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REVERSIBILITY OF HISTOPATHOLOGICAL CHANGES IN THE OVARIES IN ACUTE MURINE SCHISTOSOMIASIS MANSONI AFTER NIRIDAZOLE TREATMENT

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Abstract. Atrophy of the corpus luteum cells, and nuclear alterations of the interstitial cells, are constantly found in the ovaries of mice with acute *Schistosoma mansoni* infection. In order to investigate whether these pathological changes are reversible, 2 months after infection of mice niridazole was administered orally for 10 days in a daily dose of 100 mg/kg body weight. Uninfected and infected untreated groups served as controls. The frequency of normal ovaries was much greater in animals cured of infection than in the infected controls. In some animals, unilateral ovariectomy was performed; the histopathological changes in the ovaries removed before treatment were absent in the remaining ovaries 4 weeks after treatment. These findings indicate that ovarian alterations in murine schistosomiasis mansoni can be reversed by adequate antischistosomal treatment.

In a previous study, acute and chronic murine schistosomiasis mansoni were found to cause atrophy of the corpus luteum cells and formation of the interstitial cells into "wheel cells" in the ovaries.[1] It seemed important to investigate whether these ovarian changes were irreversible, or could be cured by adequate antischistosomal treatment.

MATERIALS AND METHODS

Ninety CF_1 outbred albino mice, about 47 days old with an average body weight of 24 g, were divided into three groups. Thirty mice served as uninfected controls (Group 1). The remaining 60 animals were each infected with 180 cercariae of *Schistosoma mansoni,* using the ring method.[2] Thirty of the infected mice were later treated with niridazole (Group 2), and the remaining 30 served as infected, untreated controls (Group 3).

Two months after the day of infection, unilateral ovariectomy was performed on ten mice of each group. The ovaries were weighed, fixed in Bouin's solution, embedded in paraffin, and the sections were stained with hematoxylin-eosin-saffron.

Three days after the unilateral ovariectomy, niridazole (CIBA-Geigy Pharmaceutical Co.) treatment of mice of Group 2 and of ten uninfected controls was begun. Niridazole was used in a suspension in water with "Tween 80" 0.1%,[3] pH 7.0,[4] and was given orally with a probe in a daily dose of 100 mg/kg body weight for 10 days.[3,5,6]

Four weeks after treatment, i.e., 15 weeks after the beginning of infection, all mice in the three groups were killed. During the 15 weeks of the study the body weight was determined weekly.

At the end of the experiment the mice were perfused by the method of Smithers and Terry[2] in order to recover the adult worms. The length of the small intestine was determined in situ,[7] and the small intestine, liver, spleen, and ovaries were dissected and weighed. The small intestine and the liver were used for determination of the total egg count by the digestion method,[8] and the concentration of eggs was calculated. The ovaries and one piece of liver from each mouse, and one piece of small intestine from four animals in each group, were fixed in Bouin's solution, embedded in paraffin, and the sections were stained with hematoxylin-eosin-saffron.

RESULTS

The body weight of the uninfected and infected control animals increased gradually. However, the body weight of the mice which were later treated with niridazole (Group 2) began to decrease from the 6th week after infection, and ovariectomy of the emaciated infected mice caused a significant mortality. It is probable that an intercurrent infection caused the rapid deterioration of the animals in this group. During and after treatment the body weight of the remaining 12 mice increased rapidly and reached the level of the other groups at the 13th week after infection. Niridazole treatment did not prevent a grad-

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TABLE 1

Pathophysiological studies in mice infected with Schistosoma mansoni *for 15 weeks and cured of infection, and in two control groups**

