 50 mg/kg.



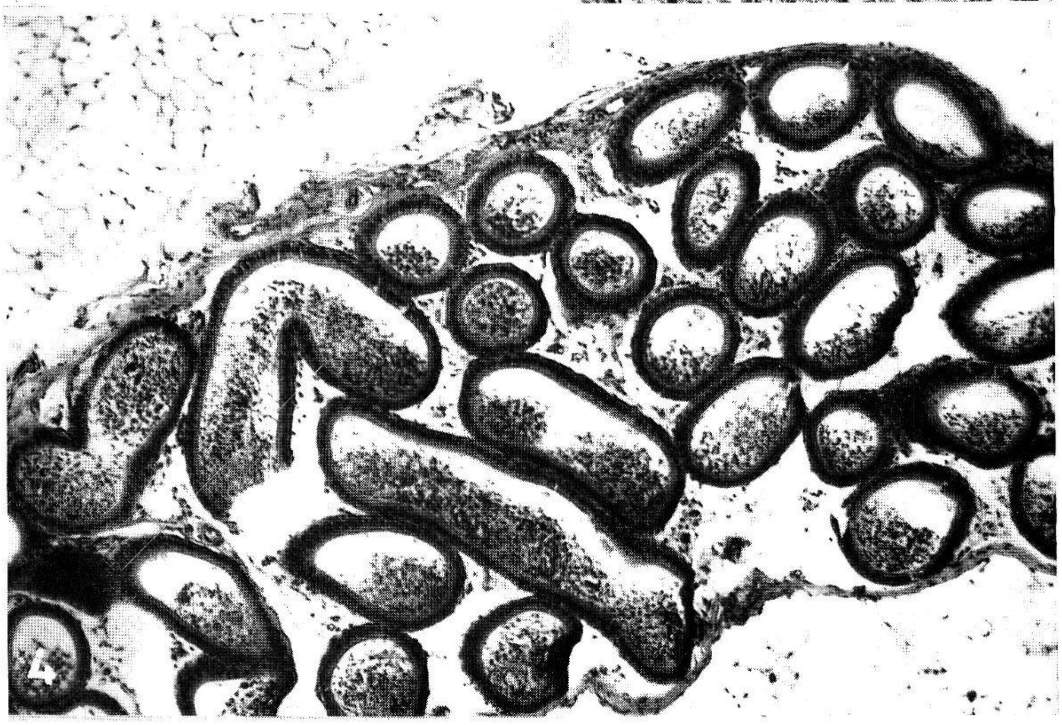


Fig. 3. A marked reduction in the number of mature sperms and disintegration of testicular parenchyma, as seen in mice given 50 mg/kg.

Fig. 4. Large number of mature sperms seen in the seminiferous tubules of an untreated mouse.

