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Histopathological and immunological studies on the experimental schistosomiasis japonica, with special reference to the chemotherapeutic effects on the involving tissues

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Introduction

It is generally accepted that hepatosplenic schistosomiasis are produced at least by four factors or by a combination of these factors; 1) eggs deposited in the tissues, 2) metabolites of the parasite or "toxins", 3) the dead or dying worms, and 4) malnutrition. Therefore, it seems logical to conclude that effective therapy, by eliminating these factors, would avoid the development of the disease.

In schistosomiasis mansoni, however, Coutinho *et al.* (1944) emphasized the role of the dead worms in the area of necrobiosis of hepatic cells, from the results of necropsy of a patient who died during antimonial treatment. Tavares & Menezes (1945), using guinea pigs infected with *Schistosoma mansoni*, found that dead worms promoted necrosis of the liver parenchyma after treatment with antimonials. These findings were confirmed also with treated guinea pigs by Coelho *et al.* (1949). Furthermore, several authors have also suggested that liver damage is produced by dead schistosomes after receiving specific therapy (Meleney *et al.*, 1953; Gillman, 1957; Menezes, 1967; Mousa *et al.*, 1967; Striebel, 1969).

On the other hand, there have been very few investigations and descriptions on the pathogenesis of schistosomiasis japonica in relation to chemotherapy. Nishi (1923) studied the effects of sodium antimony tartrate on oviposition and deposited eggs. Vogel & Minning (1947) pointed out that the parasites were either fixed by connective tissue without dying due to antischistosomal drug, or elicited an inflammatory reaction capable of trapping the parasite in the blood vessels.

Recently, Lambert (1964) reported that niridazole (Ambilhar®), a nitrothiazole derivative, is very effective against *Schistosoma japonicum* as well as *S. mansoni*, which was later confirmed by Yokogawa *et al.* (1966a, b, 1968a, b, 1969) and Pesigan *et al.* (1966).

It is of great interest to know how the drug would affect the parasites and eggs in the involved tissues and cause histological changes to the tissues. It is also necessary to establish the basis for accurate diagnosis or evaluation of "cure" of the disease, since schistosome eggs cannot always be detected in feces of patients in endemic areas where the disease is going to cease, like in Japan.

No serological tests currently available for schistosomiasis have been utilized to estimate the results of treatment. Oliver-Gonzalez and his collaborators (1955) reported that circumoval precipitin (COP) reaction becomes very slight or lacking after adequate therapy in *S. mansoni* infection.

For these reasons, the purpose of this study is to investigate the histopathological changes of the involved tissues with *S. japonicum* after treatment with niridazole and to confirm, from the results, the value of circumoval precipitin reaction as a method of evaluation of cure.

Materials and Methods

Thirty-nine hamsters were subcutaneously

infected with about 50 cercariae of *S. japonicum* from 5–10 naturally infected snails, *Oncomelania nosophora*, collected at the bank of Chikugogawa river, Kyushu, one of the endemic areas of schistosomiasis in Japan. The animals were divided into four groups (Groups A–D) consisting of 9–11 hamsters; Group A, B and C were respectively given per os 50, 100 and 200 mg/kg of niridazole in a single dose every other day for 10 times, starting 10 weeks after infection. Group D was not treated as the control. Up to 30 weeks after treatment the animals of each group were autopsied by total exanguination from orbital artery at stated intervals (Table 1). The macroscopic observation of the organs and number of worms recovered from portal and mesenteric veins were noted. The organs removed were fixed with 10% formalin and microscopical sections were stained with hematoxylin-eosin, Verhoeff-van Gieson and Mallory-azan methods.

In the second series, eight adult rabbits weighing about 2 kg were subcutaneously injected with a single dose of 300–1,000 cercariae. The rabbits were divided into three groups, E, F and G, the first two groups to receive niridazole therapy, and Group G to be left as an untreated control. The treatment was commenced from the 60th day of infection. A daily dose of 100 mg/kg of the drug was orally given to Group E for 10 consecutive days and to Group F every other day for 10 times, respectively (Table 1). The animals were autopsied at 6 and 12 months after treatment, except rabbit No. RA 1 which died due to an unknown cause at 8 days after the end of treatment.

For stool examinations the AMS III method (Hunter *et al.*, 1948) for the hamsters and direct smear method for the rabbits were performed respectively in the whole course of treatment. The miracidial hatching test was applied for the evaluation of the treatment in the rabbits.

The antisera of the hamsters were collected by means of the orbital bleeding technique (Riley, 1960). COP tests were successively followed up in each animal after treatment. The schistosome eggs for COP test were isolated from the liver and intestine of mice or hamsters experimentally infected with *S. japonicum* by Trypsin digestion method (Yokogawa & Sano, 1966).

The details of the methods used for the COP test and for classification of the type of precipitates have been described elsewhere (Yokogawa & Sano, 1966; Yokogawa *et al.*, 1967).

Results

1. Schistosomicidal activity of niridazole

Hamsters: The results of stool examina-

Table 1 Experimental animals and date of autopsy

Animals (No. of cercariae infected)	Groups	No. of animals treated (Sex)	Daily dose of niridazole	No. of animals cured	Date of autopsy
Hamsters (50 cercariae)	A	9(f)	50 mg/kg	0	At 4 days, 1, 2, 7, 12 and 30 weeks after treatment
	B	9(m)	100 mg/kg	0	
	C	10(m)	200 mg/kg	4	
	D	11(f)	control	0	Before* treatment, at 4 days 1, 2, 4, 7, 12, 20, and 22 weeks after treatment
Rabbits (300–1,000 cercariae)	E	3(m & f)	100 mg/kg (consecutive)	3	At 8 days, 6 and 12 months after treatment
	F	2(f)	100 mg/kg (every other day)	2	At 6 and 12 months after treatment
	G	3(m & f)	control	0	Before* treatment, at 6 and 12 months after treatment

* "Before" means at 70 days after infection in hamsters and at 60 days in rabbits.
The animals which died during the course of treatment were excluded from this table.

Table 2 Schistosomicidal activity of niridazole in hamsters infected with *S. japonicum*

No. of case	EPG before treatment	EPG after treatment						
		3 days	1w.	2w.	4w.	7w.	12w.	30w.
Group A								
HA 1	840	78*(7p+3f)						
2	166	52*	32	4*(9p)				
3	1,285	78*	44*	88	12	20	36(?)	
4	147	16*	2	2	14	10	12(2p)	
5	316b	0	6(5p+4m+13imm)					
6	557b	70	50*	144	134(?)			
7	705	260*	6	4	222	6(11p)		
8	149	20*	0	2	66	6(2p)		
9	194	2	16	4	24	6(6p)		
Group B								
HA 11	298b	4*(3p+26imm)						
12	1,423b	6*	6	0(9p+2f+1imm)				
13	845b	10*	2*	0	314	28	650(7p+9imm)	
14	206	6*	2	0	30	16	356	120(5p)
15	638b	2	0	0	18	16	138	130(4p)
16	308d	0	8(8p+2m+2f+10imm)					
17	37(2p)†							
18	1,400	14*	0	0	306	2(8p+1f)		
19	1,228	22*	0	0	10	40(?)		
Group C								
HA 21	997d	0(4m+2dead m)						
22	101	0	2*	0(none)				
23	65	2	0	0	0	6	16	0(none)
24	377	0	0	0	8	18	8(?)	
25	41	0	0	0	2	2	26	0(none)
26	269(3m+2f+1dead m)†							
27	867b	10*	0(5p)					
28	620	0	0	0	0	24	8(?)	
29	234	0	2*	0	0	20	42(1p)	
30	1,277bd	0	0	0	0	0(none)		
Group D								
HA 1′	747d(8p+3imm)							
2′	1,347d(13p+5imm)							
3′	182	228(4p)						
4′	16	208	124	22(4p)				
5′	266	390	258	316	118	18	314(3p)††	
6′	129	324	94	20	230	8(4p+6imm)		
7′	69	578	520	206	408	286	440(7p+1imm)††	
8′	425b	628	42(6p)					
9′	299	252	442	40	426(4p)			
10′	534b	348	582	318	388	234	174(8p)	
11′	1,559bd	548	144	34	378	112	238(4p+2f)††	

p : pair of worms m or f : adult male or female worm imm : immature worm
* : includes degenerated ova † : died without receiving a full course of treatment
†† : died of schistosomiasis at 33 to 35 weeks after infection (?) : not autopsied
Groups A, B, C & D : See text. b or d : bloody stool or diarrhea

