Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. 82, Suppl. IV: 257-267, 1987
Intern. Symp. on Schistosomiasis

CONGENITAL AND NURSING EFFECTS ON THE EVOLUTION OF *SCHISTOSOMA MANSONI* INFECTION IN MICE

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Modification of the immune response to schistosomal infection in children or offspring born to mother R infected with Schistosoma mansoni *has been demonstrated in human and in experimental schistosomiasis. One of the hypothesis to explain this fact could be the transfer of circulating antigens and antibodies from mother to foetus through the placenta or from mother to child by milk. The results of this spontaneous transference are controversial in the literature. In an attempt to investigate these questions, we studied one hundred and twenty offspring (Swiss mice), sixty born to infected-mothers (group A) and sixty born to non-infected mothers (group B). These were percutaneously infected with 50 cercariae/mouse, and divided in six sub-groups (20 mice/sub-group), according to the following schedule: after birth (sub-groups A.I and B.I), 10 days old (sub-groups A.II and B.II) and 21 days old (sub-groups A.III and B.III). After the exposure period, the young mice returned to their own mothers for nursing. Six weeks later, the mice were killed. We obtained the following results: 1) There is transference of antibody to cercariae (CAP), adult worms (SWAP) and egg antigens (SEA) from the infected mothers to the offspring, probably through placenta and milk; 2) Offspring born to infected mothers exhibit much less coagulative hepatic necrosis and show a lower number of eggs in the small intestine and a less intense and predominant exsudative stage of the hepatic granulomas when compared with the exsudative-productive stage of the control groups. The findings suggest that congenital and nursing factors can interfere on the development of the schistosomiasis infection, causing an hyporesponse to the eggs.*

In hyperendemic areas, only few individuals develop severe hepatosplenic schistosomiasis. The percentage can reach up to 8% of the total population, according to the region (Klöetzel, 1962; Conceição et al., 1978; Santos, 1978; Pereira, 1979; Coura, 1979; Menezes & Coura, 1980; Prata et al., 1980; Bina & Prata, 1984), suggesting the interference of factors that can protect and others that can aggravate the infection. Among the protective factors the serum antibodies can have an important role in immunity, with studies in: transplacental transfer of antibodies to *Schistosoma mansoni* (S. m.) and their persistance in infants up to six months after birth (Lees & Jordan, 1968); precipitating anti-*S. mansoni* (α-S. m.) antibodies in newborn serum (Hillyer et al., 1970); and finally a high correlation between the circulating *S. mansoni* soluble antigens (CSA) levels in sera from infected mothers and from the umbilical cord of their newborn children, indicating that CSA are probably transferred through the placenta (Carlier et al., 1980). However, the difficulty of this investigation in humans lead to research in murines, with contraditory results, varying from no difference (Weinmann, 1960; Taylor et al., 1971), immunotolerance (Lewert & Mandlowitz, 1969; Hang et al., 1974), worsening of the infection (Borojevic et al., 1977), protection (Rombert et al., 1979) or partial resistance (Beltrão, 1979) and a rapid fibrotic change in the peri-ovular hepatic granuloma, suggesting an immunological modulation (Andrade & Azevedo, 1987). There are also few studies regarding the transfer of *S. mansoni* antibodies from mother to fetal and newborn mice (Bruijning & Vries, 1987). It is already known that mammary glands participate of the mucosal immune system (Losonsky & Ogra, 1981; Hanson, 1982) and that breast milk can be the mean of transmission of antigens and antibodies from mother to child, as was demonstrated by the presence of two circulating *S. mansoni* antigens, parasite "M" antigen and antigen "4", in milk

Research supported by CNPq (No. 402956/84).
[+] CNPq fellowships (No. 822868-86.5).
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Abbreviations used in this paper: SEA: soluble egg antigens; SWAP: soluble worm antigenic preparations; CAP: cercaria antigenic preparations.

from infected (S. m.) mothers (Santoro et al., 1977).

The purpose of the present work was to study the congenital effects and also the possible interference of the breast-feeding on the development of the infection.

MATERIAL AND METHODS

One hundred and twenty offspring (Swiss Webster mice), sixty born from egg-positive *S. mansoni* infected mothers (group A) and sixty born from non-infected mothers (group B) were percutaneously infected with recently shed 50 cercariae/mouse of *S. mansoni* (Belo Horizonte isolate) and divided in six sub-groups (20 mice/sub-group), according to the following schedule: after birth (sub-group A.I and B.I), 10 days old (sub-groups A.II and B.II) and 21 days old (sub-groups A.III and B.III). After the exposure period, the young mice returned to their own mothers for nursing. Six weeks later (42 days of infection), blood was obtained for serological study and the mice were killed (Fig. 1). The mothers were also infected percutaneously with 50 cercariae from the same strain when they were 5 days old and were mated on the 50th day of age. The small intestine of each offspring was cut into halves from the pilorus to the ileo-cecal valve and the total egg count was done in the first 2 cm from the proximal end of both intestinal halves, according to the slide-slide pressing technique of Brener (1956), modified by Conceição (1985). The statistical value of the egg counts was calculated by the Student's t-test. Liver fragments and the remaining intestines prepared by the Swiss roll technique (Lenzi & Lenzi, 1986) were placed in Millonig's fixative (Carson et al., 1973) and treated by standard histological methods. Tissue sections were stained with hematoxylin and eosin, Lennert's giemsa, Gomori's silver reticulin, alcian blue pH 1.0 and 2.5 and picrosirius (plus polarization), in order to study the peri-ovular reaction.

