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OVARIES AND ADRENALS IN MURINE SCHISTOSOMIASIS MANSONI

I. Histopathological Changes of the Ovaries in Acute and Chronic Infection

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Abstract. Acute and chronic infections with schistosomiasis mansoni in mice were found to cause a reduction of the ovarian weight and atrophy of the corpus luteum cells, followed by lymphocytic and stroma cell infiltration. Finally, the corpora lutea disappeared completely. Acute schistosomiasis caused arrested development of the corpora lutea. Both acute and chronic schistosomiasis led to the formation of "wheel cells" in the interstitial tissue of the ovaries. A threshold level of intensity of disease was found to be necessary for these pathological changes. With less severe schistosomiasis, the morphology of the corpora lutea remained normal. The more intensive and long-lasting the infection, the greater became the atrophy of corpora lutea. The various factors which could have caused these pathological alterations are discussed in the light of available literature, and it is suggested that a pituitary hypofunction, and particularly a lack of luteinizing hormone effect, may play a role in the pathological transformation of the ovarian tissue.

The pathology of the organs most involved in the immunopathological processes in experimental schistosomiasis (liver, small intestine, spleen, kidney) has been well studied. However, information on the effects on the endocrine organs is scanty and there have been no reports on effects of murine schistosomiasis on the histology and function of the ovaries.

Retarded growth in schistosome infection has been known since 1928,[1] and delayed puberty and menstruation, and early onset of menopause, as a consequence of human schistosomiasis mansoni and haematobium are symptoms known since 1934.[2] Several authors have confirmed and completed these observations in regard to infection with *Schistosoma mansoni* and *S. haematobium* in Egypt,[3–5] and in schistosomiasis mansoni in South America.[6–11] Most of them believed that hypofunction of the ovaries was caused by decreased gonadotropic hormone excretion by the pituitary gland. In Chinese literature, an analogous condition caused by infection with *S. japonicum* has been called "schistosomiasis dwarfism."[12] Ko found infantile ovaries associated with dwarfism and sexual infantilism at autopsy of a patient with Asiatic schistosomiasis.[13] More details on the involvement of the ovaries in schistosomiasis can be found in a recent review of the literature.[14]

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The observations on changes in the function of the ovaries in human schistosomiasis, and the lack of information on the morphology and function of the ovaries in murine schistosomiasis, prompted me to carry out research studies in young mice.

MATERIALS AND METHODS

Outbred albino mice (Oncins—France 1, strain CF 1) were used as the definitive host. The intermediate host, *Biomphalaria glabrata*, originated from a cross between the susceptible Puerto Rican and nonsusceptible Brazilian strains.[15] The descendants of Cross No. XXIX-M were used.

Experiment I

Twenty-one female mice, 10 weeks old (the age of sexual maturity), were used. The mice were infected subcutaneously with 150 cercariae each by the methods of Peters and Warren.[16] The experiment lasted until all mice died as a result of schistosomiasis (between 65 and 158 days after infection). Dissection was performed as soon as possible after death. The ovaries and small pieces of the liver were fixed in 10% formalin. The ovaries were weighed and embedded in paraffin, as were the small pieces of the liver. The sections were stained with hematoxylin-eosin-saffron. The larger parts of the liver were used for determination of the density of schistosome eggs.

The liver tissue was weighed and digested in 5% potassium hydroxide solution (in a ratio of 1 part tissue:9 parts potassium hydroxide) for 16 hours at 37°C. After this time the schistosome eggs became suspended in the fluid. The suspension was measured, then shaken for 10 seconds, and immediately thereafter three 0.1-ml samples were taken out in order to count the eggs under a stereomicroscope (magnification 40×) on an object glass with longitudinal engravings inside quadrangular walls made from a mixture of dye, glycerine, and yolk. The mean of three determinations served for the calculation of the number of eggs in the suspension. From this, the number of eggs in 1 g tissue was calculated.