Table 3 Schistosomicidal activity of niridazole in hamsters infected with *S. japonicum*: Showing mean EPG and percentage reduction of EPG* in each group

Group of hamsters	Before treatment	After treatment						
		3 days	1w.	2w.	4w.	7w.	12w.	30w.
Group A	484.3	64.0 (86.8)*	19.5 (95.9)	35.4 (92.8)	78.7 (83.7)	9.6 (98.0)	24.0 (95.1)	
Group B	709.2	8.0 (98.9)	2.6 (99.6)	0 (100.0)	135.6 (80.8)	20.4 (97.1)	381.3 (46.2)	125.0 (82.4)
Group C	536.7	1.3 (99.8)	0.5 (99.9)	0 (100.0)	1.7 (99.7)	11.7 (97.8)	20.0 (96.3)	0 (100.0)
Group D	506.6	389.3 (23.3)	275.8 (45.6)	136.6 (73.0)	324.7 (35.9)	131.6 (74.0)	291.5 (42.4)	

$$\text{*Percentage reduction of EPG} = \frac{(\text{EPG before treatment}) - (\text{EPG after treatment})}{\text{EPG before treatment}} \times 100$$

Table 4 Schistosomicidal activity of niridazole in rabbits infected with *S. japonicum*

No. of rabbit	No. of cercariae infected	Dose of niridazole	Stool examination before and after treatment					
			before	3 days	1m.	3m.	6m.	12m.
Group E								
RA 1	300	100 mg/kg× 10 every days	+	−(2 dead worms)				
RA 2	400		+	−	−	−	−(none)	
RA 3	500		+	−	−	−	−	−(none)
Group F								
RA 4	400	100 mg/kg× 10 alternate days	+		−	−	−(none)	
RA 5	400		+		−	−	−	−(none)
Group G								
RA 1′	1,000	untreated control	+(112p+41m)					
RA 2′	400		+	+	+	+	+(69p+43m)	
RA 3′	300		+	+	+	+	+	+(5p+41m)

+ or −: positive or negative for schistosome eggs. ( ): No. of schistosomes recovered by necropsy.

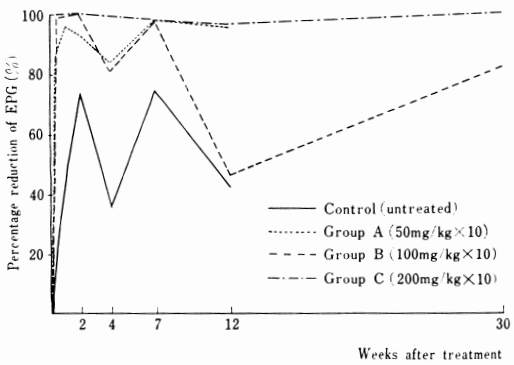

Fig. 1 Percentage reduction of EPG in hamsters treated with niridazole.

tions before and after treatment and the number of worms recovered at autopsy are summarized in Tables 2-4 and Fig. 1.

Eggs per gram feces (EPG) before treatment, namely 70 days after infection, ranged from 16 to 1,559 in the groups of hamsters. Though the levels of egg excretion in feces varied with the individual and the day of stool examination, EPG in treated groups rapidly decreased after treatment. However, degenerated eggs appearing in the feces during the treatment disappeared within one or two weeks after the completion of the treatment, and egg counts of hamsters of Group A and B showed a tendency to increase again

after two or four weeks. While in Group C which received 200 mg/kg of the drug every other day for 10 times, four out of six hamsters remained negative for schistosome eggs for more than four weeks after treatment. They became eventually negative for eggs by 30 weeks through a transitory positive period.

Except animal No. HA 26 which died due to pneumonia after the third administration of the drug, no worm was recovered from four out of seven animals in Group C at autopsy.

In the control group the level of egg counts was rather settled during the course of the observation. The worm burden in control hamsters was ranging from 3 to 13 copulated adult worms. No disintegrated worms were observed.

From these results of autopsy, it was obviously recognized that the daily dose of 200 mg/kg of niridazole was completely effective for hamsters.

Rabbits: A daily dose of 100 mg/kg of niridazole was given to five rabbits for 10 days. No egg was detected from the animals and the miracidial hatching test remained negative after treatment. Two disintegrated worms were microscopically found in the intrahepatic veins of the sectioned tissues of No. RA 1, which died at 8 days after treatment.

2. Pathological changes of the involved tissues after treatment

1) Hamsters

a. Control hamsters

Two control hamsters (Nos. HA 1′ and 2′) were sacrificed 70 days after infection, prior to the beginning of administration of niridazole. The number of worms recovered is shown in Table 2. Before autopsy, these two animals excreted a number of *S. japonicum* eggs with a diarrheal stool.

The pathological changes at autopsy were as follows: The liver was coloured dark brown and was slightly enlarged. It showed a fine superficial granularity and partially cirrhotic appearance. There were a number of gray-white milliary lesions on the surface of the liver. An extensive granuloma formation was present in the loop of the colon, which adhered to the right ovary. The left flexure of colon adhered to pancreas tissue, where egg lesions were also found. Enlargement of mesenteric lymph nodes was noticed.

Microscopically, the parenchymal structure of the liver lobules was rather well preserved, but the central and interlobular veins and sinusoids were enormously dilated. In certain sections, it was found that the wall of the sinusoids plugged with embryonated eggs was partially vanished. Many well-formed pseudotubercles around mature eggs with intact miracidium inside were seen in the portal spaces, consisting of layers of neutrophils, epithelioid cells and histiocytes in the inner side of the lesion, and of a layer of fibroblasts, lymphocytes and plasma cells in the outer side. Fibrosis associated with the granuloma was proved by Verhoeff-van Gieson and Mallory-azan staining. The giant cells were occasionally seen in the egg lesions. An acute inflammatory response or an abscess formation around mature eggs, however, was also observed adjacent to the granuloma as shown in Photo 1. Mucoid secretory products homogeneously stained by eosin were frequently included within some viable mature eggs (Photo 1 and 14). The marked round cell infiltration occurred in the portal tracts. Schistosome pigment was found in increased Kupffer cells and in large phagocytic cells in the portal spaces and pseudotubercles.

In the gastrointestinal tract, the loop and the left flexure of colon were most severely involved. A number of active egg lesions were seen in the submucosa, which increased in thickness, and a few in the mucosa. The lesions were characterized by the diffuse cellular inflammatory infiltrate, composed mainly of neutrophils, lymphocytes and plasma cells. The egg lesions occasionally developed to granulomatous pseudotubercles with peripheral fibroblastic proliferation (Photo 2).

Eosinophils were scarcely observed in the egg lesions or the tissues. In general, no inflammatory reaction was seen around immature eggs in the mucosa. There were

micro-ulcers in the mucosa. Subacute exudative inflammation occurred in the subserosa of the colon, with marked infiltration of plasma cells and lymphocytes (Photo 3).

In the other organs, lung sections showed chronic interstitial or septal pneumonia with a few egg lesions and congestion of blood vessels. The capillaries were enormously dilated and adhesion of a number of neutrophils to the endotherial surface was seen. A few immature worms were rarely found in the lumen of some vessels. A moderate hyperplasia of the lymphatic follicles and a cellularity of the red pulp in the spleen were recognized. The schistosome pigment was captured into the fixed and free macrophages. Mesenteric lymph nodes showed follicular hyperplasia, and the sinuses were enlarged and filled with inflammatory cells. Only mature eggs gave rise to well-developed granulomatous pseudotubercles ; but no granuloma formation was elicited by immature eggs, most of which were destroyed leaving egg shell remnants as so-called ghost forms. The egg lesions were also encountered in the pancreas and the omentum.