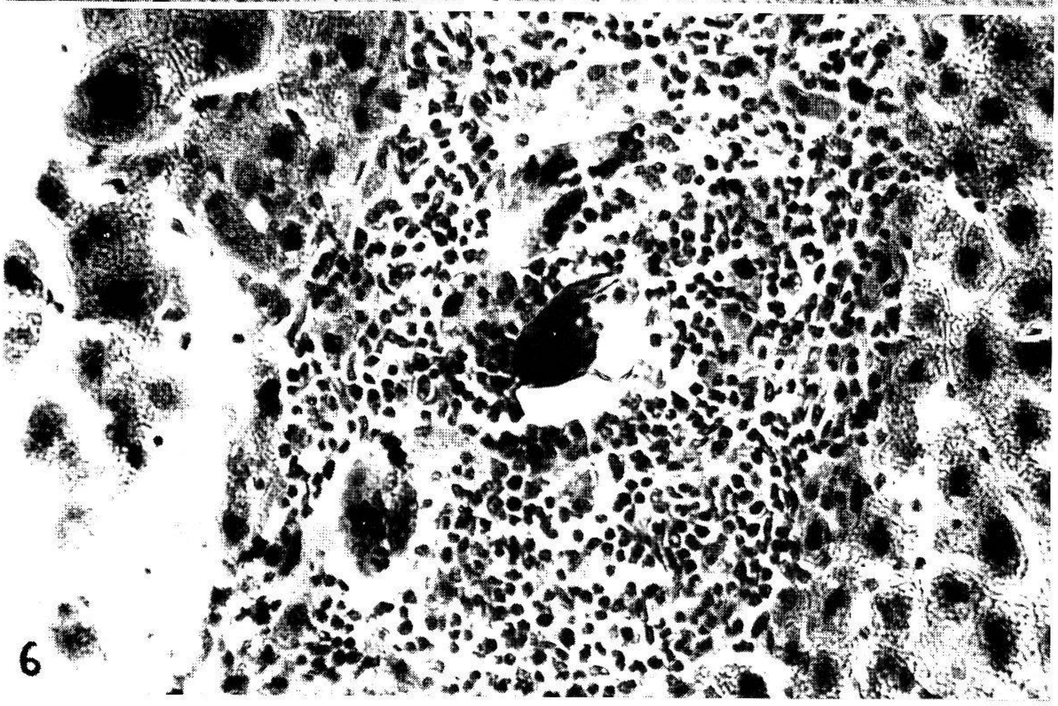
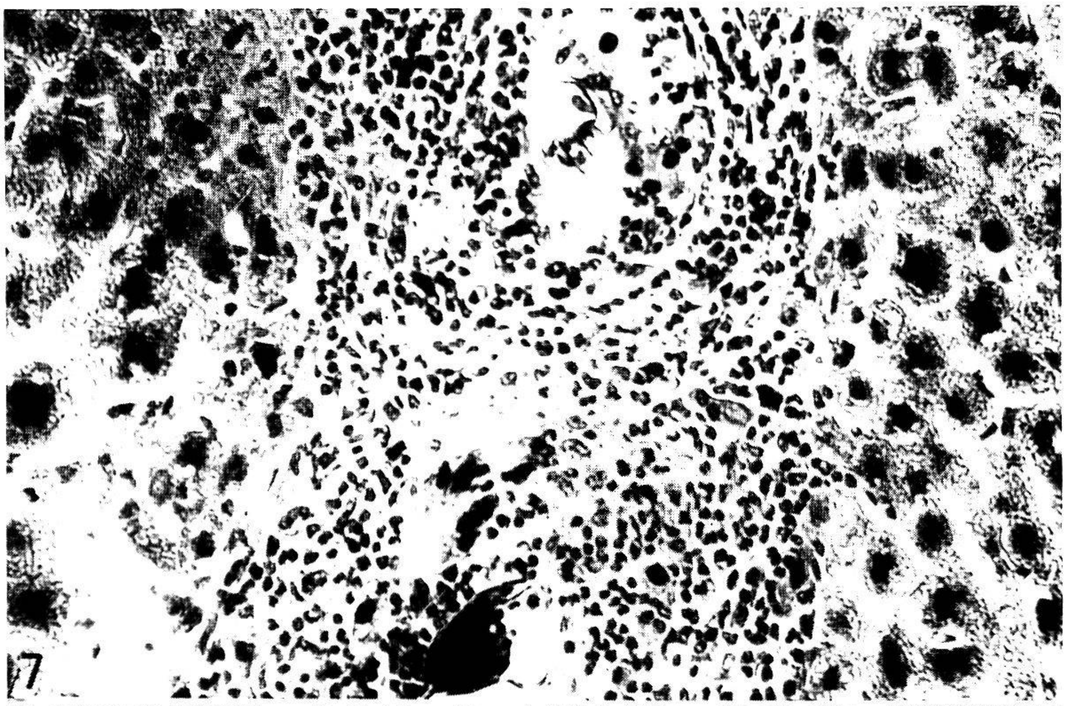


Fig. 5-8. Granulomas in livers of non-treated mice (controls) and treated mice receiving 100 mg/kg of CIBA 32644-Ba for ten days. (Autopsied 8 weeks after infection.)

Fig. 5. Acute cell responses with neutrophils, lymphoid cells and histiocytes seen around eggs. The eggs contain apparently viable miracidia. (Non-treated mouse.)

Fig. 6 *and* 7. Cell responses with lymphoid cells, plasma cells, epitheloid cells and fibroblasts seen around the egg. The egg contains apparently degenerated miracidia. Remarkable degeneration of liver parenchyma is noted. (Treated mouse.)