Parameter	Group (no. mice)		
	Uninfected (27)	Infected, treated, and cured (8)	Infected and untreated (18)
Body weight (g)	30.2 ± 0.5	30.5 ± 0.7	29.9 ± 0.6
Liver weight (mg)	1,769 ± 52 (5.9 ± 0.2)†	2,431 ± 123 (8.1 ± 0.3)	2,839 ± 160 (9.5 ± 0.5)
Spleen weight (mg)	129 ± 6 (0.4 ± 0.02)	193 ± 16 (0.6 ± 0.05)	606 ± 89 (2.0 ± 0.31)
Weight of small intestine (mg)	774 ± 27 (2.6 ± 0.1)	1,823 ± 190 (5.9 ± 0.6)	2,598 ± 150‡ (8.7 ± 0.6)
Length of small intestine (cm)	46.5 ± 0.7	40.8 ± 1.4	37.2 ± 0.9‡
No. of eggs/g			
Small intestine	—	14,222 ± 2,167	24,458 ± 3,353‡
Liver	—	11,268 ± 2,055	13,379 ± 2,503

* All measurements are given as mean ± standard error.
† Figures in parentheses = percentage of total body weight.
‡ Seventeen determinations.

ual increase in the body weight of ten uninfected controls.

Adult worms were recovered from each infected mouse and also from 4 of the 12 animals in the infected and treated group. Only eight mice in this group (67%) from which no worms were recovered after perfusion were considered as cured.

Table 1 indicates a significant difference in all parameters between uninfected controls and infected and cured mice; similar differences were found between infected and cured and infected untreated mice except in the absolute liver weight and the concentration of eggs in the liver. These pathophysiological findings, and the significant difference in the egg count/g small intestine between infected and cured and infected untreated animals, provide corroborative evidence for the success of treatment.

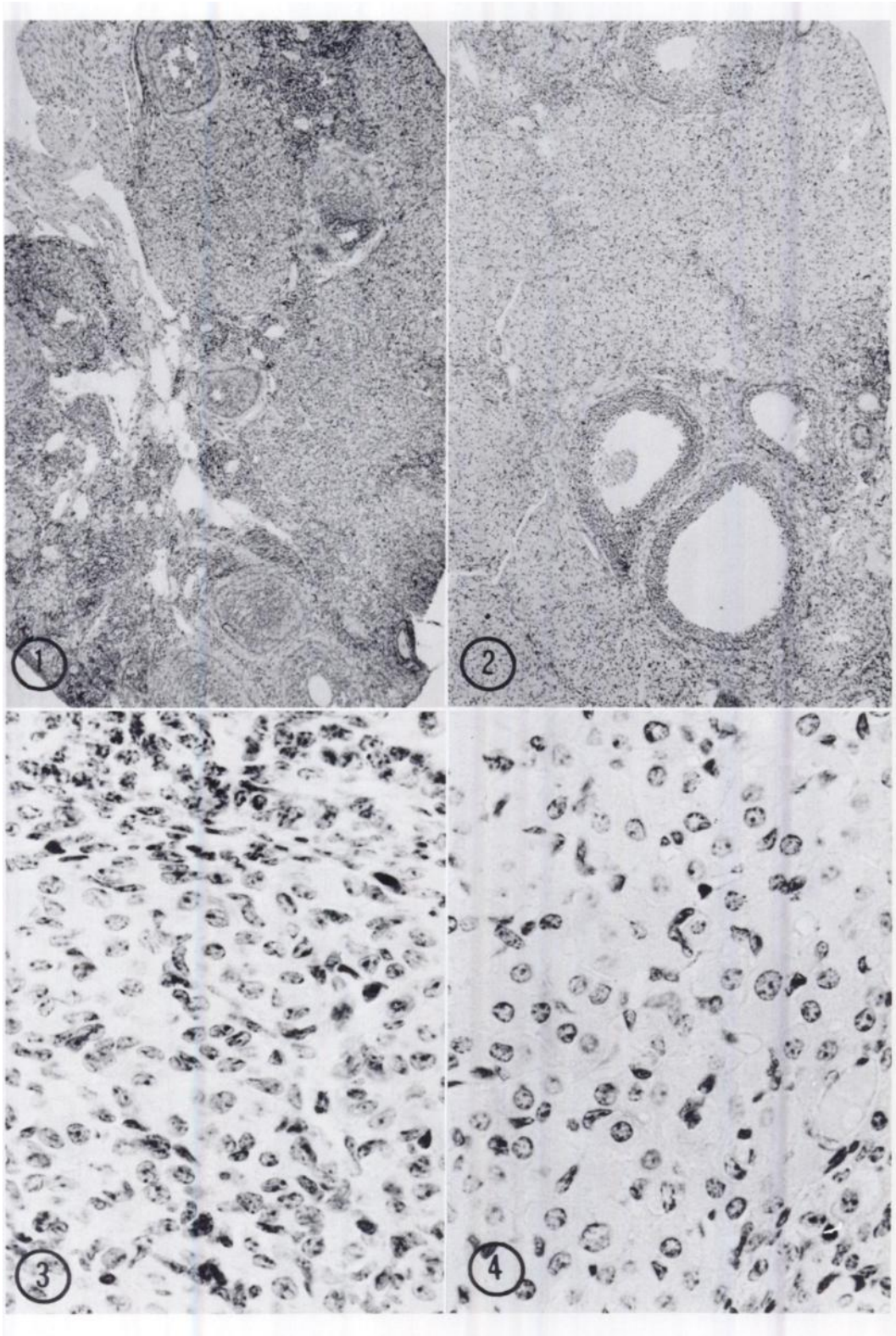
Table 2 shows that the weight of the ovaries in the intact mice was only a little greater in the infected and cured animals than in the infected controls. In mice in which unilateral ovariectomy had been done, 6 weeks later the ovaries which remained in situ showed a statistically insignificant growth in each group. This growth was greater in the infected and cured group than in the infected untreated animals; consequently, the weight of the ovaries of the infected and cured animals was nearer to that of the uninfected controls than to that of the infected controls.