These pathological findings stated above are merely those of the control hamsters corresponding to the time of the beginning of the drug therapy in the treated groups. Therefore, follow-up observations on the control group corresponding to the time of the autopsy of the treated groups must be necessary to distinguish time factors or effects of naturally endowed ability of healing on the involving tissues of the host animals from the direct action or effects of niridazole. The results will be summarized below.

Follow-up study on pathology of cotrol hamsters

The control hamster No. HA 8′ was autopsied 14 weeks after infection (at one week after the end of the treatment). The liver and intestine were more severely involved than those of animals Nos. HA 1′ and 2′. Pigmentation and granularity on the liver surface predominantly increased ; a intense granuloma formation occurred in lower small intestine as well as in the colon. These gross pathological findings had a tendency to develop in the course of infection. The liver became cirrhotic and the intestine adhered to adjacent organs, e.g. abdominal wall, right ovary, pancreas and so on, in the long duration of infection. Furthermore, the ascites in the peritoneal cavity of control animals at least 20 weeks after infection.

The egg lesions in the liver and colon increased in number and in intensity with the lapse of time and reached the maximum about 15 week after infection. Severe ulceration and thickening of the submubosa due to increased fibrous granulomas occurred in the colon. Small intestines were also markedly involved at these periods ; numerous basophilic immature eggs freshly deposited in the mucosa and submucosa.

The liver sections showed the serious development of fibrosis along the portal tracts and the sinusoids at 25 weeks after infection. Thus, in later stages of infection the fibrous tissues eventually partitioned the liver parenchyma. In later stages, there were also, indeed, occasionally active egg lesions around intact mature eggs and inflammatory cell infiltration in the portal spaces as long as infection and oviposition went on. Kupffer cells increased in number with more extensive accumulation of schistosome pigment.

The calcification of eggs first appeared in the fibrous granuloma of the colon at 13 weeks after infection, with different stages of degenerated eggs ; pycnosis of the embryo cells occurred in some eggs and granular or hyalin degeneration in the others. Neutrophils and macrophages invaded schistosome eggs through broken egg shell, and then egg contents were replaced by epithelioid cells or occasionally multinucleated giant cells. However, the first occurrence of calcified eggs in the liver was one week later than in the colon.

From 13 weeks after infection eosinophils increased in number in all control animals ; they were generally more predominant in the colon than in the liver in early stages of infection but not so in later stages.

Not only in the liver and the intestine but

also in the other organs, a new formation of active egg lesions was continuously produced, while the egg lesions were transformed into the fibrous granulomas. Severe involvement frequently occurred in the lungs and pancreas, that is pneumonia in the lungs and diffuse fibrosis with parenchymal atrophy in the pancreas were caused by embolization of eggs.

Thus, three control hamsters (Nos. HA 5′, 7′ and 11′) could not survive until the end of this study (Table 2). They died of schistosomiasis within 35 weeks after infection.

b. Treated hamsters

Hamsters that died during the course of treatment

Two hamsters (Nos. HA 17 and HA 26) died of pneumonia after the third or fourth administration of niridazole. No significant difference in macroscopical and microscopical findings between these two hamsters and controls was noticed. Hamster No. HA 17 harboured 2 pair of living worms in the portal veins; No. HA 26 harboured 6 uncoupled adult worms including one dead male.

In the liver, the active egg lesions and inflammatory cell infiltration with mostly round cells in the portal spaces were still present. Sinusoids in the periphery of the lobules were dense with inflammatory cells. The portal and central veins showed congestion as in control. The accumulation of phagocytic cells taking pigment into their bodies were much predominant in the periphery of the egg lesions. A great deal of schistosome pigment was present freely in blood vessels as well as in Kupffer cells and phagocytes. This dirty blood with schistosome pigment was also seen in the vessels of the other organs, such as brain, heart, lung, kidney, spleen and abdominal lymph nodes, for instance.

In spite of these resemblances to the controls, some eggs deposited in the liver had already been degenerated and dissolved. It should be noted that phagocytic cells of host tissues, neutrophils, histiocytes, and monocytes, invade into the eggs through disintegrated egg shell and replace the peculiar cells of miracidium.

In the alimentary tract, a few eggs in the process of degeneration were also observed, but a number of well-developed eggs and freshly deposited immature eggs predominated. The submucosa of the colon was severely involved due to the presence of active egg lesions. Inflammatory responses in the subserosa of these treated animals, however, were evidently slight, as compared with those of control.

In the lungs of both animals, interstitial pneumonia caused by eggs was observed. Congestion accompanied with a diffuse cellular inflammatory infiltration was present. A number of neutrophils adhered to the endotherial surface of the thick-walled capillaries.

The fibrous reaction associated with the egg lesions in pancreas was more intensely seen than in that of the control. The pigmentation of the spleen and abdominal lymph nodes was also more predominant than that in the control animals, and the number of free macrophages slightly increased. However, there were neither egg lesions nor egg remnants in these organs.

Furthermore, the various organs and tissues of these hamsters were examined in detail concerning to the toxic effect of niridazole, because its toxicity might be the cause of the death of the animals. No toxic effect of niridazole itself on any organs and tissues could be revealed.

From these findings, it seems likely that these animals died due to a certain phenomenon analogous to the Herxheimer reaction in syphilis.

Hamsters given a complete treatment

One or two animals of each group were autopsied at 4 days, 1, 2, 4, 7, 12 and 30 weeks from the completion of treatment. In groups A and B given a daily dose of 50 and 100 mg/kg of niridazole, respectively, no findings of improvement or repair of the involved tissues due to the effect of treatment were noticed at autopsy.

On the organs and tissues of the hamsters of Group C which were considered to be parasitologically cured, however, the egg

lesions were markedly diminished and the involved tissues were completely repaired during the course of examination. Four days after treatment, there were no yellowish-white punctiform zones on the surface of the liver and very few in the intestine of hamster No. HA 21, which harboured 6 degenerated male worms without intestinal pigment. A number of black and opaque eggs were seen in the tissues of the liver and the colon by the press preparation of the small specimens.

The liver sections showed that most of the egg lesions became fibrous granulomas with degenerated and/or calcified eggs. As the destruction and resolution of egg substance advanced, neutrophils, macrophages with schistosome pigment, and epitheliod cells invaded the inside of the eggs (Photo 4). The macrophages took a lot of schistosome pigment into their bodies accumulated in the portal spaces and around the egg tubercles, especially in the peripheral zones of them. The round cell infiltration was still present in the portal tracts and occasionally around granuloma. Fine fibrosis occurred in the same degree as shown in the liver of control animals 10 weeks after infection.

A number of egg remnants and calcified eggs were seen in the submucosa of the colon. Some degenerated eggs were replaced by epithelioid cells or giant cells (Photo 5). No newly deposited immature eggs could be found anywhere. However, the inflammatory reactions still remained around degenerated eggs, with the cellular inflammatory infiltrate mainly composed of neutrophils and lymphocytes. A number of phagocytes taking dark brown pigment into their bodies were wandering in the egg lesions. A few eosinophils were scattered among these inflammatory cells.

The fibrous proliferation and the inflammatory cell infiltration were gradually diminished with the lapse of time in the portal spaces and egg tubercles in the hamsters receiving successful treatment.

Though a few egg remnants were seen in the wall of colon at 2 weeks after treatment, many eosinophils were recognized among the residual inflammatory cells. They appeared also in liver, spleen, pancreas, lungs, and especially predominant in lymph nodes.

At 7 weeks after treatment, an evidently diminished graunloma formation and periportal fibrosis were replaced with regenerated liver parenchyma (Photo 6). The portal and central veins were less dilated than those of the control animals. No congestion of portal system was observed. The round cell infiltration in the portal spaces was scarcely recognized. As shown in Photo 6, the increased phagocytes filled with dark brown pigment showed swelling.

In the colon of animal No. HA 30, a number of degenerated eggs were still present, evidently without a fresh deposition of basophilic immature eggs. The capillaries and venules were congested and dilated, with a slight infiltration of inflammatory cells around them. However, thickening of the wall of the colon was not so increased as that of control animals. The inflammatory reactions did not occur in the subserosa. A few eosinophils were seen in the capillaries of the mucosa and around the degenerated eggs in the submucosa.

