Placental Morphology of Newborns at Risk for Congenital Toxoplasmosis

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It has been demonstrated (1,2,3,4,5) that the congenital transmission of toxoplasmosis may be responsible for discrete or severe disseminated lesions of the newborn, depending on the time of intrauterine infection.

According to Eichenwald (6), the clinical aspects of neonatal toxoplasmosis may be so varied, that it should be taken into consideration in the differential diagnosis of obscure neonatal disease. Thus, laboratory diagnosis may render valuable aid in the early detection of the protozoal infection. As the placenta is considered a fetal organ, it may be used as a fetal biopsy, permitting the etiologic diagnosis.

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With the purpose of evaluating the incidence of congenital infection by *Toxoplasma gondii* in our community, 1032 newborns, selected at random from a total of 1.131 births in the Maternidade Clovis Corrêa da Costa—Instituto Fernandes Figueira (August, 1978–February, 1980) were examined (7). Within 3–5 days after birth, blood samples were taken for indirect immunofluorescence test (IF). Three hundred and seventy seven (36.5 per cent) IF-IgG and IF-IgM seronegative cases and 655 (63.5 per cent) IF-IgG seropositive cases reacting from 1 in 16 to 1 in 1024 were found. In 15 such cases (1.4 per cent), sera were reactive in both the IgG and IgM classes, 11 cases being IF-IgM 1 in 16, and four cases IF-IgM 1 in 64. These cases were considered at potential risk for congenital infection. The serological and clinical data relative to the newborns are given in Table 1.

TABLE 1
Neonates at risk for congenital toxoplasmosis, presenting positive indirect immunofluorescent test for toxoplasmosis (IF-IgG and IF-IgM). The clinical and laboratorial data

Number	Sex	Weeks of pregnancy	Weight (g.)	Antibody Anti-toxoplasma IF-IgG	IF-IgM	Clinical data
1	F	39	2600	1:256	1:64	Hepatosplenomegaly
2	M	39	3400	1:1024	1:64	Hepatosplenomegaly Chorioretinitis
3	F	38	2300	1:64	1:64	Low-birth-weight Microcephaly
4	M	38	3650	1:256	1:16	Normal
5	M	39	3600	1:1024	1:16	Normal
6	M	39	3675	1:64	1:16	Unilateral congenital Cataracts
7	M	38	3070	1:256	1:16	Normal
8	F	39	3300	1:64	1:16	Normal
9	M	38	2775	1:64	1:16	Normal
10	M	38	2800	1:256	1:16	Normal
11	M	39	2900	1:64	1:16	Down's Syndrome
12	M	39	2300	1:256	1:16	Normal
13	F	39	2500	1:256	1:16	Normal

In 13 IgG and IgM positive cases the morphologic study of the placenta was made and constitutes the basis of the present paper.

Material and Methods

All placentas obtained from the obstetric delivery rooms of the Maternidade Clovis Corrêa da Costa were examined grossly by the Benirschke and Driscoll's technique (8). The microscopic studies were done in the case of premature and small-for-date infants, in cases of stillbirth, neonatal death or morbidity, chronic or subacute fetal distress (meconium impregnation) and in the presence of gross abnormality of the placenta. In this investigation the screening procedure used for the selection of placentas was based on the results of gross examination and the serological as well as clinical data of the neonates.

The weight of the organ with membranes and umbilical cord was taken in the delivery-room; in the laboratory, weight was measured after removing the membranes, cord and adherent clots, generally some hours after the placenta had been delivered.

The placentas were fixed in 10 per cent formol. For microscopic examination six sections were made: one from the margin, another from near the center, making sure that the latter contained a representative sample of chorionic vessels and two or three blocks of abnormal areas. A roll of the membranes and a longitudinal and a cross section of the umbilical cord were also examined. Routinely the hematoxylin-eosin stain was employed; the periodic-acid of Schiff and Giemsa's stain were used for better staining of the microorganisms and the cellular elements.

Results

Table 2 shows the data obtained on macroscopic examination. The weight of the placenta was appreciably altered by drying; so, the values obtained at the time of birth are greater.

In eight specimens (cases 4,5,6,7,8,10,11,13) a hematogenous infection was suggested by the appearance of the membranes and the cord. It was characterized by mild opalescence of the membranes, modification of the normal texture of Wharton's jelly and of thickening of umbilical vessels (Fig. 1,2). Subacute or chronic fetal distress was diagnosed by meconium impregnation of the membranes and umbilical cord in four cases (1,3,6,11). On the maternal surface of the placenta calcification was detected in eleven cases. In three specimens, nothing worth noting was found on the placental disk, cord or membrane.

