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DISTRIBUTION OF THE A, B AND H BLOOD GROUP ANTIGENS IN NORMAL OVARIAN TISSUE

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ABSTRACT

The distribution of A, B and H antigens in normal ovaries of 92 patients was studied. The age of the patients varied from premature (1000 grams of weight) to adults of 73 years. The study was done using the Red Cell Adherence (RCA) test described by Kovarik, Davidsohn and Stejskal in 1968. We found that only the coelomic epithelium, the *rete ovarii* and the mesonephric embyional rest contain the corresponding isoantigens.

We consider these findings important because we expect that the benign neoplasms arising from these structures contain the blood group substances, while the malignant neoplasms lose them. These findings could possibly be of diagnostic value.

INTRODUCTION

Our aim has been to study benign and malignant neoplasms of the ovary using the red cell adherence (RCA) test introduced by Kovarik,

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Davidsohn and Stejskal (11). We hoped to develop a reliable criterion for the diagnosis of these neoplasms.

Davidsohn demonstrated the loss of the isoantigen A, B and H in some carcinomas (2, 3, 11). Other investigators demonstrated the presence of A, B and H antigen in carcinoma: Glynn claimed to have demonstrated the A, B and H antigen in epithelial cells of gastric carcinoma (5, 6) and Hakkinen demonstrated an antigen similar to group A substance in the gastric secretion of patients with such lesions in the stomach (7, 8). Szulman (17) demonstrated isoantigens A and B in carcinoma of the cervix.

We undertook to investigate the distribution of A, B and H isoantigens in normal ovarian tissue. Hartman (10), Szulman (16, 17), Weinberg (18) and Stejskal (15) have demonstrated the existence of antigens A, B and H identical with those present in red blood cells in many normal tissues and in secretions of some organs. Previous studies have shown that mucinous cysts of the ovary contain large quantities of the three antigens (13, 16). However, these antigens have not been investigated in normal ovarian tissue. This is what we decided to study because it is essential to establish in which benign lesions blood group antigens are present and in which malignant ovarian neoplasms they are not demonstrable. According to our observations in benign lesions, the cells preserve their antigen-producing ability or it may even be increased. On the other hand, in malignant lesions, the production of antigens is reduced or even lost entirely.

MATERIALS AND METHODS

Materials.

The tissues were obtained from 92 patients, 80 of them adults. The ages of these patients varied from 19 to 73 years. We studied also ovaries of six premature infants (weighing 1000, 1100, 1120, 1140, 1600 and 1900 gm). Three newborn infants weighed 2800, 3000 and 3500 gm. We also studied ovaries of two infants ages 3 and 7 months and one of a child 3 years old. The ovaries of the infants and children were obtained from autopsies and the causes of death were prematurity (two of them due to preeclampsia), bronchopneumonia and sepsis.

The indicator red blood cells of groups A, B and O were obtained from the Charles Hymen Blood Center of the Mount Sinai Hospital Medical Center where acid-citrate-dextrose (ACD) is used as an anticoagulant. The age of the red cells did not affect the quality of the reaction. A 1%

suspension of red blood cells is prepared in Tris buffered saline with a pH of 7.45.

Pooled human anti-A and anti-B sera with an agglutination titer of 1:512 were used for the detection of the isoantigens A and B (11). *Ulex europeus* extract in Tris buffered saline pH 7.45 with an agglutination titer of 1:64 to 1:128 was used for the detection of the H antigen.

All tissues were fixed in 10% formaline and embedded in paraffin. Serial sections of five-microns-thick were cut. The blood groups of the patients are determined by testing the red blood of the patient in the usual fashion or by doing the RCA test on tissue sections (11). Two consecutive sections are used, one for the red cell adherence test (3, 11) to investigate in which normal structure of the ovary the isoantigens are present; the second section is stained with hematoxylin and eosin for comparison.

Methods.

1– The ovarian paraffin sections are deparaffinized and washed in Tris Buffer Saline (TBS) for 5 minutes.

2– Slides of Group A, B and O are placed side up in a separate moist chamber. The tissue sections are covered with the corresponding antiserum at room temperature for 15 minutes.

3 - The slides are placed in a slide rack and staining dish. They are washed for 45 minutes with Tris Buffer Saline. The TBS is changed three times at 15 minutes intervals.

4– The slides are returned to the moist chamber. Each section is then covered with the indicator erythrocyte suspension for 10 minutes.

5– A set of Petri dishes are prepared with minimal amounts of Tris Buffer Saline and two supporting pieces of wooden applicators. One Petri dish for each slide. The slide with sedimented erythrocytes are turned upside down and placed immediately on the two supporting applicators; so they just touch the Buffer solution.

6– After 10 minutes the indicators erythrocytes that did not react specifically with homologous immune-serum sink to the bottom of the Petri dish.