To the study of the hepatic specimens, a very detailed protocol was employed, analysing the cellular and extracellular matrix components within the granulomas and portal space, besides the vascular and lobular changes. The granulomas were classified according Li Hsü et al. (1972) and the differentiation between exsudative and exsudative-productive was done mainly by Gomori's silver reticulin.

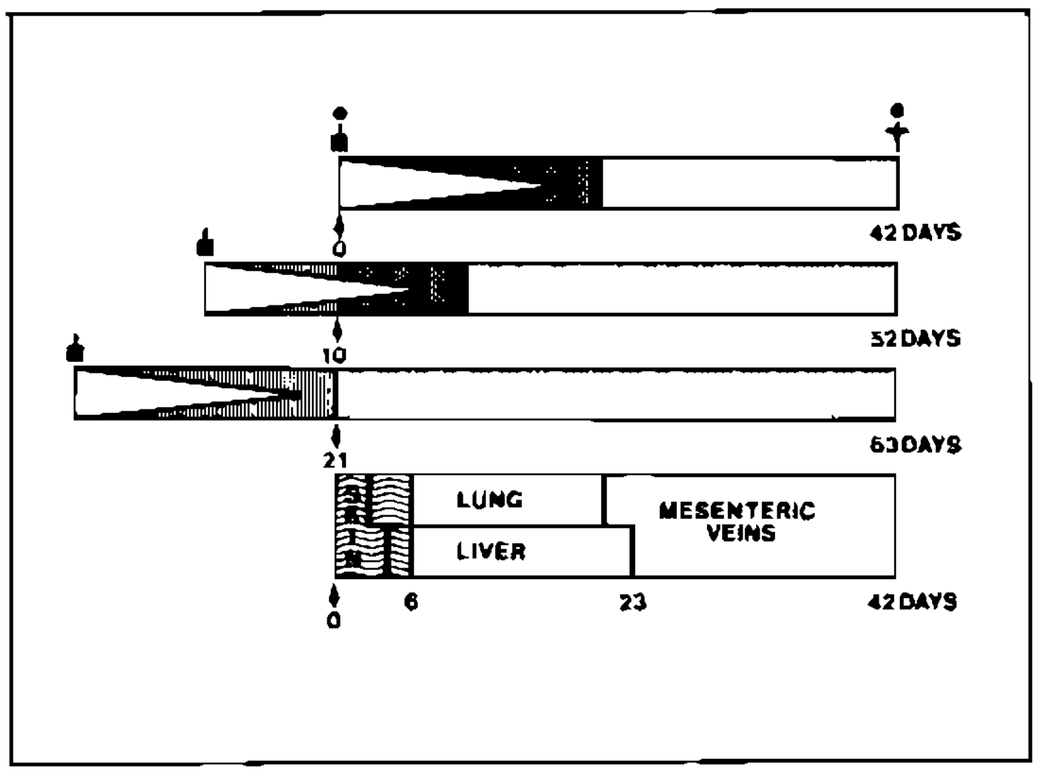

Fig. 1: graph of experimental design applied to offspring of infected *(S. mansoni)* and non infected mothers, showing the infection period (▨), nursing period (▥), closure evolution (▷), day of birth (▲), day of infection (↑) day of necropsy (†), time of serum obtention (●), location of the parasites during their migration.

The peri-ovular reaction in the rolls of small intestine (distal half) was classified and quantified in three patterns (Lenzi et al., 1986): 1 – no reaction (NR); 2 – small or incipient reaction (SR); and 3 – defined granuloma (G). The SR or small reaction was considered when few cells were attached to the eggs or there was an inflammatory halo different in intensity around the eggs, usually rich in eosinophils, not forming yet a granuloma, and characterizing a pregranulomatous reaction.