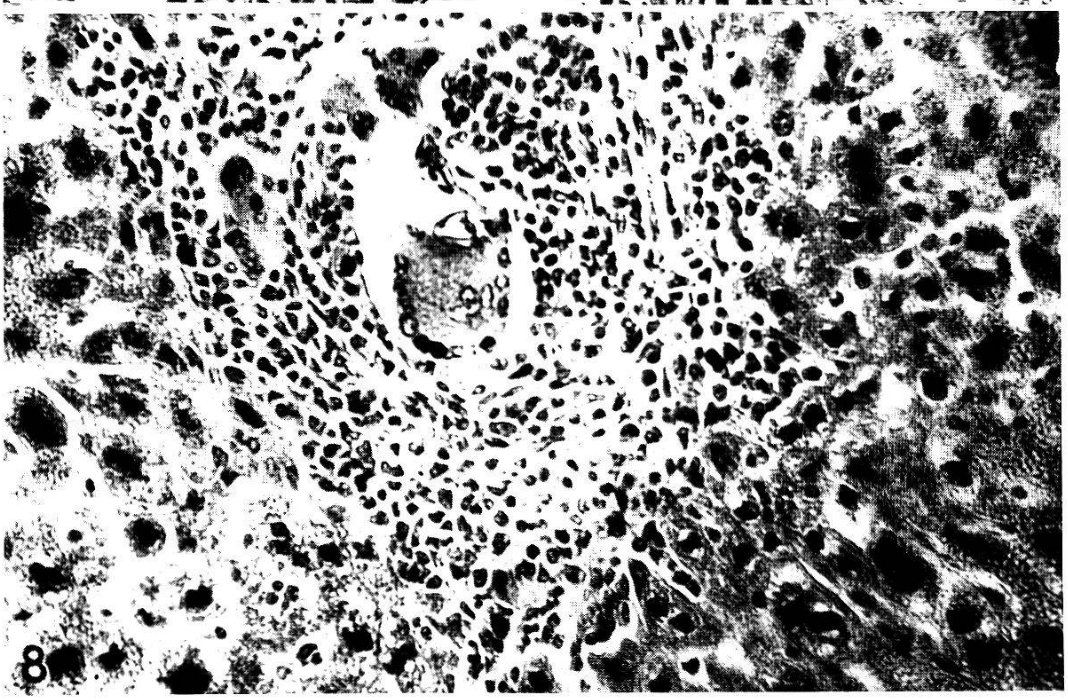


Fig. 8. A multinucleated giant cell engulfing the egg. A surrounding epitheloid cell response is seen. (Treated mouse.)

are administered daily for 10 days, the drug is extremely effective against both the mature and immature worms.

Conclusion and Summary

Daily doses of 50 mg/kg and 100 mg/kg of CIBA 32644-Ba were administered for 10 days to mice experimentally infected with *Schistosoma japonicum*, starting treatment either 14 or 28 days after the infection. No effect was noted in the groups treated with 50 mg/kg but a markedly good effect was noted in the group which received 100 mg/kg, starting 28 days after the infection.

The results of toxicity tests carried out with this drug showed that in mice treated with 25 mg/kg and 50 mg/kg, spermatogenesis was inhibited in some animals. However, these changes could be seen to be reversing 21 days after the cessation of drug administration.

Conclusion et résumé

Des doses de 50 mg/kg/jour et 100 mg/kg/jour de CIBA 32644-Ba ont été administrées pendant 10 jours à des souris infestées expérimentalement par *Schistosoma japonicum*. Le traitement fut commencé 14 et 28 jours après l'infestation.

Aucun effet n'a été observé chez les animaux traités à raison de 50 mg/kg/jour ; par contre une activité remarquable fut observée chez les animaux traités 28 jours après l'infestation, avec 100 mg/kg/jour.

Les tests de toxicité ont montré une inhibition de la spermatogénèse chez un certain nombre de souris traitées avec 25 et 50 mg/kg/jour ; cependant les altérations histologiques observées au niveau du testicule se sont montrées réversibles 21 jours après l'arrêt du traitement.

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