TABLE 2

The mean weight ± confidence interval of the ovaries, and the frequency of normal and atrophied corpora lutea in mice infected with Schistosoma mansoni *for 15 weeks and cured of infection, and in two control groups*

Group	No. of mice	Weight of ovaries (mg)				Corpora lutea	
		Two ovaries	One ovary		Increase in 6 weeks (%)		
			At unilateral ovariectomy*	At dissection		Normal	Atrophied
Uninfected	27	30.9 ± 3.9 (18)†	13.2 ± 3.5 (9)	17.8 ± 5.5 (9)	35	27	0
Infected and cured	8	18.1 ± 6.1 (4)	10.8 ± 5.9 (4)	16.7 ± 10.0 (4)	55	6	1‡
Infected and untreated	18	17.6 ± 4.4 (12)	11.0 ± 3.9 (6)	13.9 ± 5.3 (6)	26	3	15

* Unilateral ovariectomy was performed 2 months after the day of infection and mice were killed 6 weeks later. (The ovariectomy was done 3 days before the start of treatment in the "infected and cured" group.)
† Figures in parentheses = number of mice.
‡ In the eighth mouse of this group both normal and atrophied corpora lutea were present.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In six of the eight infected and cured mice, the corpora lutea were normal. In one mouse both normal and atrophied lutea were present, and in another a slight atrophy was found. On the other hand, in the infected controls only three of 18 ovaries showed a normal histological picture. In 15 animals the corpora lutea presented generally a very severe atrophy accompanied by alteration of the interstitial cells. In two ovaries no corpora lutea were present. The follicles growing in these ovaries, and the lack of this phenomenon in the uninfected controls, make it probable that schistosomiasis, not a lack of spontaneous ovulation,[9] caused the lack of corpora lutea. In three severely infected mice among these 15 animals, newly formed corpora lutea were present in a very early stage of development. One of these mice was the only animal in the infected untreated group in which the interstitial cells were not altered.

The most striking proof of successful treatment of the ovarian changes was provided by histological examination of ovaries in mice from which one ovary was removed 2 months after infection, when the disease was well developed, and the other ovary was dissected 6 weeks after niridazole treatment. Figures 1–4 show the histological picture of the ovaries from the same mouse before and after treatment. Two months after infection the ovary shows atrophy of the corpora lutea and a typical alteration of the corpus luteum and of the interstitial cells, as described earlier.[1] Six weeks after treatment the corpora lutea of the ovary which remained in situ were normal, and the corpus luteum cells and the interstitial cells presented no signs of alteration. According to my previous experience, the histopathological changes in schistosomiasis are always present in both ovaries and never affect only one. Therefore, the present finding can be considered to be a result of the treatment.