The sera were studied by immunoenzymatic electro-transfer blot according to the following procedure: sodiumdodecylsulphate (SDS) – solubilized proteins of antigenic preparations from schistosome eggs (SEA), worms (SWAP) and cercariae (CAP) were subjected to electrophoresis in SDS-polyacrylamide slab gels (dimensions: 16 mm x 14 mm) in a gel with 10% acrylamide. The electrophoresis was performed in the discontinuous buffer system described by Laemmli (1970) in an apparatus according to Plate I, Studier (1973) at room temperature with 60 V and 20 mA for about 5 hours. The molecular weight estimation of polypeptide chains was made by running marker of known molecular weight (MW-SDS-200 KIT SIGMA – Stock No. SDS-6H). The electrophoretical transfer procedure of proteins onto nitrocellulose filter sheet was adapted from Towbin et al. (1979) and Moriearty (1984). The blotting was run overnight at 12 V and 85 mA at 4 °C in a transfer buffer con-

taining 9.0 g Tris-Base and 43.23 g glycine. The chemicals were dissolved in 3 l water with 20% (V/V) methanol.

The filters sheets with the adhered proteins were cut in strips and washed in a quench of Phosphate buffered saline (PBS) with 0,5% (V/V) Tween 20 with Bovine Lactoalbumin and in a wash buffer (WB) of PBS 0,05% (V/V) Tween 20 for 1 h and 30 min *respectively*, at room temperature. The strips of nitrocellulose were incubated with schistosomiasis mice sera (diluted for 1/200 in WB) overnight at 4 °C with agitation. After antibody incubation, strips were washed 6 x 15 min in WB with agitation at room temperature. They were incubated with the second (probe) antibody (peroxidase-conjugated anti-mouse polyvalent immunoglobulins at 1:500 dilution in WB [Sigma nº A-0412]) at 4 °C in agitation, for 1 h and 30 min. The strips were washed 6 x 15 min in WB at room temperature and exposed to the substrate solution of 15 mg 3-3'-diaminobenzidine/60 ml citrate-phosphate buffer, pH 5.0/40 μl H_2O_2 for direct visualization and identification of proteins recognized by sera. The blots were then dried between filter paper.

RESULTS

Histologically, in all group, livers showed the classical aspects of acute murine schistosomiasis. The egg lesions were characterized by non-reactive or weakly reactive stage, the exsudative stage, and the initial phase of exsudative-productive stage, where reticulin fiber and collagen type I and III appeared. Eosinophils were the most proeminent cells, together with monocytes/macrophages.

A zone of hepatocellular necrosis was often seen within or around the egg lesions. Portal and incomplete septal fibrosis were observed in some portal space related to egg lesions. In the offspring of infected mother there was predominance of exsudative granulomas, specially when the mice were infected on day first and 21 th, and they exhibited much less coagulative necrosis than the offspring of non-infected mothers (Table I).

The results of the egg count are shown in the Table II and Fig. 2. In the subgroup A.III the mean of the number of eggs was significantly lower when compared to the mean found in the sub-group B.III.