Microscopic examination

In all positive cases inflammatory lesions were seen on the membranes, villous plate and umbilical cord (Table 3). On the membranes of the placental disk and chorion, focal infiltrates of leucocytes (lymphocytes, polymorphonuclears and eosinophils) were frequent and commonly localized around the chorionic vessels. These presented focal areas of myolysis and endothelial hyperplasia. Migration of fetal leucocytes was commonly observed. In two cases

TABLE 2
Macroscopic examination

Cases	Placental weight (g.) DEL. R.	LAB.	Membranes Transparency	Meconium Staining	Umbilical cord	Gross diagnosis
1	625	448	+	+	Meconium staining Edema	PFD
2	580	425	–	–	Normal	Normal
3	385	280	–	+	Meconium staining	PFD
4	425	350	–	–	Thickened vessels	PHI
5	720	515	+	–	Normal	PHI
6	630	505	+	+	Meconium staining Thickened vessels	PFD PHI
7	705	434	+	–	Necrosis (?)	PHI
8	475	358	+	+	Normal	PHI
9	490	373	–	–	Normal	Normal
10	680	480	+	+	Necrosis (?)	PHI
11	570	390	+	+	Meconium staining Thickened vessels	PHI PFD
12	530	445	–	–	Normal	Normal
13	515	347	–	–	Necrosis (?)	PHI

PFD—prolonged fetal distress
PHI—probable hematogenous infection
DEL. R.—Delivery-room
LAB.—Laboratory

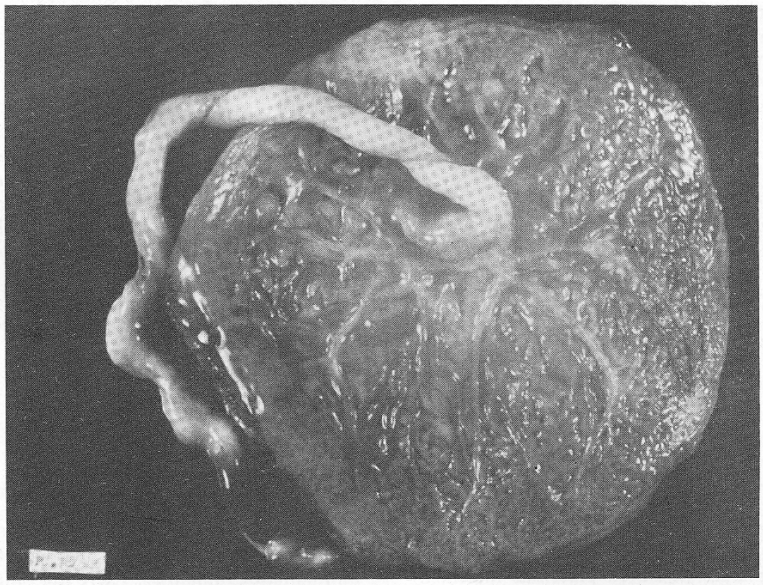

FIG. 1. Fetal surface of the placenta. Note areas of hypotransparency of the membranes and thickened chorionic vessels

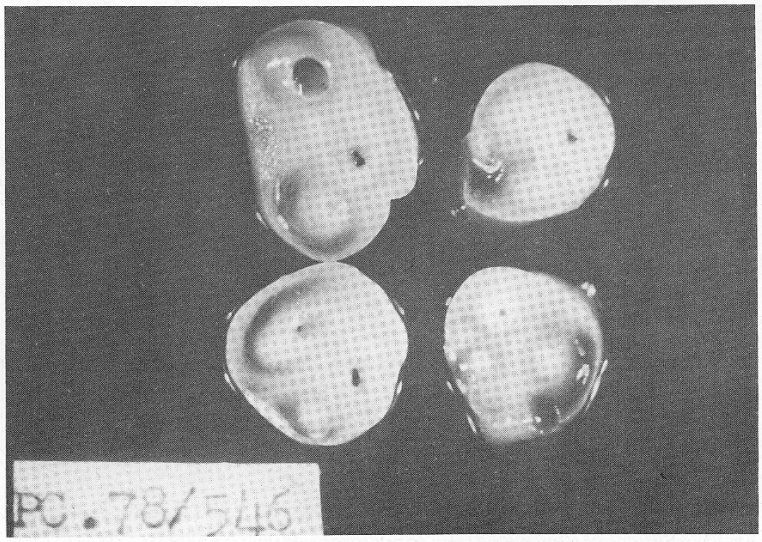

FIG. 2. Sections of the umbilical cord exhibiting hyaline white perivascular areas