Experiment II

Forty female mice, 7 weeks of age (the period of puberty) were divided into two equal groups. The first group served as uninfected controls. Mice in the second group were infected percutaneously with 220 cercariae each, using the ring method of Smithers and Terry.[17] After 2 months the mice were killed. After dissection the ovaries were weighed, fixed in Bouin's solution, embedded, and stained as in experiment I. The number of corpora lutea (CL) was counted in the histological preparations. The total number of eggs in the liver and the small and large intestine was determined, and the density of eggs in the liver was calculated.

RESULTS

The *body weight* of 20 control mice in experiment II showed a considerable increase in 2 months, from 22.0 ± 0.2 (S.E.) to 29.5 ± 0.4 g, an increase of 34.1%. The 20 infected mice showed a significantly smaller increase, from 22.0 ± 0.2 to 23.0 ± 0.9 (4.5%) in the same period.

In both experiments the liver and the small intestine of the infected mice showed the typical *macroscopical picture* of murine schistosomiasis. The ovaries of the infected mice were generally smaller than those of the controls, their surface was rounded, and the typical CL were missing.

The *weight of the ovaries* of the infected mice was significantly lower than that of the uninfected controls (in 20 controls, 29.1 ± 1.3 mg; in 20 infected mice 2 months after infection, 18.7 ± 0.7 mg; and in 11 infected mice which died 65–158 days after infection, 17.2 ± 3.2 mg).

By *microscopic examination*, neither eggs nor granulomas were found in the ovaries of the infected mice.

Atrophy of the corpora lutea

In comparison with the CL of the controls (Fig. 1a), the luteal cells in infected mice were smaller. The acidophily of the cytoplasm was decreased and the outlines were no longer sharp. The density of the nuclei had increased (Fig. 1b). This change of CL was always diffuse and affected each corpus luteum. The intensity of the atrophy was higher in the mice which died from schistosomiasis (experiment I) than in those which were killed 2 months after infection (experiment II). In advanced stages of the atrophy, at first mainly lymphocytic cell infiltration and later stroma cell proliferation was evidenced among the CL cells (Fig. 1c). The nuclei of the corpus luteum cells were often smaller than in the controls; they were polymorphous and sometimes it was difficult or even impossible to distinguish the CL cells from the stroma cells when the atrophy was advanced (Figs. 2a, b). In two cases the stroma cells replaced the CL, which had totally disappeared. However, the development of the follicles had not stopped and antrum formation and secondary oocytes could also be seen in these two cases.

The severity of atrophy and infiltration of CL by lymphocytes and stroma cells could arbitrarily be classified into six groups, and the intensity of atrophy was compared with the intensity of cellular infiltration. A regression analysis with a coefficient as high as 0.78 demonstrated a close relationship between the two pathological changes.

The severity of atrophy in experiments I and II is compared in Table 1. Atrophy of the CL was significantly higher in the mice which died from chronic schistosomiasis than in those which were killed 2 months after the infection. A similar statistical comparison of the cellular infiltration of CL between the two experiments leads to the same conclusion. A regression analysis of the severity of the atrophy and the time between infection and death of 11 mice which died at different points of time following infection presents a coefficient as high as 0.56, showing that the intensity of the atrophy tends to become more pronounced with time. It seems that the duration of schistosomiasis is a definite factor in producing the pathological changes in the CL. One explanation may be that the disease becomes more intense with time after