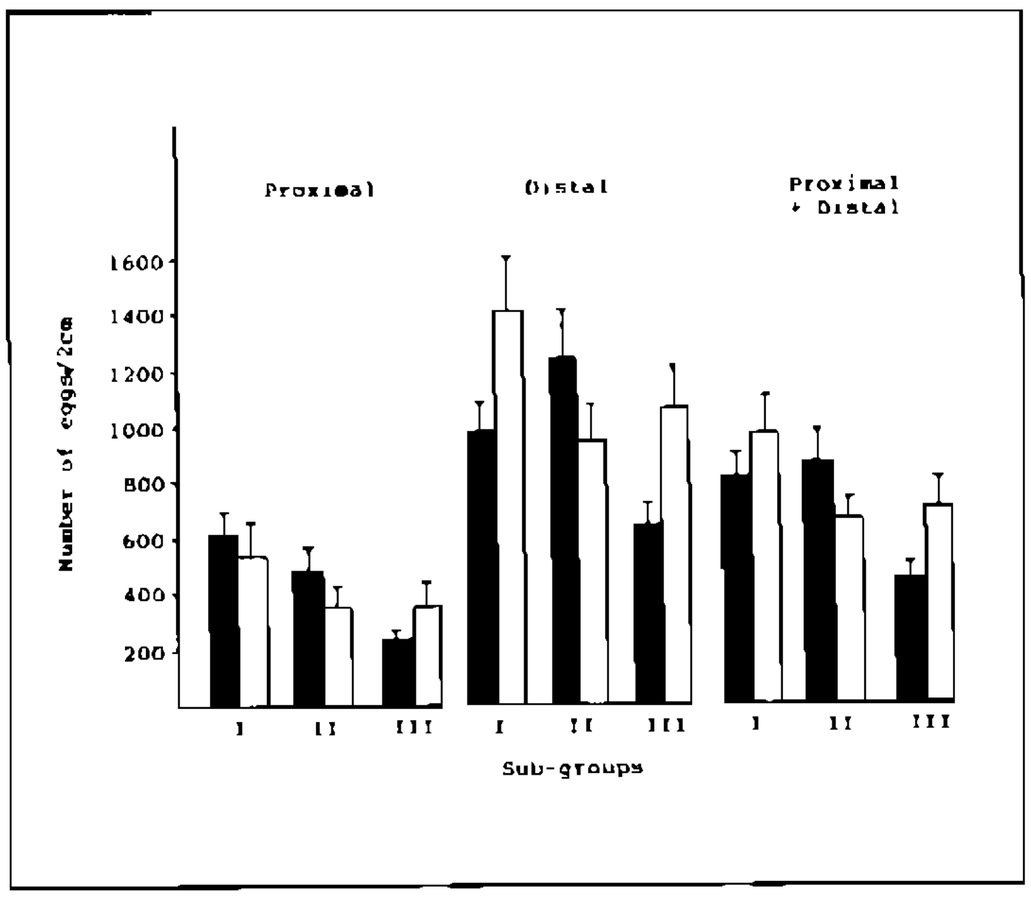

Fig. 2: Egg count in the small intestine Mean ± SE (n = 20).

The quantification of the peri-ovular reaction patterns in the rolls of small intestine (distal half) resulted in the following data: subgroup A.I.: NR: 63%; SR: 27%; G: 10%. Subgroup B.I.: NR: 58%; SR: 32%; G: 9%. Subgroup A.II.: NR: 70%; SR: 24%; G: 6%. Subgroup B.II.: NR: 56%; SR: 41%; G: 3%. Subgroup A.III.: NR: 66%; SR: 31%; G: 3%. Subgroup B.III.: NR: 52%; SR: 36%; G: 12%. In all sub-groups the quality and intensity of the peri-ovular reaction was approximately the same, except in the sub-group B.III, where the percentage of G was higher, the inflammatory reaction stronger, with granulomas more advanced in their maturation process, when compared to the paired sub-group.

The results of the search of antibody in the sera of non-infected and infected offspring against SEA, SWAP and CAP by immunoenzimatic electro-transfer blot are depicted in the Figures 3-5. The larger amount of transferred antibodies against CAP and SWAP from the infected mother to the offspring was detected in 10 days old animals, and the smallest quantity on day 21st, after the intestinal closure. The intestinal closure appeared to abolish selectivelly the antibody against SEA – 31 Kd. In the sera collected 6 weeks after infection, we only observed less antibodies against SEA and CAP in offspring of infected mothers, infected on day 21st.

Histopathological results on other anatomical organs (except liver and intestine) are not presented in this paper.

TABLE I

Summary of the principal histopathological results

Mother	Findings	Offspring		
		Time of the infection		
		Day 0	Day 10	Day 21
Infected	Granuloma stage	$E > EP_{(T)}$	$EP_{(T)} \geqslant E$	$E > EP_{(T)}$
	NECROSIS:			
	– Granulomatous	–/+	–	–/+
	– Parenchyma	–/+	–/+	–/+
	Cells:			
	– Mast cell	–	–/+	–/+
	– Epithelioid	–/+	–/+	–
	– Giant	–	–	–
Control	Granuloma stage	$EP_{(T)} > E$	$E \geqslant EP_{(T)}$	$EP_{(T)} > E$
	NECROSIS:			
	– Granulomatous	+++	+++	–/+
	– Parenchyma	+++	+++	+++
	Cells:			
	– Mast cell	–	–/+	–/+
	– Epithelioid	–/+	–/+	–/+
	– Giant	–	–	–/+

E = Exsudative
$EP_{(T)}$ = Exsudative-productive (In transition E → P)
– = Not detected
–/+ = Rare
+++ = Frequent

TABLE II

Egg count in the small intestine*

Mothers	Offspring	Proximal	Distal	Proximal + Distal
Infected	A.I	611 ± 88	990 ± 107	801 ± 75
	A.II	484 ± 94	1248 ± 175	866 ± 115
	A.III	238 ± 31**	645 ± 76**	441 ± 52**
Control	B.I	543 ± 121	1411 ± 199	977 ± 134
	B.II	365 ± 72	949 ± 132	657 ± 88
	B.III	357 ± 72	1067 ± 150	712 ± 100

* Mean ± SE (N = 20 mice/group).
** $p < 0.01$.
Age of mice when infected:
A.I and B.I: after birth (before breast feeding)
A.II and B.II: 10 days old (during breast feeding)
A.III and B.III: 21 days old (after weaning).