TABLE 3
Microscopic examination

Cases	Membranes infiltrate Macrophagic	Leucocytic	Vasculitis Chorion, villi, decidua, cord	Villitis and Villous dismaturity	Microorganisms
1	+	+	+	+	Chorion cord
2	+	+	+	+	Chorion villus
3	+	+	+	+	Villus cord
4	+	+	+	+	Chorion cord
5	−	+	+	+	−
6	+	+	+	+	−
7	+	+	+	+	−
8	+	+	+	+	−
9	+	+	+	+	−
10	+	+	+	+	−
11	+	+	+	+	−
12	+	+	+	+	−
13	−	+	+	+	−

microorganisms with the morphologic appearance of *Toxoplasma gondii* were seen. The presence of pigmented macrophages in eleven placentas indicated prolonged fetal distress.

Villous plate. Lesions were found in the chorionic stem and in the terminal villi. Areas of trophoblastic necrosis and subepithelial accumulation of histiocytes occurred beneath the syncitial layer (Fig. 3); disruption of the stroma was associated with coagulation necrosis. Dismaturity of the villi was common, and was represented by lack of syncytiotrophoblast differentiation, persistence of cytotrofoblast, hypercellularity of villous stroma and central localization of nondilated capillaries. There was mild focal edema of the villi; hydropic villi were not observed. Villitis was always present, constituting the hallmark of the hematogenous infection. It was usually focal, more frequent in the infradecidual area, but sometimes present in the middle of the villous plate and even at the subchorionic level. It was represented by increased numbers of inflammatory cells, predominantly histiocytes or by necrosis of all the cellular elements of

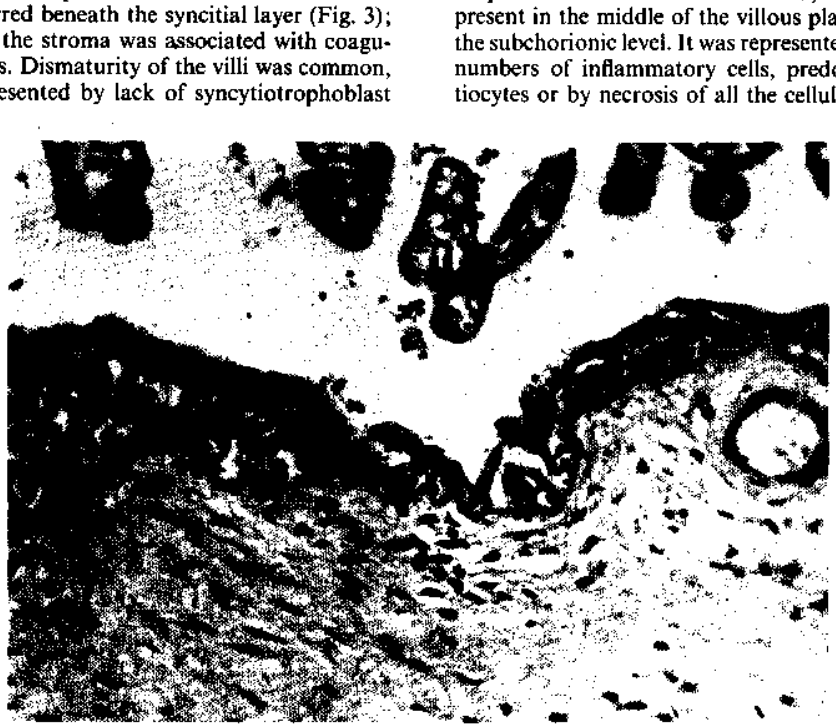

FIG. 3. Surface of a chorionic stem with necrosis of the trophoblast and presence of subepithelial cellular infiltrate (Hematoxylin-Eosin ×400)