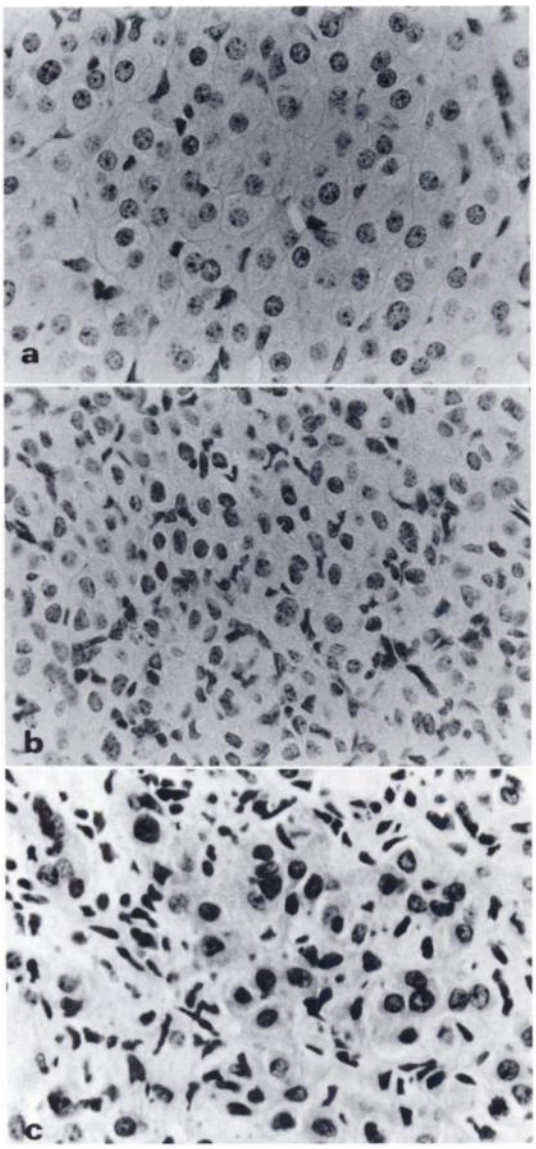

FIGURE 1. **a.** Corpus luteum of an intact mouse; **b.** Corpus luteum of an infected mouse showing slight atrophy of the corpus luteum. This mouse was killed 2 months after infection; **c.** Corpus luteum of an infected mouse showing very severe atrophy with a heavy infiltration of the corpus luteum by stroma cells. This mouse died 82 days after infection. Hematoxylin & eosin, ×70.

the infection. Indeed, the mean number of eggs in 1 g liver in experiment I was higher than in experiment II, but the difference was not statistically significant. (In experiment I, 150 cercariae and in experiment II, 220 cercariae were used for infection of each mouse.)

TABLE 1

Frequency and proportion of the different grades of atrophy of the corpora lutea in mice infected with Schistosoma mansoni*

Grade of atrophy§	Experiment I†		Experiment II‡	
	Frequency	Proportion	Frequency	Porportion
None	0	0	4	0.20
Slight	0	0	8	0.40
Medium	1	0.09	3	0.15
Severe	6	0.55	5	0.25
Very severe	2	0.18	0	0
Complete	2	0.18	0	0

* $\chi^2 = 15.80$; degrees of freedom = 5; $P > 0.01$.
† Eleven animals which died from schistosomiasis between 65 and 158 days after infection.
‡ Twenty animals killed 2 months after infection.
§ None, corpora lutea without atrophy; very severe, indistinct cell borders; differentiation between corpora lutea and surrounding stroma is very difficult; complete, no corpora lutea.

The total number of eggs recovered from the liver, the small, and the large intestine, and the mean number of eggs in 1 g liver were used as parameters for the estimation of the intensity of schistosomiasis. There was a major difference in the egg burden of the organs between four infected mice without CL atrophy and those with CL atrophy in experiment II (Table 2). There was also a tendency towards a relationship between the intensity of atrophy and the intensity of disease. A similar examination of the mean number of eggs in 1 g liver in experiment I yielded the same results. The conclusion could be drawn that a threshold level of intensity of schistosomiasis is necessary for the atrophy of the CL. The morphology of the CL remained normal when the disease had not reached a certain severity. The more severe the disease, the more severe the degree of CL atrophy.