Hamsters Nos. HA 23 and 25 sacrificed at 30 weeks after treatment had livers of smooth surface with spotted pigmentation. The press preparations from the livers showed neither degenerated eggs nor worms in the tissues. No egg lesion was found in the alimentary tract macroscopically, and no worm was obtained from the portal and mesenteric veins.

Microscopically, very few egg shell remnants and calcified eggs were seen in the liver. No noticiable granuloma formation was found even around accumulation of the degenerated eggs. The inflammatory infiltration and the connective tissue proliferation completely disappeared; the liver parenchymal cells regenerated well (Photo 7). Schistosome pigment in phagocytic cells diminished more than 7 weeks after treatment.

In the loop of colon of hamster No. HA 25, there was no pathological change; egg remnants were never found. On the contrary, in the colon and small intestine of No. HA 23, calcified eggs and noncalcified egg shell

remnants were still recognized. A few eosinophils were scattered in the tissues. Regeneration of muscle fibers, however, occurred in the muscular layers of the intestine.

2) Rabbits

a. Control rabbits

Three control rabbits of Group G were autopsied one by one at 60 days, 8 months and 14 months after infection. The worm burden of these animals ranged from 51 to 265 worms shown in Table 4.

Though rabbit No. RA 1′ autopsied at 60 days after infection could not always be regarded as an appropriate control because of its large worm load, it was possibly useful for understanding the basic pattern of the pathological changes in early stages of schistosomiasis in rabbits.

Rabbit No. RA 1′ had a liver of a hard consistency with a number of yellowish-white punctiform zones all over the surface. The most severe involvement in the alimentary tract was found in the lower part of small intestine. A gross, irregular granularity and thickening occurred in the intestinal wall.

The liver sections showed many typical egg lesions with intense infiltration of round cells, caused by viable eggs or egg remnants. In one large granuloma, the abscess formation was associated with giant cells (Photo 8). Liver parenchymal cells adjacent to these egg lesions occasionally fell into necrobiosis, possibly due to oppression of fibrous tissues and deficiency of blood supply. Some other parenchymal cells were scattered to be embeded in fibrous tissues like an islet. Compared with the lesions that occurred during the course of infection on hamsters, eosinophils markedly increased in number in the egg lesions of rabbits. As shown in Photos 9 and 10, there was a thick band of fibrous tissue in the portal tracts and around the egg lesions, with moderate canalicular and angiomatoid hyperplasia accompanied by arteritis. However, it was difficult to find out the direct relation between the changes of the wall of the portal veins and the presence of the parasite in them. Sinusoids were seriously dilated and Kupffer cell proliferation occurred.

In the other organs, there was no difference between the histopathological observations of the control rabbit and those of control hamsters sacrificed at 70 days after infection.

In later stages of the infection, the liver of control rabbits increased its consistency, nodular aspect, and pigmentation; the alimentary tract was involved more seriously both in the small intestine and in the colon. At 14 months after infection, the wall of the colon of rabbit No. RA 3′ became so thin that its contents could be seen. From this finding it was proved microscopically that the bottom of the ulcer had reached under muscularis mucosa of the colon. Moderate ascites was proved in the peritoneal cavity of rabbit No. RA 2′. It was most prominent in the liver sections that the proliferated fibrous tissues completely partitioned the lobules, with the formation of pseudolobules. The active egg lesions with the large accumulation of intact mature eggs were still present in the broad zone of fibrosis, consisting of layers of neutrophils, epithelioid cells and histiocytes in the inner side, and of layers of lymphocytes, plasma cells, fibroblastic cells in the outer side. In the colon of animal No. RA 3′, a serious abscess formation was also seen at 14 months after infection, associated with intense infiltration of eosinophilic granulocytes.

b. Treated rabbits

In rabbit No. RA 1, which died due to an unknown cause at 8 days after the completion of treatment, yellowish-white punctiform zones were scarcely present on the surface of the liver. The liver sections showed two disintegrated worms in the portal veins, which were enveloped by fibrous tissue associated with accumulation of mononuclear and polymorphonuclear leukocytes (Photo 11). The cuticular layer of the worm was destroyed and necrobiosis of the somatic cells occurred. Serious thrombophlebitis occurred in the veins; diffuse necrobiosis of the hepatic cells was observed. This animal was considered to have died probably due to the Herxheimer type reaction.

At six *months after treatment*, the liver of

rabbit No. RA 2 was adequately improved; the consistency was normal and the surface was smooth with normal colour. It should be noted that an active regeneration of liver papenchymal cells evidently occurred at the periphery of the lobules; many binuclear cells with slightly basophilic cytoplasma were seen at the periphery (Photo 12). The fibrous tissues markedly diminished. And yet, non-calcified egg shells still remained, though rare; and eosinophils were also seen, both in the liver and in the colon.

The liver sections of rabbit No. RA 3 sacrificed at 12 months after treatment showed neither hyperplasia of connective tissues nor perilobular partitioning (Photo 13). A few egg shell remnants were present in the liver, but not in the intestinal tract. There was no inflammatory cell infiltration in these tissues.

3) Summary of histopathological study

The liver and intestine of the animals used were seriously involved due to schistosome eggs in early stages of the infection. The pathological findings of the liver of the control animals are characterized by minute abscess or granuloma formation with thrombophlebitis and an intense round cell infiltration in the portal tracts. Hyperplasia of connective tissues is observed in the portal spaces and around the egg granuloma. Ulceration due to egg lesions is seen in the mucosa of the intestine. Eosinophilia is more prominent in rabbits than in hamsters. In later stages, thick bands of fibrous tissue partition the lobules of the liver; thus bilharzial cirrhosis is initiated. Severe ulceration and irregular thickening of the intestinal wall occur.

On the contrary, if the animals are given effective dose of the drug in early stages of infection, the development of granuloma formation and the progress of fibrosis are evidently discontinued. The proliferated fibrous tissues are replaced with regenerated hepatic cells. The inflammatory reactions in the egg lesions or portal spaces of treated animals markedly diminish by the completion of the drug therapy.

In general, the intact mature egg has a well-developed miracidium inside, which is tightly jointed with the egg shell by septal membrane. The nuclei of the innate miracidial cells are well stained with hematoxylin. The mature egg frequently includes mucoid substance inside of the egg shell (Photo 14).

On the other hand, in the treated animals, many degenerated eggs appear at the end of treatment. Some of them show pycnosis of the nuclei of miracidial cells and granularity of egg content (Photo 15a), hyalin degeneration (Photo 15b), or calcification which generally begins from the peripheral zones (Photo 15c); and others remain only as non-calcified egg shells, which are invaded by neutrophils and macrophages, and replaced gradually by epithelioid cells and fibroblasts (Photo 15d). The egg shell remnants are present in liver tissues for more than one year after treatment. Of course, these degenerated eggs are also seen in untreated animals with the lapse of time of infection. It is, however, precisely observed that they appear earlier and more predominantly in treated animals than in untreated ones.

3. Circumoval precipitin (COP) test

The results of circumoval precipitin test on the sera of each hamster of four groups are given in Tables 5 and 6. The figures in these tables mean the percentage of eggs that formed precipitates around the egg shells. The precipitates around the egg showed various shapes and dimensions: Type I means small precipitates in form and in number; type II, large globular, ligulate or massive precipitates; and type III, bandform or segmented precipitates. Most precipitates of type II or III were observed in the sera of 30% or higher.