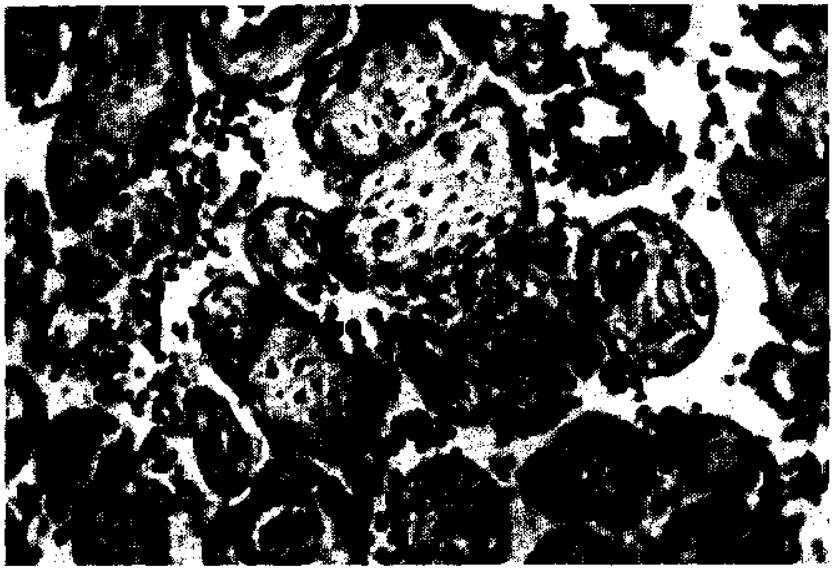

FIG. 4. In the middle of the villous plate proliferative-necrotic villitis is observed (Hematoxylin-Eosin × 400)

the chorionic villus (Figs. 4,5,6). Nodules of conglomerated villi exhibiting trophoblastic necrosis allied to intra and intervillous lymphohistiocytic infiltrate with vascular involvement were observed (Fig. 7). In the trophoblastic and stromal cells of the villi structures resembling trophozoites and cysts of toxoplasma were visualized (Figs. 8, 9). The most characteristic change in the decidua basalis and capsularis was a lymphoplasmocitoid vasculitis. In the umbilical cord vasculitis was constant; it was manifested by scanty and focal leukocytic infiltrations and hyalinization. In these areas, microorganisms resembling toxoplasma cysts were seen in four cases. A few cysts were associated with a karyolytic nucleus, indicating the intracellular origin (Fig. 10).

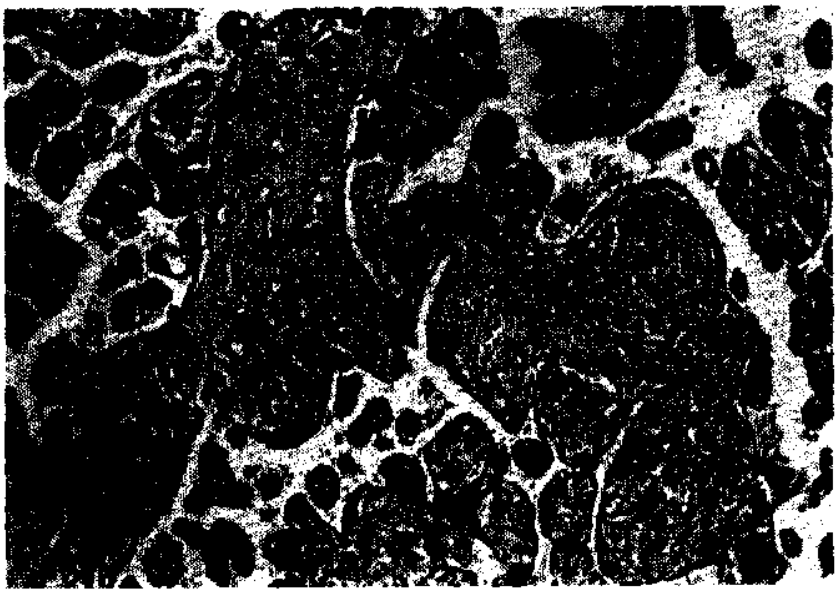

FIG. 5. Dismaturity of the villi. Presence of intravillous inflammatory cells and vascular involvement. (Hematoxylin-Eosin × 125)