Histopathologic examination of the liver of mice with advanced schistosomiasis in experiment I showed hepatic fibrosis in 5 of 12 mice. The intensity of atrophy of the CL was higher in the mice with hepatic fibrosis than in those mice which had no hepatic fibrosis and which had generally died earlier in the infection.

Arrested development of the corpora lutea

According to Allen[18] and Snell,[19] it is possible to differentiate between young and old CL of mice. Based on the descriptions given by these authors and our own observations, the CL of the control, uninfected mice in experiment II could be

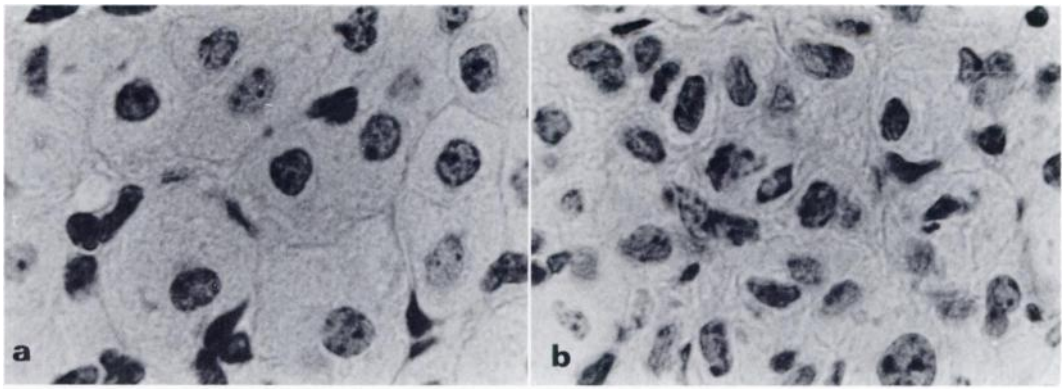

FIGURE 2. **a.** Corpus luteum of an intact mouse; **b.** Corpus luteum of an infected mouse showing atrophic changes of the luteal cells. This mouse was killed 2 months after infection. Hematoxylin & eosin, ×180.

divided into four stages of development according to the site of CL in the ovary, the basophilia or eosinophilia of the cytoplasm, the size of the cytoplasm and of the nucleus, the outlines of the cells, the chromatin content of the nucleus, and the possible leukocytic or stroma cell infiltration.

A comparison between the control and the infected group (Table 3) showed that the development of newly formed CL had entirely stopped in the ovaries of 18 of 20 infected mice. In two infected mice the development of CL had not stopped and the CL were normal. The average number of CL was higher in the controls than in the infected mice but the difference was not significant.

"Wheel cell" formation in the nucleus of the interstitial cells

The nuclei of the interstitial cells in the ovaries of the infected animals in both experiments were smaller than those in the controls. Their form was polymorphous and the most striking phenomenon was presented by the intense basophilic spots of chromatin (Figs. 3a, b). The nuclear membrane was maintained, but sometimes a few free chromatin granules were visible. In the mice with chronic schistosomiasis, these altered interstitial cells could only be seen in small groups among the growing stroma cells. In the oldest mouse, which died 158 days after infection, only one or two interstitial cells were present in the ovaries which

TABLE 2

Relationship between degree of atrophy of corpora lutea and the number of schistosome eggs in the liver and the small and large intestines of 20 mice infected with 220 cercariae of S. mansoni *and killed 2 months later (experiment II)*

Degree of atrophy	No. mice	Mean no. of eggs/organ ± SE			Mean no. eggs in 1 g liver ± SE
		Liver	Small intestine	Large intestine	
None*	4	9,200 ± 827 (5,100–13,300)†	15,600 ± 2,482 (4,100–25,300)	1,700 ± 268 (500–3,100)	4,600 ± 335 (3,000–6,000)
Slight	8	23,900 ± 2,101 (13,100–41,600)	57,300 ± 4,517 (32,500–93,000)	11,600 ± 1,297 (6,400–23,400)	13,800 ± 1,744 (6,200–29,200)
Medium	3	25,300 ± 3,287 (14,900–37,500)	68,200 ± 6,418 (36,900–93,300)	17,300 ± 2,706 (3,400–25,500)	12,200 ± 1,386 (6,400–18,700)
Severe	5	25,400 ± 1,789 (11,500–32,100)	69,100 ± 5,031 (34,900–97,000)	16,700 ± 850 (12,900–22,800)	17,300 ± 984 (9,600–20,900)

* Corpora lutea without atrophy.
† Range.