7 Each slide is then moved aside on the supporting applicators to an area of clear Buffer. Then the slide is examined in the microscope and compared with the section stained with Hematoxylin Eosin.

Controls of RCA Reaction.

1– In every test done the reaction is controlled by including a section of Uterine-Cervix (of the same blood group) of which the stratified epithelium is rich in its corresponding antigen. This is a positive control.

2– Every tissue has built-in controls. The vascular endothelial cells contain the antigen so they give a positive reaction regardless of the tissue. This serve as positive control. The connective tissue do not contain the antigen. It is always negative, providing a built-in negative control.

3– The 1% suspension of erythrocytes is tested for agglutination with the antiserum that is used on the section.

RESULTS

Ovarian Cortex.– The ovaries of adult patients are lined by columnar or cuboidal germinal epithelium. In it we could demonstrate isoantigens A, B and H. In all cases where the epithelium was present the RCA test was positive and represented by a line of indicator red cells along the epithelium (Fig. 1). In adult patients with chronic oophoritis the germinal epithelium is more prominent. In these cases the RCA test was positive in the epithelium and negative in the adjacent inflammatory cells and in the connective tissue (Fig. 2).

A frequent finding in adult ovaries were germinal inclusion cysts lined by coelomic germinal epithelium in which we saw occasionally metaplastic changes in the form of endometrial (Fig. 3) or tubal epithelium (Fig. 4). In the germinal cyst the RCA was strongly positive. In some of them we found secretion in the lumen which gave a strong positive reaction.

The germinal epithelium of the ovaries of the premature and mature infants and of children is continuous, fine and delicate. We were able to confirm it through the study of serial sections in which the coelomic epithelium was absent from one section to another. The isoantigens A, B and H were also demonstrable in the germinal epithelium of the surface of these ovaries and in the epithelium extending along the hilus of the organ and in the border of the mesoovarium where they could be demonstrated by a positive RCA test. This concept is contrary to the opinion

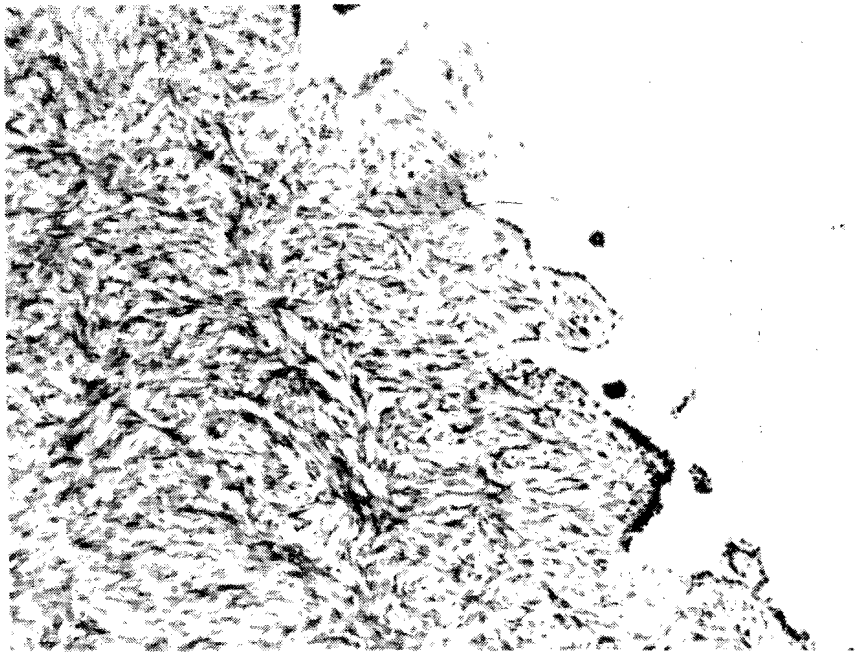


Fig. 1A. Hematoxylin and eosin. Cortical ovarian stroma lined by germinal epithelium . Magnification: 100 X.