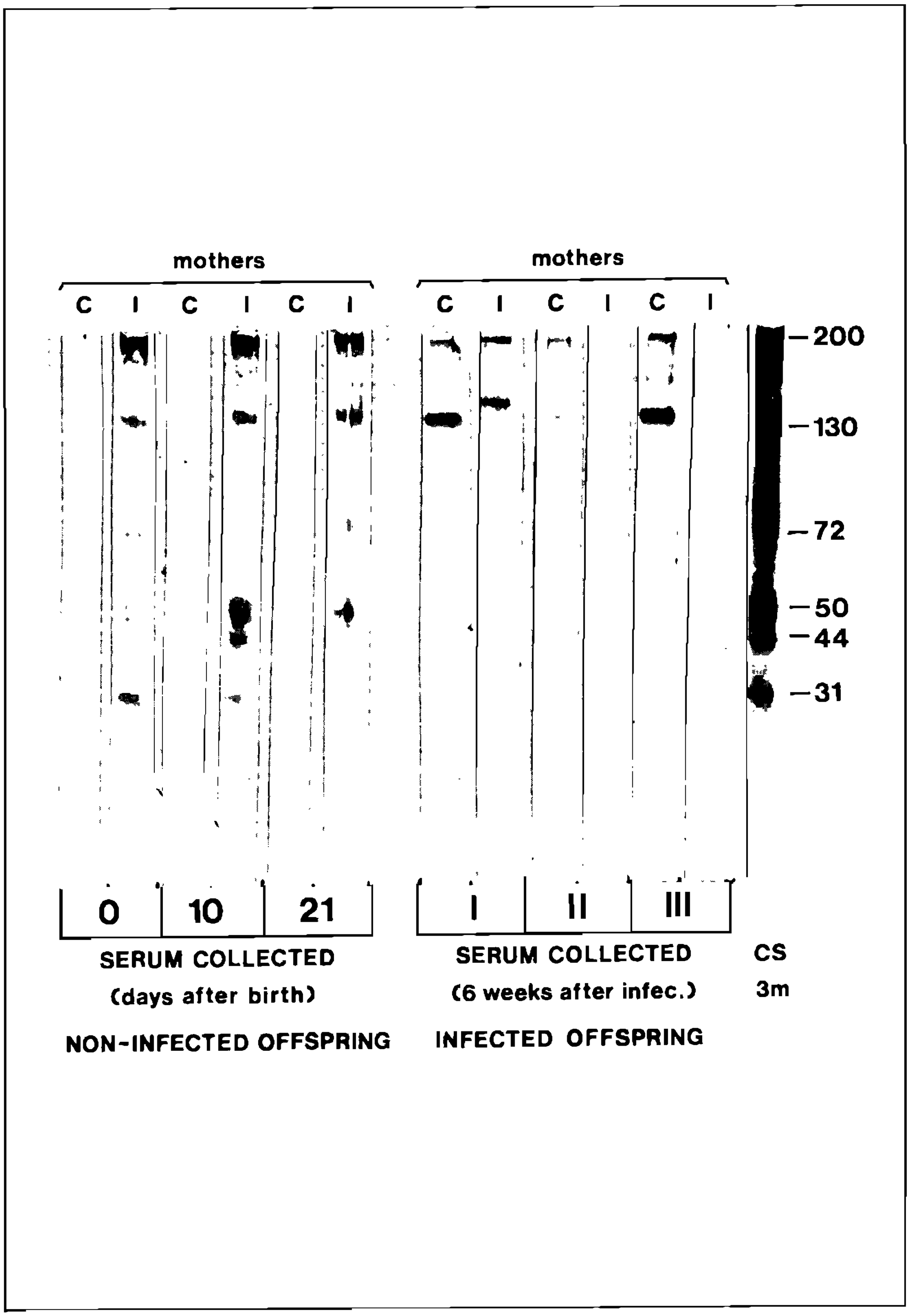

Fig. 3: antibodies anti-SEA in offspring of infected (I) *(Schistosoma mansoni)* and control (C) mothers in mice (Immunoenzymatic electro-transfer blot). CS: chronic serum of mouse after 3 months of infection.