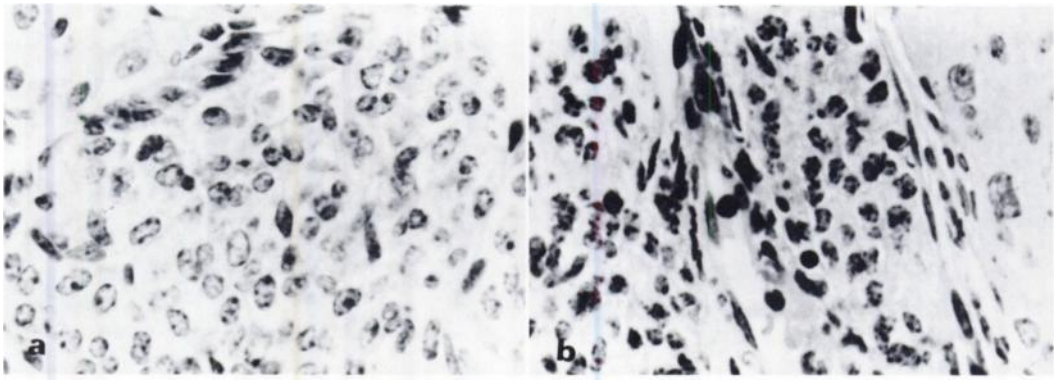

FIGURE 3. **a.** Interstitial cells in the ovary of an intact mouse. Corpus luteum cells are present in the left upper corner; **b.** "Wheel cells" in the ovary of an infected mouse which was killed 2 months after infection. Corpus luteum cells are present at the right border. Hematoxylin & eosin, ×115.

had already been partly transformed into a fibrous tissue.

The two infected mice which had normal interstitial cells, normal CL, and a normal development of CL had a lower intensity of infection than the 18 mice with altered interstitial cells, arrested development, and atrophy of CL (Table 4). The intensity of the infection seems to be an important factor, not only in the atrophy of CL, but also in arresting their development and in the pathological alterations of the interstitial cells.

DISCUSSION

Atrophy of the CL, arrest of their development, and alteration of the nuclear chromatin of the interstitial cells were found in murine schistosomiasis. No reports of similar observations were found in the literature.

The altered corpus luteum cells were sometimes so similar to the stroma cells which infiltrated the CL that it was impossible to differentiate between them. More extensive cytological examinations might confirm or refute the supposition that the corpus luteum cells may transform into stroma cells during their pathological involution.

Alterations of the interstitial cells in the ovary were first described by Selye et al.,[20] in rats following hypophysectomy. These authors called the cells, which resembled plasma cells in the arrangement of nuclear chromatin, "wheel cells." Achheim found the same type of cell in the interstitial gland of the ovary of old rats.[21] I found no data in the available literature regarding the presence of these "wheel cells" in the interstitial tissue of the ovaries of mice.

The results of these experiments demonstrate that the presence or absence of ovarian changes

TABLE 3

Frequency and proportion of corpora lutea with different stages of development in both ovaries of uninfected mice and mice infected with 220 cercariae of S. mansoni *and killed 2 months later (experiment II)*

Stage of development	Group					
	Twenty uninfected mice		Two infected mice with normal corpora lutea		Eighteen infected mice*	
	Frequency	Proportion	Frequency	Proportion	Frequency	Proportion
Very early	31	0.05	6	0.13	0	—
Early	79	0.12	1	0.02	0	—
Developed	503	0.79	34	0.76	485	0.98
Involution	24	0.04	4	0.09	9	0.02
Total	637	1.00	45	1.00	494	1.00

* Sixteen mice with atrophied and two with normal corpora lutea.