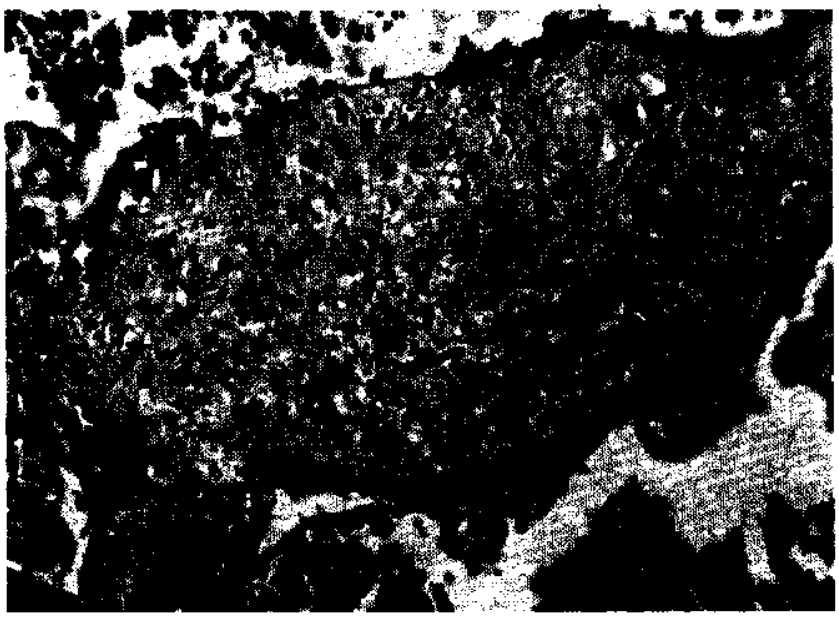

FIG. 6. Enlarged villus with areas of superficial necrosis, proliferation of local cells and histiocytic infiltration (Hematoxylin-Eosin × 500)

FIG. 7. Nodule of conglomerated villitis and intervillitis (Hematoxylin-Eosin × 500)

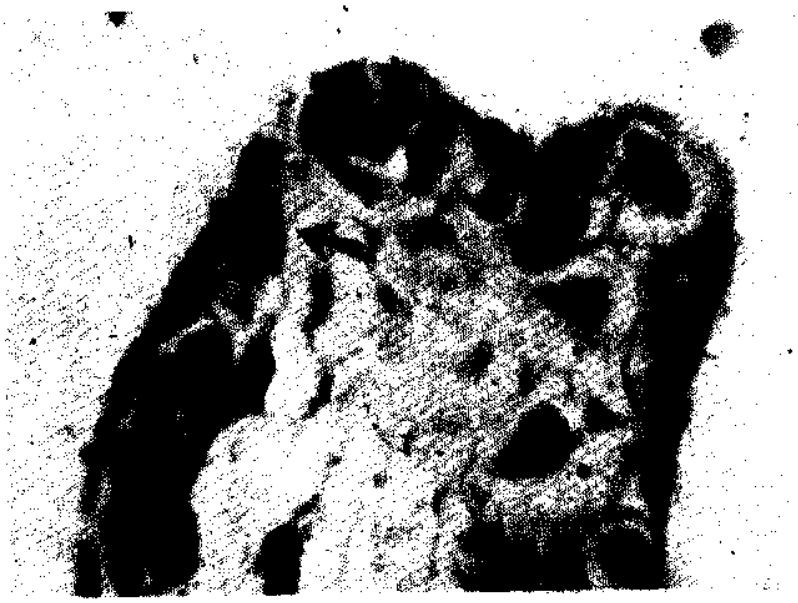

FIG. 8. Presence of intracellular protozoa in a trophoblastic cell of a villus (Hematoxylin-Eosin × 1200)

FIG. 9. Structures resembling Toxoplasma organisms are observed in relation to villous cells (Hematoxylin-Eosin × 1200)

FIG. 10. In the intervillous space a parasited cell associated with a karyolytic nucleus can be seen. (Hematoxylin-Eosin × 1200)

Comments

Congenital toxoplasmosis is sometimes difficult to diagnose clinically and histologically, since it can present in diversified forms. Benirschke and Driscoll (8) maintain that the infection of the fetus may be anticipated and diagnosed on examination of the placenta. Altshuler (9) pointed out that there is a tendency to overlook the importance of placental examination in the diagnosis of toxoplasma infection, though the histopathologic assessment of this organ may be contributory.

The present study was undertaken to determine if newborns presenting antibodies directed against *Toxoplasma gondii* had distinctive placental morphology. All of 13 newborns serologically suspected of having toxoplasma infection had shown relatively satisfactory clinical condition at birth. However, in all the associated placentas histologic examination permitted the diagnosis of hematogenous infection. Similar lesions in different stages of evolution were seen. In all of the specimens evidence of the hematogenous route of toxoplasma to the placenta was suspected by the presence of disseminated vascular lesions and focal or disseminated villitis. A prolonged search through many sections permitted identification of both tachyzoites and cyst forms of *Toxoplasma gondii* in four cases. The organisms in groups were seen near inflammatory foci on the membranes umbilical cord or villi, respectively according to frequency. The variation in sites of infection may be a reflection of the degree of local parasitemia. None of the special stains used (Giemsa, periodic-acid of Schiff) were superior to hematoxylin-eosin preparations.