By serial COP determinations in the same animals, it was evidently shown that the percentage of positive eggs and type of precipitates are reduced after treatment in proportion to dose of niridazole. Particularly, in Group C which included cured hamsters, the percentage of positive eggs markedly decreased to 10% at 16 weeks after treatment, and below 10% at the 30th week (Table 6 and Fig. 2). On the contrary, in the untreated

Table 5 COP test on the sera of hamsters treated with niridazole: Showing percentage and type of COP

No. of hamster		Before treatment	Immediately after treatment	Weeks after treatment					
				2w.	4w.	7w.	12w.	16w.	30w.
Group A									
HA	1	37.0(Ⅲ)	30.0(Ⅱ)						
	2	50.0(Ⅲ)	53.3(Ⅲ)	44.4(Ⅲ)					
	3	76.5(Ⅲ)	50.0(Ⅲ)	46.2(Ⅲ)	50.0(Ⅲ)	43.0(Ⅱ)	16.7(Ⅰ)		
	4	56.0(Ⅲ)	50.0(Ⅲ)	30.0(Ⅱ)			45.5(Ⅱ)		
	6	67.7(Ⅲ)		59.2(Ⅲ)	24.0(Ⅰ)				
	8	44.4(Ⅲ)		38.5(Ⅱ)	60.0(Ⅲ)	30.0(Ⅰ)			
	9	61.9(Ⅲ)		55.6(Ⅲ)	41.1(Ⅱ)				
Group B									
HA	11	35.8(Ⅲ)	35.6(Ⅱ)						
	12	41.8(Ⅱ)	34.6(Ⅱ)	28.9(Ⅱ)					
	13	62.1(Ⅲ)	45.2(Ⅲ)	41.2(Ⅱ)		42.9(Ⅱ)	54.4(Ⅲ)		
	14	30.6(Ⅱ)	25.9(Ⅱ)	32.2(Ⅱ)		29.5(Ⅱ)	22.3(Ⅰ)		22.2(Ⅰ)
	15	37.5(Ⅱ)	33.4(Ⅱ)	37.8(Ⅱ)		32.7(Ⅱ)	32.5(Ⅱ)	17.2(Ⅰ)	
	16		53.0(Ⅲ)	30.9(Ⅱ)					
	18	61.6(Ⅲ)	47.6(Ⅲ)	40.0(Ⅱ)		40.9(Ⅱ)			
	19		48.2(Ⅲ)	39.3(Ⅱ)		37.5(Ⅱ)			
Group C									
HA	21	40.4(Ⅱ)	31.8(Ⅱ)						
	22	69.2(Ⅲ)	44.4(Ⅱ)	14.3(Ⅰ)					
	23	38.9(Ⅱ)	39.3(Ⅱ)	36.4(Ⅱ)		22.2(Ⅰ)	26.9(Ⅰ)	4.4(Ⅰ)	7.5(Ⅰ)
	24	58.1(Ⅲ)	34.6(Ⅱ)	55.6(Ⅱ)		42.3(Ⅱ)	27.3(Ⅰ)	14.3(Ⅰ)	
	25	35.9(Ⅱ)	26.1(Ⅰ)	20.4(Ⅰ)		8.3(Ⅰ)	11.1(Ⅰ)	10.3(Ⅰ)	0.0(—)
	27	31.1(Ⅱ)	42.6(Ⅱ)	24.0(Ⅰ)					
	28	42.8(Ⅱ)	19.1(Ⅰ)	41.3(Ⅱ)		35.3(Ⅱ)	25.0(Ⅰ)	12.5(Ⅰ)	
	29	28.2(Ⅱ)	35.7(Ⅱ)	43.5(Ⅱ)		68.0(Ⅲ)	30.4(Ⅱ)		
	30	31.1(Ⅱ)	60.0(Ⅲ)	55.6(Ⅲ)		6.7(Ⅰ)			
Group D									
HA	3′	48.3(Ⅲ)	37.5(Ⅱ)						
	4′	47.5(Ⅲ)	44.2(Ⅱ)	38.2(Ⅱ)					
	5′	41.8(Ⅱ)	39.5(Ⅱ)	58.0(Ⅲ)		61.7(Ⅲ)	45.8(Ⅲ)	60.0(Ⅲ)	
	6′	72.0(Ⅲ)	62.5(Ⅲ)	83.4(Ⅲ)		70.6(Ⅲ)			
	7′	60.0(Ⅲ)	45.0(Ⅱ)	57.2(Ⅲ)		43.7(Ⅱ)	30.8(Ⅱ)	53.3(Ⅱ)	
	8′	48.2(Ⅲ)	57.1(Ⅲ)	37.5(Ⅱ)					
	9′	70.7(Ⅲ)	41.7(Ⅱ)	45.4(Ⅱ)					
	10′	47.7(Ⅲ)	64.3(Ⅲ)	66.7(Ⅲ)		62.5(Ⅱ)	61.6(Ⅱ)		
	11′	55.8(Ⅱ)	43.5(Ⅱ)	51.8(Ⅱ)		43.5(Ⅱ)	57.1(Ⅱ)	46.7(Ⅱ)	

Group A: 50 mg/kg×10 every other days
Group B: 100 mg/kg×10 every other days
Group C: 200 mg/kg×10 every other days
Group D: Untreated

Table 6 COP test on the sera of hamsters treated with niridazole: Showing the average percentage of positive eggs in each group

Groups	Daily dose in mg/kg	Before treatment	Immediately after treatment	Weeks after treatment					
				2w.	4w.	7w.	12w.	16w.	30w.
A	50 mg/kg×10 days	56.2	45.8	45.7	43.8	36.5	31.1	···	···
B	100 mg/kg×10 days	44.9	40.4	35.8	···	36.7	36.4	17.2	22.2
C	200 mg/kg×10 days	41.7	37.1	36.4	···	30.5	24.1	10.4	3.8
D	Control	53.6	48.4	54.4	···	57.4	48.8	53.3	···

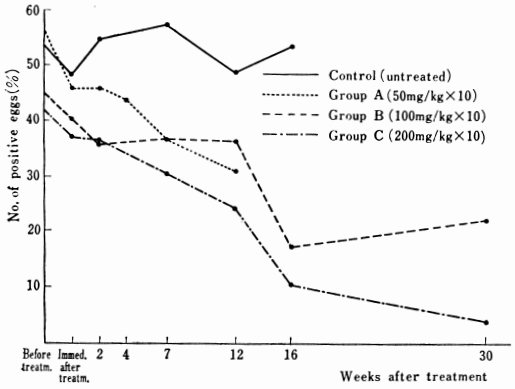

Fig. 2 COP test on the sera of hamsters treated with niridazole: Showing the average percentage of positive eggs in each group.

group, the COP test maintained high levels of the positive reaction up to 26 weeks after infection.

Discussion

1. Histopathological changes of the involved tissues after treatment

The World Health Organization's Scientific Group on Research in Bilharziasis (Chemotherapy) (1964) has emphasized that long-term studies, using various modern techniques for the detection of liver damage or pulmonary impairment, are urgently needed in order to increase our knowledge of the pathogenesis of bilharziasis in relation to chemotherapy.

In this study, a few attempts have been made to clarify, with reference to treatment with niridazole, the pathogenesis of schistosomiasis japonica, chemotherapeutic effects on the involved tissues and the criteria of estimation of "cure". It is evidently proved that, if effective dose of niridazole is given to hamsters and rabbits in early stages of the infection, the development of ganuloma formation and the progress of fibrosis are prevented due to the schistosimicidal and ovicidal effects of the drug.

Nishi (1923) has reported that oviposition of *S. japonicum* are suppressed by treatment with sodium antimony tartrate and that various stages of degeneration occur in deposited eggs: granular and hyalin degeneration, destruction of egg shell, and calcification. He has also pointed out that these findings of degenerated eggs really accord with the observations described in non-treated host tissues by Nakayama (1910).

The same findings are also observed in the present experiments. However, degeneration of eggs is apparently accelerated in the treated animals; the eggs deposited in the liver and the colon have already been degenerated and dissolved after the third or fourth administration of niridazole. In the control, calcified eggs are first seen in tissues at 13 weeks after infection. It has been noted that schistosome eggs not excreted die in the host tissues 3 or 4 weeks after oviposition (Vogel, 1942; Gönnert, 1955a). By observing the activity of succinic dehydrogenase, Oda (1959) has also found that the survival period of eggs in tissues is about 3 weeks, and that calcification of eggs can be observed 40 days after the death of egg.