TABLE 4

Comparison between mice with altered interstitial cells and mice with normal interstitial cells in regard to the severity of pathological changes 2 months after infection with 220 cercariae of S. mansoni *(experiment II)*

Group*	Number of mice	Corpora lutea frequency of		Mean number of eggs/organ			Mean number of eggs/g liver
		development	atrophy	Liver	Small intestine	Large intestine	
AIC	18	0	16	22,700 ± 4,576† (11,100–41,600)‡	58,800 ± 11,835 (24,800–97,000)	13,000 ± 3,702 (2,000–25,500)	13,600 ± 3,355 (5,600–29,200)
NIC	2	2	0	6,100 (5,100–7,100)	6,100 (4,100–8,000)	750 (500–1,000)	3,300 (3,000–3,600)

* AIC, altered interstitial cells; NIC, normal interstitial cells.
† Confidence interval, $P = 0.095$.
‡ Range.

depends on the intensity of the disease. In experiments on 53 mice, in which light infections were also used, ovarian alterations were found only in those animals in which the mean number of eggs/g tissue exceeded 8,400 in the small intestine, 6,000 in the large intestine, and 5,100 in the liver (unpublished data).

It is not yet clear whether these histopathological changes in the ovaries are specific for schistosomiasis or whether they can also occur in association with other infectious diseases in mice as alterations of a nonspecific, degenerative character. No analogous observations were mentioned in the literature on the pathology of the ovaries in infectious diseases of mice.[22, 23] Although the question of the specificity of these lesions remains unanswered, it can be stated that an acute massive infection of mice with cercariae of *Schistosoma mansoni* causes significant histopathological alterations in the ovaries.

The pathogenesis of the ovarian changes in murine schistosomiasis is not yet known. Raised total plasma-estradiol levels were found in cases of hepatic cirrhosis in humans.[24] Abdel Aziz et al.[25] reported a significant increase in the estradiol fraction of the urinary *estrogens* in patients with bilharzial hepatosplenomegaly. According to Ghalioungui et al.,[26] a selective pituitary inhibition can be caused by the retained estrogens in bilharzial cirrhosis.

Flerkó[27] and Tiboldi[28] observed the disappearance of CL and an impediment in the development of the follicles after long-lasting estrogen treatment in rats. However, in murine schistosomiasis only the luteinizing hormone–corpus luteum system might have been affected and the follicle-stimulating hormone–follicle system probably remained intact.

Nutritional factors are considered very important in the various ways in which bilharziasis can affect the human endocrine system.[4, 29, 30]

An analogy can be drawn with the retardation followed by infantilism observed in heavy hookworm infection,[31] although in areas where other parasitic infections and nutritional deficiencies are the rule it is impossible to attribute any sign or symptom to hookworm disease alone.[32]

Between 1940 and 1975 several scientists have studied the effects of malnutrition on experimental schistosomiasis in mice, rats, and guinea pigs, but they did not consider the possible endocrine changes in the host. The role of disturbed absorption caused by severe intestinal schistosomiasis as a factor responsible for the ovarian changes cannot be excluded. Ishii found changes in the pituitary gland in experimental schistosomiasis japonica.[33] Ko saw atrophic and fibrotic changes in the pituitary at autopsy of a female schistosomiasis dwarf.[13] No information could be found on the morphology and function of the pituitary gland in human or experimental schistosomiasis mansoni.

The pathology of the ovaries in the present experiments suggests that hypofunction of the pituitary, and primarily a lack of luteinizing hormone effect, may play a role on the appearance of ovarian changes in massive infection of mice with *S. mansoni*.

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