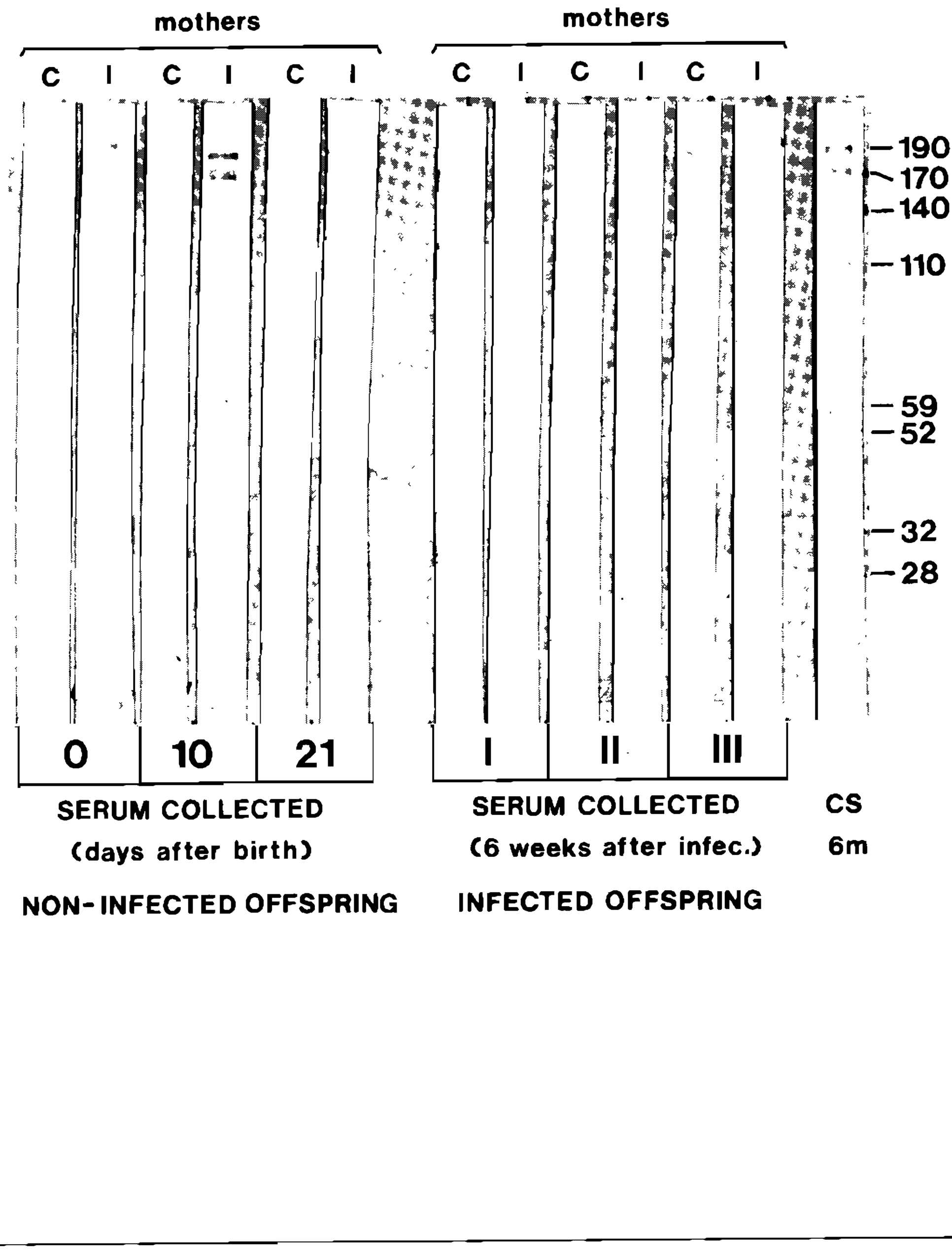

Fig. 4: antibodies anti-CAP in offspring of infected (I) *(Schistosoma mansoni)* and control (C) mothers in mice (Immunoenzymatic electro-transfer blot). CS: chronic serum of mouse after 6 months of infection.