In all specimens there was a relative immaturity of the terminal villi and focal or diffuse villitis. The presence of focal necrosis in trophoblast was relatively common; invasion of the cell by the microorganism was rarely seen. This supports the hypothesis that the trophoblastic epithelium is the primary site of placental invasion (10).

We would like to stress the absence of gross edema and villous hydrops, described in some cases of toxoplasmosis (11).

The presence of focal calcification of villi, with deposition of calcium salts on the trophoblastic cells was fairly frequent. Gross examination revealed calcification in 11 placentas. Beckett (12) points out that it is not clear whether calcification is of significance in association with toxoplasma, since it is often seen in the human placenta.

Probably the maternal infection had developed in the last trimester of pregnancy, as the newborns

presented mild or no clinical symptoms and the placental lesions were discrete. Fetal and placental lesions depend upon the stage reached in the evolution and repair of the inflammatory process at the time of delivery (2). Benirschke and Driscoll (8) emphasize that the infection of the fetus by *Toxoplasma gondii* may be expressed in the placenta with varying degrees of severity. In 22 perinatal autopsies with toxoplasmosis we could observe that the placental and fetal lesions were more intense than in the reported series and varied according to the duration of the intrauterine infection; the most severe and disseminated lesions were observed in the cases of well established fetal involvement.

Desmonts and Couvreur (2) verified a close correlation between positive placental isolation of microorganisms and congenital infection since the children involved usually had serologic or parasitologic evidence of toxoplasmosis.

Our data are in keeping with that of Glasser and Delta (13), who consider placental toxoplasmosis indicative of fetal infection. They emphasize that prompt treatment of the infant may prevent or reduce the degree of permanent damage.

Summary

Gross and histologic examination of 13 placentas related to newborns serologically suspected of congenital toxoplasmosis exhibited lesions compatible with hematogenous infection. In four cases microorganisms having the morphologic features of trophozoites and cysts of *Toxoplasma gondii* were observed. We would like to emphasize the importance of gross examination of the placenta, which permitted the diagnosis of prolonged fetal distress and or probable hematogenous infection in ten cases.

References

1. Alford CA, Stagno S, Reynolds DW. Congenital toxoplasmosis: Clinical, laboratory and therapeutic considerations, with special reference to subclinical disease. Bull N Y Acad Med 1974; 50: 160–81.
2. Desmonts G, Couvreur J. Congenital toxoplasmosis. N Engl J Med 1974; 290: 1110–16.
3. Saxon SA, Knight W, Reynolds DW, Stagno S, Alford CA. Intellectual deficits in children born with subclinical congenital toxoplasmosis. A preliminary report. J Pediatr 1973; 82: 792–7.
4. Stagno S. Congenital toxoplasmosis. Am J Dis Child 1980; 134: 636–7.
5. Stray-Pedersen B, Lorentzen-Styr AW. Infants potentially at risk of congenital toxoplasmosis. Am J Dis Child 1980; 134: 638–42.
6. Eichenwald HF. A study of congenital toxoplasmosis with particular emphasis on clinical manifestation,, sequels and therapy. In: Siim JC, ed: Human toxoplasmosis. Copenhagen: Munksgaard, 1952.
7. Coutinho SG, Garcia AGP, Amendoeira MRR, Assumpção MRE, Albano N. Detection of newborns at risk for congenital toxoplasmosis in Rio de Janeiro 1982. In Press.
8. Benirschke K, Driscoll SG. The pathology of the human placenta. Berlin: Springer-Verlag, 1967, 274.
9. Altshuler G. Toxoplasmosis as a cause of hydranencephaly. Am J Dis Child 1973; 125: 251–2.
10. Elliott WG. Placental toxoplasmosis. Report of a case. J Clin Pathol 1970; 53: 413–7.
11. Bain AD, Bowie JH, Beverley JKA, Beattie CP. Congenital toxoplasmosis simulating haemolytic disease of the newborn. J Obstet Gynaecol Br Emp 1956; 6: 826–32.
12. Beckett RS, Flynn FJ. Toxoplasmosis. Report of two new cases, with a classification and with a demonstration of the organisms in the human placenta. N Engl J Med 1953; 249: 345–50.
13. Glasser L, Delta B. Congenital toxoplasmosis with placental infection in monozygotic twins. Pediatrics 1965; 35: 276–83.