Since Majima (1888) reported the first human case of the hepatic fibrosis caused by eggs of *Schistosoma japonicum*, a number of publications have emphasized the importance

of the role of eggs in the production of hepatosplenic schistosomiasis. Symmers (1903) noted the pathological picture of cirrhosis in "pipestem fibrosis", attributing it to the effects of eggs.

Nakayama (1910) described that the action of schistosome eggs on tissues is only mechanical stimulation similar to the lesions caused by glass beads in the tissues. Fujinami (1916), Kiyono & Murakami (1917), Nishi (1923) and Day (1933) presented that schistosome egg lesions could not be explained only by mechanical stimulation of eggs but should also be attributed to the metabolic products from the miracidium in the egg. Faust & Meleney (1924) reported that mucoid material seen within the well-embryonated egg (see Photo 14) acts as a pyogenic toxin in host tissues.

By the injection of living and heat-killed schistosome or *Ascaris* eggs into the portal vein of albino rats, Ushiyama (1953) found that living schistosome eggs elicite more intense lesions than the others.

Hashem (1947) showed that all stages of evolution of bilharzial hepatic fibrosis can well be explained by the local effects of eggs impacted in the portal venules. He also proposed that the disproportion between the degree of fibrosis and the number of eggs deposited would be caused by diffusion of toxins from disintegrated eggs. Recently, Scorza & Scorza (1967), who share these points of view, have observed not only the appearance of granulomata in the portal tracts, but also the hyperplasia of the reticular and collagenous tissue situated between portal spaces with granulomata in rabbits merely injected with live eggs of *S. japonicum*.

On the so-called toxic substance, Smith & Lichtenberg (1967) have demonstrated that it is not toxin but the secretory products of cephalic glands of mature miracidium, consisting of cystine- and cysteine-rich protein, and that it reacts with tryptophan-rich host globulin to form many acidophilic radiating precipitates, which were previously described as clavate substance by Nakayama (1910) and Nishi (1923). This substance has been recognized to be leukotactic, resulting in a micro-abscess forming around the egg (Hsü *et al.*, 1969). As many leukocytes in the micro-abscess are eventually fragmented, Hsü *et al.* (1969) have also postulated that the secretion itself or its resulting antigen-antibody complex may contain proteolytic enzymes.

Lichtenberg & Sadun (1968) have reported, using primate species infected with *S. mansoni* cercariae, that portal fibrosis is correlated with heavy egg deposition in the portal triads and intrahepatic portal radicles, accompanied by granulomatous as well as diffuse inflammation. Thus there seems to be little doubt that the eggs are the main pathogenic agent in schistosomiasis.

In these respects, it was confirmed from the results of the present experiments that in treated animals the inflammatory reaction is diminished due to death of eggs, and that, if relapse of oviposition does not occur, fibrous proliferation ceases and is replaced with regenerated tissues at least 7 weeks after the completion of treatment.

However, from another point of view that the granulomatous reaction of the host to the schistosome eggs plays a major role in the development of hepatosplenic schistosomiasis, Domingo *et al.* (1967) have recognized that granuloma formation by injected viable eggs of *S. mansoni* is suppressed by immunosuppressive drugs. Lichtenberg (1964) has postulated that the protective role of the granuloma in schistosomiasis is to sequester potentially harmful antigens produced by the schistosome egg. Conversely, he has also suggested that the large concentration of antigen in the granulomas might promote central necrosis, thereby leading to scar formation during the healing phase. These multiple small scars have been shown to develop to typical fibrosis of the Symmers type (Warren, 1966). Therefore, the granuloma in schistosomiasis seems to be actually a harmful reaction for the host. It is a matter of interest that Domingo *et al.* (1967) have suggested that if granuloma formation could be suppressed in the early stages of

the disease, the development of overt hepatosplenic disease might be averted.

In the chemotherapy of schistosomiasis, it is well known that toxic effects, resembling the so-called Herxheimer reaction in syphilis, occur not only in humans but also in experimental animals treated with various drugs. A number of authors have recognized that, regardless of the type of compound used, many of the effects observed may be due to the death and disintegration of the parasites. Schubert (1948) and Standen (1953) have asserted that a shifting of worms into the liver is a major characteristic of the response of schistosomicidal drugs.

Mousa *et al.* (1967) have pointed out the importance of the role of the shifted worms as the real origin of Symmers fibrosis, in relation to the fact that the incidence of periportal pipestem bilharzial fibrosis of the liver has increased since antimony treatment was introduced in Egypt in 1919.

Coutinho *et al.* (1944) and Day (1948) have described hepatic lesions due to dead worms. Tavares & Menezes (1945) have reported that dead worms may produce liver parenchymal necrosis, and that the necrotic lesions are initially invaded by eosinophils and later replaced by fibrous tissue. Meleney and his co-workers (1953) have observed in guinea pigs and rabbits that dead worms stimulate thrombosis and an intensive perivascular reaction that leads to scar formation and recanalization of the vessels.

Gönnert (1955b) has attributed the tissue reaction to the toxic matabolic produts, the break-down of the parasite and its eggs. However, he has observed that after the successful treatment, extensive regeneration occurs in the liver leading to a normalization of its structure and regression of granulomas.

Gillman (1957) has concluded that the cirrhosis of bilharziasis is the final result of the progressive obstruction of the intrahepatic portal branches, induced by endo- and periphlebitis caused by the eggs and especially by the dead worms. Menezes (1967) has emphasized that the worms not only obstruct the vascular lumens, but cause destruction of the vessel walls, inducing an inflammatory reaction with proliferation of the subintimal connective tissue which extends to the fibrous tissue of the portal triad.

Furthermore, Menezes (1963) has produced lesions similar to Symmers fibrosis in the livers of rats and rabbits injected with lyophilized adult *S. mansoni* worms.

Hewitt & Gill (1960, 1962) and Geake (1962) have claimed that a pronounced "lung shift" appears to be a common response to treatment. Dead schistosomes carried as emboli to the lungs produce necrosis of the pulmonary arteries and focal necrotizing pneumonia in the immediate vicinity (Elwi, 1967; Menezes, 1967).

In the present studies, dead worms which were ensheathed by fibrous tissue associated with round cell infiltration, were observed in the intrahepatic veins of rabbit No. RA 1. Thrombophlebitis and somewhat diffuse necrobiosis of hepatic cells around the veins occurred. Striebel (1969) has stated that such a necrobiosis is subsequently delimited from the intact liver cells by a concentric connective tissue reaction.

In two hamsters, No. HA 17 and HA 26, which died during the course of treatment, it seemed likely that a great deal of the schistosome pigment seeing fixed in macrophages or freely in blood might be derived from parasite intestine or disintegration and resolution of worm body. The shifting of worms into the liver or lung was not found at necropsy.

It is true that the parasites obtained after the successful treatment are white and opaque because of the release of their intestinal pigment. Striebel (1969) has asserted, based on the finding that ovary cells and vitelline cells of females are also expelled en masse, that this output of sexual products may possibly account for the fact that Dodin *et al.* (1966) succeeded in demonstrating the presence of free circulating antigen in the serum of human patients seven days after the beginning of treatment with niridazole. Capron *et al.* (1969) have recognized the existence of "immunological rebound phenomenon" in

patients of schistosomiasis retreated with niridazole one year after the initial treatment. They have presented that antischistosomal therapy with niridazole has the immunological consequence of increasing the production of precipitating antibodies after the 15th day following the beginning of treatment.

From these observations and discussions, it would be supposed that a kind of allergic reaction to mass antigenic substances might be brought about rather immediately due to some unknown mechanisms. This reaction might occur very rarely and transiently; the animals exempted from intense reaction could survive longer than untreated control animals.

The World Health Organization's Scientific Group on Research in Bilharziasis (Chemotherapy) (1964) has stated that this phenomenon seems to differ from the Herxheimer reaction, mainly in that it occurs at some critical sites (e.g., liver, lungs) with the aggravating circumstances that (a) the size of the dead agent (worm) is relatively large; (b) the number of worms in both natural and artificial infections may be very high; and (c) the tissue of the organ may be already damaged by the same or another etiological agent.