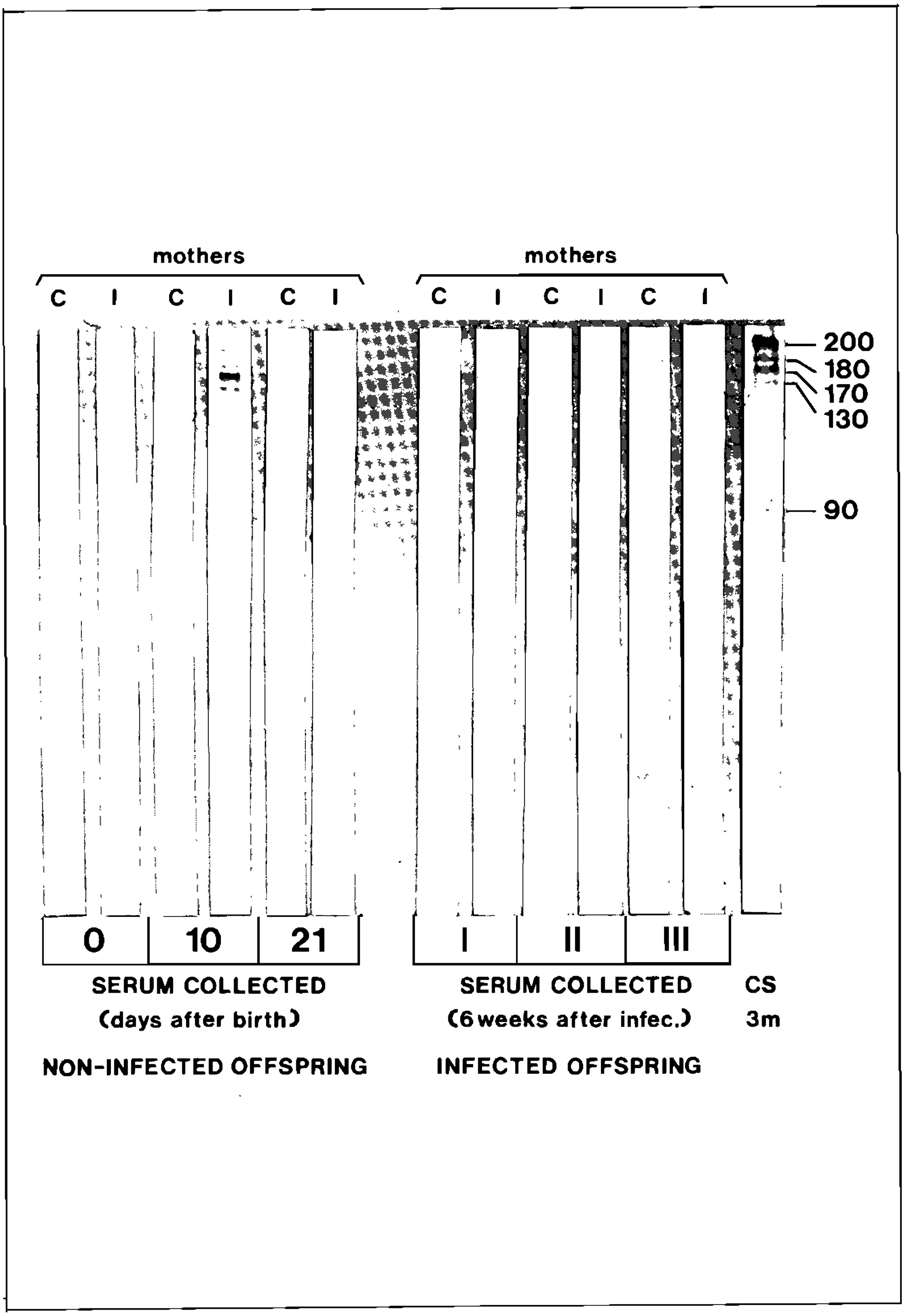

Fig. 5: antibodies anti-SWAP in offspring of infected (I) *(Schistosoma mansoni)* and control (C) mothers in mice (Immunoenzymatic electro-transfer blot). CS: chronic serum of mouse after three months of infection.

DISCUSSION

Before we discuss our results it is important to emphasize two different situations that occur in endemic areas: First, as mentioned in the introduction, only few individuals develop chronic severe hepatosplenic schistosomiasis; second, clinical findings of classic acute toxemic form are not seen in residents of endemic areas, but in persons, specially young, who eventually visit them (Bina & Prata, 1984). These two situations have different mechanisms, one aggravating the disease, and the other attenuating it, and both can be influenced by congenital or genetic (prenatal) or nursing (post-natal) factors.

Tavares-Neto (1987), in a very interesting study, has observed that the frequency of children having severe diagnosis varied according to their parent's diagnosis. When both parents presented a mild diagnosis the frequency of severe diagnosis in the children was similar to that of the general population (5.9%). When only the male parent had severe diagnosis the frequency was 48.6%. Finally, when father and mother presented severe diagnosis the frequency was 37.5%. The relative risk of children having severe diagnosis in these types of couples were respectively 1, 3, 17 and 11, aproximate values. Thus, concluded Tavares-Neto, in the evolution towards the hepatosplenic form there would have been influences of maternal effect, predisposing in its nature, which could be said to be due to the environment, since they had similar influences on their sons and daughters. On the other hand, continues the author, notwithstanding the maternal effects, the recurrence of the severe form in siblings, when the mother did not have a diagnosis, was high (n = 20), showing evidence of influence of the genetic component. If the maternal effect meant the environmental influences only, and if there were a parallelism between the esquistosomotic infection and the hepatosplenic disease, there would be a higher agreement of the averages of eggs found in the mothers with those found in children; however, higher agreement of those averages was observed in the male parents and their children. According to Tavares-Neto, these observations reinforce the contribution of the genetic component in the development of the hepatosplenic form of the mansoni schistosomiasis, although it is necessary to make a distinction of facts that are involved in the infection process and in the schistosomiasis itself. The advantage of experimental studies is to allow the separation of these two aspects. In our present study, which shows partial results, we focused on some parasitological, pathological and serological consequences due to the occurrence of transplacental and breast milk transfer of antibodies and antigens related to schistosomes, from mother to offspring. In fact, offspring of infected female mice, mainly when infected on day 1 and 21 of age show a less intense and predominant exsudative stage of hepatic granulomas when compared with the predominant exsudative-productive stage of the control groups, indicating a slowdown in the granuloma maturation or a delay in the egg deposition. But what really called our attention was the less frequent necrosis presented by all the offspring of infected mothers within the granulomas and in the parenchyma (Table I). Otherwise, the results of the egg count and the quantification of the peri-ovular reaction patterns, showed that offspring from infected mother, specially when infected after the nursing period (subgroup A.III) presented a significant decrease in the number of eggs retained in the intestine, a less intense peri-ovular inflammatory reaction, and less antibodies against SEA and CAP when compared to the control group. Therefore these animals are less susceptible or more resistant to the infection although they presented a less intense inflammatory reaction to the eggs in the intestine and liver, indicating that host granulomatous hypersensitivity can be suppressed without lessening immunity. The data confirm the report of Lewert and Mandlowitz (1969). However it is too premature to try to explain them by an immune tolerance to the eggs, associated with protective mechanisms against other stages of the parasites. In fact, it is known that immunity on reexposure to schistosome cercariae is directed toward the worms, which are antigenically distinct from the eggs, granulomatous hypersenstivity might be specifically suppressed with egg antigens without alteration of immunity to reinfection (Hang et al., 1974). In schistosome infection, the host might benefit from such tolerance or hyporesponsiveness, since many of the pathological manifestations of schistosomiasis are due to hypersensitivity responses by the host rather than direct damage by the parasite (Lewert, 1970). It was also shown that the antigen responsible for the hyporesponsiveness in the offspring apparently emanates from the eggs (Hang et al., 1974). In our study it was possible