In the infected untreated mice, the histopathological alterations of the corpora lutea were more marked in the ovaries which were dissected at a later stage of the disease than in those which were removed earlier. In the uninfected control animals, no difference was found in the histological picture between the ovaries removed by unilateral ovariectomy and those which had remained in situ.

No living egg was found in the liver of small intestine in the infected and cured group, proving the efficacy of treatment.[6] In two of eight animals necrotic worms were observed in the liver, with considerable tissue reaction around them.

On the 4th day of treatment living worms were found in the tributaries of vena portae in the liver of two mice which died; in one case the worms had migrated into the small arteries of the kidney and the spleen, causing anemic infarcts in these organs.

As mentioned previously, only animals in which no worm was recovered were considered as cured, i.e., eight of 12 treated animals. However, it should be mentioned that in the four animals not considered as cured the pathophysiological parameters were between those of the infected and cured and infected untreated groups; only one of the four had living eggs in the liver and in two of them the ovaries were normal. It is therefore probable that even in these animals the treatment was not entirely inefficient, despite the presence of living worms at the end of the study.

DISCUSSION

The histopathological alterations in the ovary before treatment and absence of these alterations in the ovary after treatment in the same mouse, the much greater frequency of normal ovaries in the infected and cured group than in the infected untreated animals, and the greater compensatory hypertrophy of the ovaries after treatment than

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FIGURES 1–4. Ovaries and corpus luteum cells from the same infected mouse. **1.** Ovary removed at unilateral ovariectomy 2 months after infection (8.4 mg). The corpora lutea are relatively small and have a great density of cell nuclei. One primary, and some developing or atretic follicles are present. **2.** Secondary ovary 15 weeks after infection and 4 weeks after niridazole treatment (12.5 mg). The corpora lutea have a normal size and lipoid content. Developing and mature follicles are present. **3.** Corpus luteum cells and interstitial cells (top of figure) in the ovary shown in Figure 1. The corpus luteum cells have oval or irregular nuclei. The outlines of the cells are indistinct. The size of the nuclei of the interstitial cells is diverse, and there are intensive spots of chromatin in the nuclei. **4.** Corpus luteum cells and interstitial cells (upper left corner) in the ovary shown in Figure 2. The corpus luteum cells have normal size with light cytoplasm and round nuclei. The nuclei of the interstitial cells are similar and have the usual chromatin arrangement. Hematoxylin and eosin; 1 & 2 ×25, 3 & 4 ×250.

without treatment found in this study have proved that the ovarian changes in murine schistosomiasis are not irreversible, and can be cured by treatment with niridazole.

There is no proof at the present time whether similar alterations in ovaries freed from schistosome eggs and granulomas occur in humans with schistosomiasis. The observations of Hsüeh and Wu[10] that many patients with advanced schistosomiasis japonica who had primary amenorrhea, disturbed menstruation, or sterility had recovered normal function after appropriate antimony treatment are not sufficiently strong arguments for a similar and reversible pathology of the ovaries in human schistosomiasis, but this possibility cannot be excluded.

A general deterioration which ceased after treatment as the cause of the ovarian changes is not probable. The body weight of mice in the infected untreated group increased like that of the controls (19% in 14 weeks); nevertheless, ovarian changes were present in 15 of 18 animals.

Bond was the first to describe that in the rabbit a compensatory hypertrophy of one ovary may occur when the other has been removed.[11] This physiological phenomenon was later confirmed in other animals. In the present study, the growth of the in situ ovaries after unilateral ovariectomy, which occurred partially through compensatory hypertrophy, was observed not only in the uninfected and in the infected and cured mice, but also in the infected untreated animals. Therefore, maintenance of the effects of growth hormone and of gonadotropins[12, 13] on the growth of the ovaries can be postulated.

In the present study, in three of 15 severely infected mice new corpora lutea were found in a very early stage of development 15 weeks after infection. In a previous work,[1] an interruption in the development of corpora lutea was established 9 weeks after infection. From these new findings it can be concluded that the interruption in the development of corpora lutea in mice infected with *Schistosoma mansoni* is not permanent, and newly formed corpora lutea can appear without treatment in a later phase of the disease.

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