Conversely, Warren (1961) reported that mice treated before oviposition develop no disease, and further he (1962) has confirmed that mice treated when hepatosplenic schistosomiasis is either partially or wholly developed, rapidly undergo a reversion toward normal.

Sadun *et al.* (1969) have presented that, even the mice treated with niridazole after the onset of egg laying, there was no detectable increase in liver damage which might have been attributed to the shifting or reabsorption of dead worms. They have also stated that treatment and resultant reabsorption of dead worms did not bring about any detectable alteration of liver function; the elevation in transaminase levels might be related to the damage occurring in the lungs rather than in the liver.

After the completion of effective niridazole treatment, the present studies revealed the fact that various stages of degeneration occur in the deposited eggs due to ovicidal effect of the drug following the diminution of inflammatory reactions in the tissues and that fibrous tissues are replaced by regenerated hepatic cells by 7 weeks after treatment.

Finally, it may be given as a conclusion that effective chemotherapy can prevent the establishment of hepatic fibrosis.

2. COP reaction as a test of estimation of cure

The evaluation of treatment or estimation of "cure" in schistosomiasis is still a subject of great controversy.

Recently, as a new method for estimating the daily egg output, Bell (1963) has devised a filtration concentration staining method. Even if Bell's egg counting method is used, however, it may be extremely difficult to detect schistosome eggs in feces of such a light infection case as less than 1,000 EPD (eggs per day) (Yokogawa *et al.*, 1968a).

Pellegrino and his co-workers (1962, 1963, 1964, 1965) have asserted that the oogram method is a simple, sensitive and reliable criterion for the screening of drugs.

Newsome (1963) and Lambert (1967) have reported that the miracidial hatching test is the simplest and most reliable method of determining the abscence or persistence of active parasitic infection.

For all that, disappearance of viable eggs from stools or rectal mucosa could not always determine cure of the disease; living worms may persist after egg production ceases. Consequently, other procedures are required for realistic evaluation of treatment.

In the case of *Paragonimus westermani* infection, complement fixation test (CFT) has been recognized to be practically useful for estimation of chemotherapeutic effects (Yokogawa *et al.*, 1962).

In schistosomiasis, however, no clear-cut result was obtained with CFT (Fairley, 1919, 1926; Schofield, 1959).

Okabe & Tanaka (1958) have suggested that urine precipitin reaction becomes negative after successful drug therapy. Ishizaki *et al.* (1967) have reported, using a series of

two fold dilutions of *S. japonicum* antigen, that the threshold value of positive skin test becomes lower after successful treatment.

Concerning the prognostic value of circumoval precipitin (COP) test, Oliver-Gonzalez (1954) has pointed out that secretions or excretions from eggs, which are considered to exert a cytolytic effect and cause the formation of micro-abscess in host tissues, may be the antigenic material responsible for the formation of the precipitate in COP. This suggests that COP could possibly be of use in following the effectiveness of therapy.

Since his suggestion, many investigators have reported that the circumoval precipitin reaction reduces within several months or one year after adequate treatment, mainly with stibophen (Fuadin®) (Oliver-Gonzalez *et al.*, 1955; Rodriguez-Molina *et al.*, 1959, 1962; Sadun *et al.*, 1962; Cancio *et al.*, 1967).

The present experiment is the first one pursuing the effectiveness of treatment with niridazole in animals infected with *S. japonicum*. The anti-egg precipitin titers were predominantly reduced less than 10% within 30 weeks after the successful treatment. It should be noted that the results obtained are closely in accord with the pathological findings. Active egg lesions or cellular inflammatory infiltration completely disappear at 7 weeks after the completion of treatment, and there were no viable eggs in the tissues; thus, secretion and excretion of antigenic substances from miracidium may cease. The time lag between the pathological findings and conversion from positivity to negativity could be accounted for by an immunological memory of host immunocompetent cells.

Results obtained in this study agree with Scorza & Scorza (1961); they have advocated that a reading of 15% or more in this reaction can be considered as indicative of the presence of living eggs in host tissues.

From the above mentioned, it is suggested that the circumoval precipitin test may be of value in evaluation of cure. Further studies, however, will be required for clinical use.

Summary

In experimental schistosomiasis japonica in hamsters and rabbits treated with niridazole, the following results were obtained:

1) The degeneration of eggs deposited in tissues is accelerated during the treatment, and no freshly deposited egg is present after the successful treatment.

2) The round cell infiltration in egg granuloma and portal tracts disappears at 7 weeks after treatment.

3) The fibrosis of the liver in the earlier stages of infection is replaced with regeneration of hepatic cells within 7 weeks after treatment.

4) The disintegrating parasites which were enveloped by fibrous tissues associated with accumulation of round cells may embolize portal veins and bring about necrobiosis to the adjacent hepatic cells.

5) Anti-egg precipitin titers of hamsters are reduced below 10% within 30 weeks after effective drug therapy. The results suggest that COP test may be of value in estimation of cure.