to observe the transference of antibodies to eggs (SEA), cercariae (CAP) and adult worms (SWAP) from the infected mothers to the offspring, probably through placenta (SEA: 200, 130, 72, 50, 44, 31 Kd; CAP: 190 Kd; SWAP: 200 Kd) and through milk (SEA ?; CAP: 170, 140, 110, 59, 52, 32, 28 Kd; SWAP: 170, 130, 90 Kd). The larger amount of transferred antibodies through milk was detected in 10 days old animals, before the occurrence of the intestinal closure to the absorption of macromolecules (Ex.: Immunoglobulins), which in the mice occurs on day 16 and 17 (Lecce, 1972).

In rodents, during suckling, the small intestine acts as an organ of double function; the proximal segment that will differentiate in duodenum, because of presence of specific receptors in the glycocalyx is able to capture selectively immunoglubulins from the milk, to transport them through the cytoplasm in coated vesicles, and after fusion with the lateral cell membrane, to discharge the IgG into systemic circulation (Rodewald, 1970, 1973, 1980). It is believed that clathrin avoids its fusion with the lysosomes, and the consequent digestion of IgG by proteolytic enzymes (Wild, 1976, 1980). In the medium and distal segments, the epithelium of lactent animals is formed by lysosome-rich cells, where the milk is intracellularly digested, since the gut is not mature enough to promote the intraluminar digestion that is established during weaning (Clark, 1959; Williams & Beck, 1969). Previously to the weaning the villi epithelial cells are progressively replaced by the columnar epithelium characteristic of the adult gut. The term closure has been used to described the blocking of transit of biopolymers through the small intestine epithelium that coincides with gut maturation (Rundell & Lecce, 1973).

Suppression by maternal antibody cannot be excluded as the mechanisms involved in the hyporesponsiveness, even at 42 days after birth, a time at which no significant amounts of maternal antibody remained, because it is possible that *in utero* exposure to maternal idiotypes of schistosome specificity (Wikler et al., 1980; Kresina & Nasinoff, 1983) might well influence the subsequent antischistosomal repertoire expressed by the offspring upon subsequent exposure to infection.

The discrepancy of the data from the literature related to the outcome of the infection in offspring of infected mothers (see introduction) can be explained by the variation in the methodology of the experiments (Ex.: Time of infection, load of cercariae, *S. mansoni* isolate or strain, etc.). Hang et al. (1974) have shown that, in their experimental system, large doses of soluble egg antigens administered i. v. to pregnant mice rendered the offspring hyporesponsive, but moderate or low doses of antigen sensitized the progeny. It is also important to emphasize, that in our experimental design (Fig. 1) the day of infection varied in relation to the nursing and closure periods, changing the possible influence of breast feeding (specific and non-specific stimulant or inhibitory factors of the milk) on the development of schistosomal infection. The sequential transfer of antibodies and antigens to the offspring through placenta and milk during or after the migration and maturation process of the parasites (Fig. 1) can also break out mechanisms of antigenic competition (Taussing, 1973; Liacopoulos & Ben Efraim, 1974). In conclusion, the hyporesponsiveness to the infection, with apparent best protection observed in the offspring of infected mother, specially in the sub-group A.III, can be due to the transference of antibody and antigens via placenta and/or by milk.

Therefore, the outcome of the infection in this kind of experiment is variable, depending if it occurs in the beginning, or midle, or after the breast-feeding period. The influence of nonspecific factors of the milk (Losonsky & Ogra, 1981) on the evolution of the schistosomiasis infection deserves further studies.

ACKNOWLEDGMENTS

The authors are grateful to Dr. G. Gazzinelli for providing *S. mansoni* antigens; to Ms Luzia F. G. Caputo, Ms Vânia C. Valentim; Mrs F. Fátima Cruz and Mrs Iolanda D. Pedro for technical assistance; to Mr Genilto J. Vieira and Ms Heloísa M. N. Diniz for the preparation of figures, and to Ms Rosângela F. L. Ribeiro for the typing of the manuscript.

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