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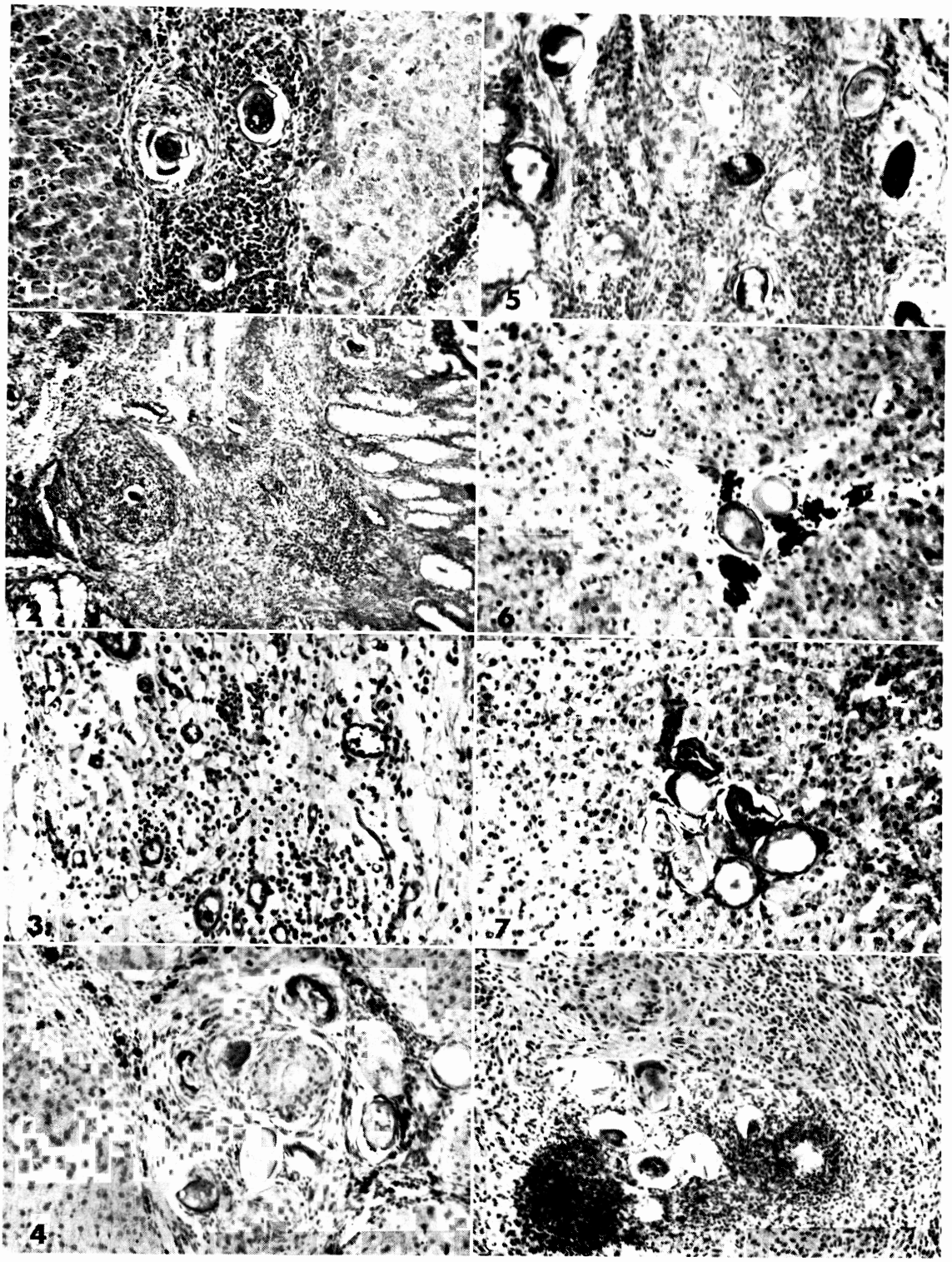
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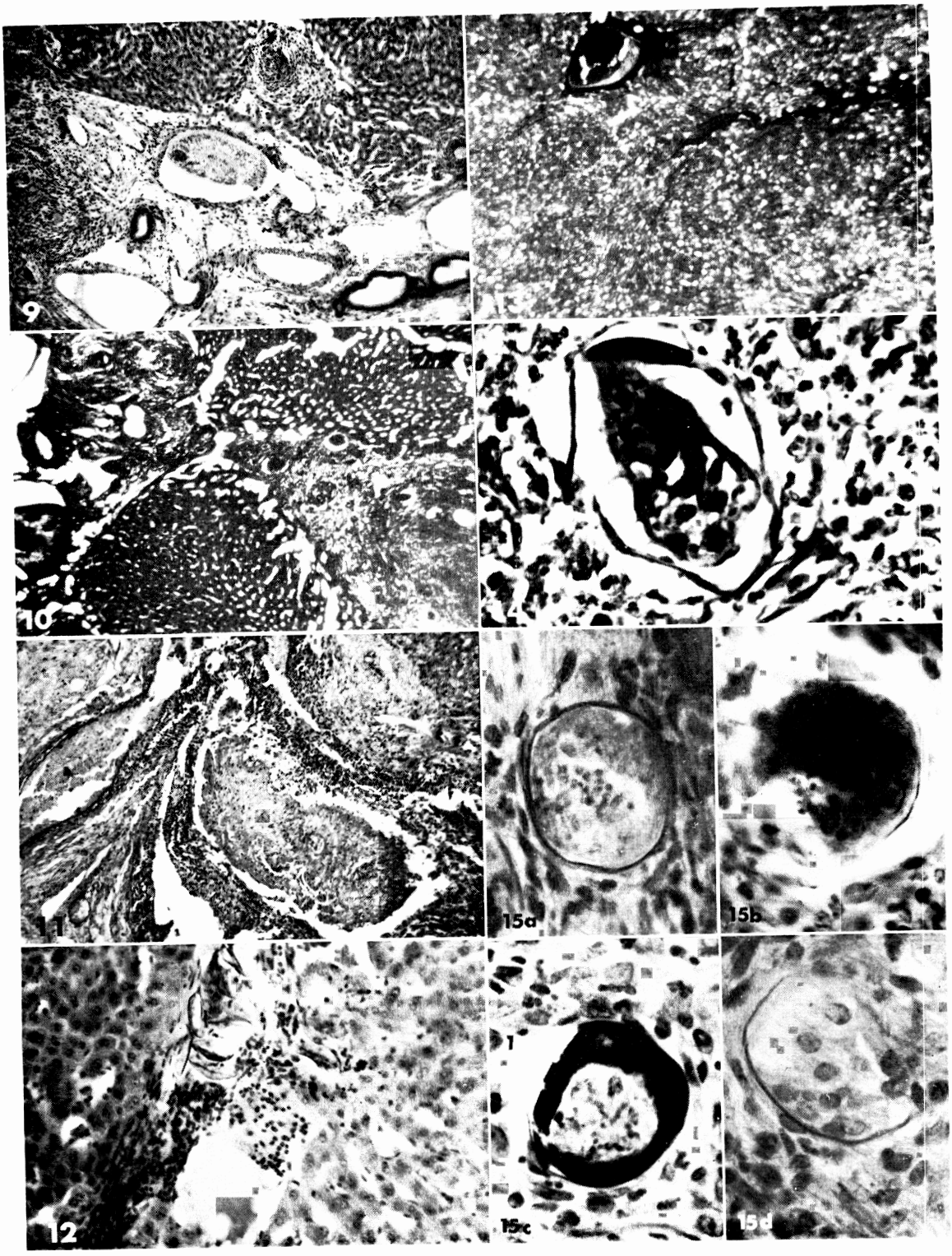
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日本住血吸虫症の治癒機転に関する実験病理学的研究

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日本住血吸虫症の治療後の組織修復機転についての病理学的研究は少ない．そこで本症感染のハムスター及び家兎に感染後60～70日目より niridazole を経口的に投与した後，前者では治療終了直後から30週迄，後者では1年後に至る迄経時的に剖検し，その病理組織像の変化を追究した．またその際 AMS Ⅲ法による検便と，免疫血清学的検査法の一つである COP test (circumoval precipitin test) とを同様に経時的に行ない治療成績の参考とした．その結果，niridazole の投与により寄生虫学的に治癒と認められる場合，治療後7週目には，1) 肝細胞の再生と線維化した虫卵結節の縮少・消失とによる局所組織の正常化，2) 円形細胞浸潤など炎症反応の消褪，3) 栓塞虫卵の石灰化ないし卵内ミラシジウムの崩壊吸収とそれを置換する宿主細胞の卵殻内侵入，など組織修復の病理学的所見が観察された．これらの所見を検便成績，COP test の結果と比較し，本症における治癒判定の問題についても考按した．

Explanation of Plates

Photo 1 Liver; hamster No. HA 1′, showing inflammatory cell infiltration and granuloma formation around mature eggs in the portal spaces. HE. ×100.

Photo 2 Colon; hamster No. HA 1′, showing micro-ulcer in the mucosa, and developing granuloma with peripheral fibrosis and diffuse inflammatory cell infiltration in the submucosa. HE. ×40.

Photo 3 Colon; hamster No. HA 1′, showing subacute exudative inflammation in the subserosa with proliferation of plasma cells. HE. ×100.

Photo 4 Liver; hamster No. HA 21, showing various stages of degenerated eggs. Note the diminution of round cell infiltration. HE. ×100.

Photo 5 Colon; hamster No. HA 21, showing epithelioid cells and giant cells which replaced embryo cells in eggs. Many calcified eggs are present. HE. ×100.

Photo 6 Liver; hamster No. HA 30, showing diminished fibrous tissues of granuloma and well regenerated hepatic cells. Phagocytes filled with schistosome pigment are seen. HE. ×100.

Photo 7 Liver; hamster No. HA 23, showing accumulation of degenerated eggs without inflammatory reactions or fibrosis. HE. ×100.

Photo 8 Liver; rabbit No. RA 1′, showing serious abscess formation with giant cells. HE. ×100.

Photo 9 Liver; rabbit No. RA 1′, showing broad zone of fibrosis with angiomatoid and canalicular hyperplasia. A well-preserved male worm is seen in the lumen of the dilated portal vein. HE. ×40.

Photo 10 Liver; rabbit No. RA 1′, showing fibrous tissue proliferation in the portal tracts and egg granulomas. Mallory-azan. ×40.

Photo 11 Liver; rabbit No. RA 1, showing thrombophlebitis due to disintegrated worm engulfed with numerous neutrophils. Note the connective tissue enveloping the worm. HE. ×100.

Photo 12 Liver; rabbit No. RA 2, showing regeneration of hepatic cells at the peripheral zones of the lobules. Note many binuclear cells. HE. ×100.

Photo 13 Liver; rabbit No. RA 3, showing well regenerated liver parenchyma. Mallory-azan. ×40.

Photo 14 Intact mature egg with mucoid substance inside. Note well-developed miracidium and septal membranes. HE. ×400.

Photo 15 Various stages of degenerated eggs. a. Granular degeneration with pycnosis. b. Hyalin degeneration. c. Calcification. d. Egg shell remnant invaded by epithelioid cells and fibroblasts. HE. ×400.