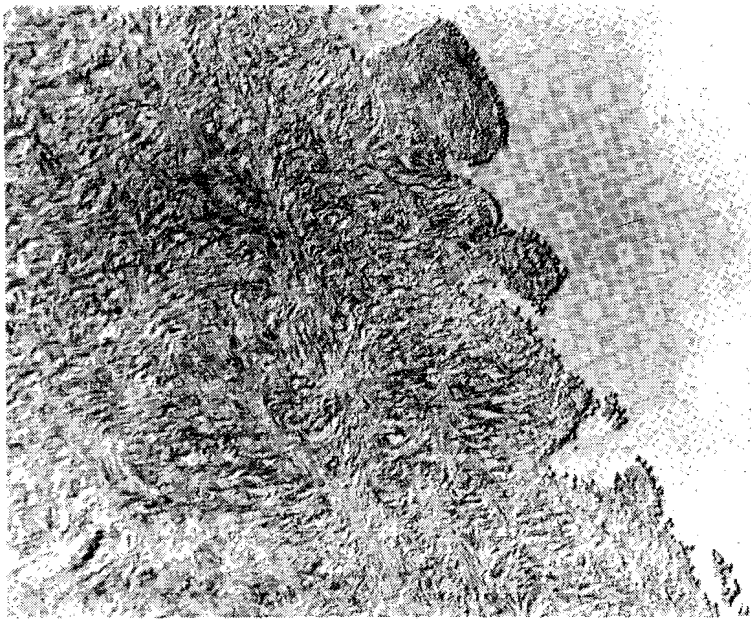


Fig. 1B.– RCA test. Positive along the germinal epithelium. Negative in the ovarian stroma. Magnification: 100 X.

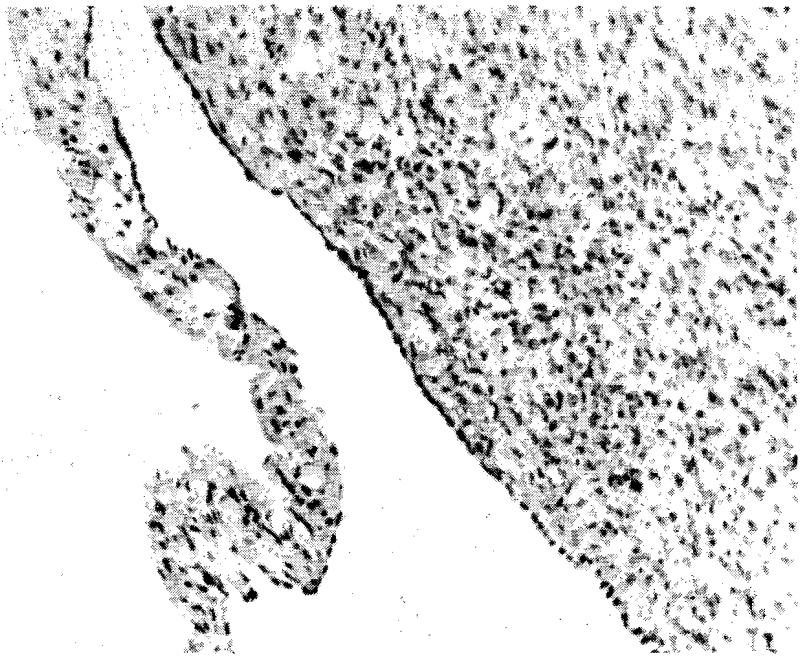


Fig. 2A.– Hematoxylin and eosin. Prominent germinal epithelium of a 40 year-old woman. Cortical stroma with marked infiltration of plasma cells and lymphocytes. Magnification: 100 X.

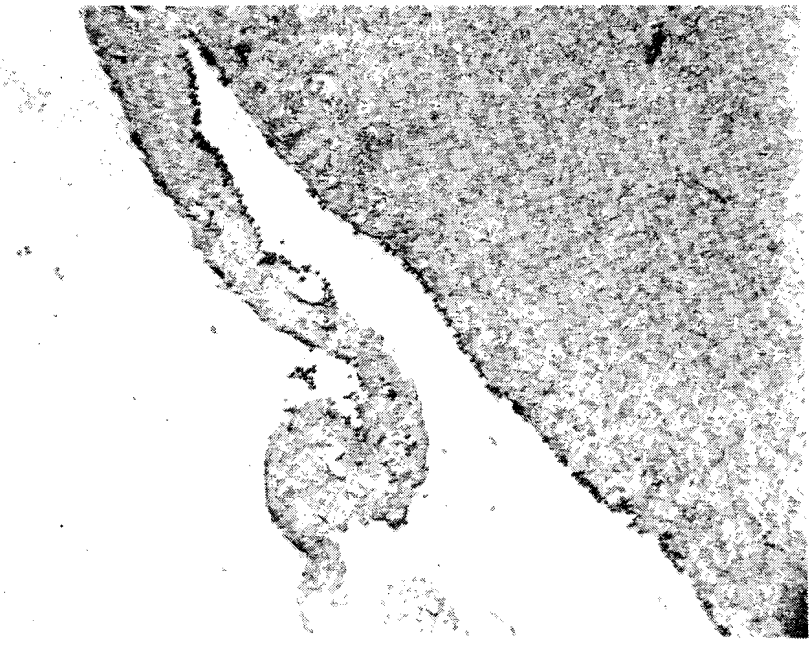


Fig. 2B.– RCA test. Positive on the coelomic epithelium. Negative on the ovarian stroma and on the inflammatory infiltrate. Magnification: 100 X.

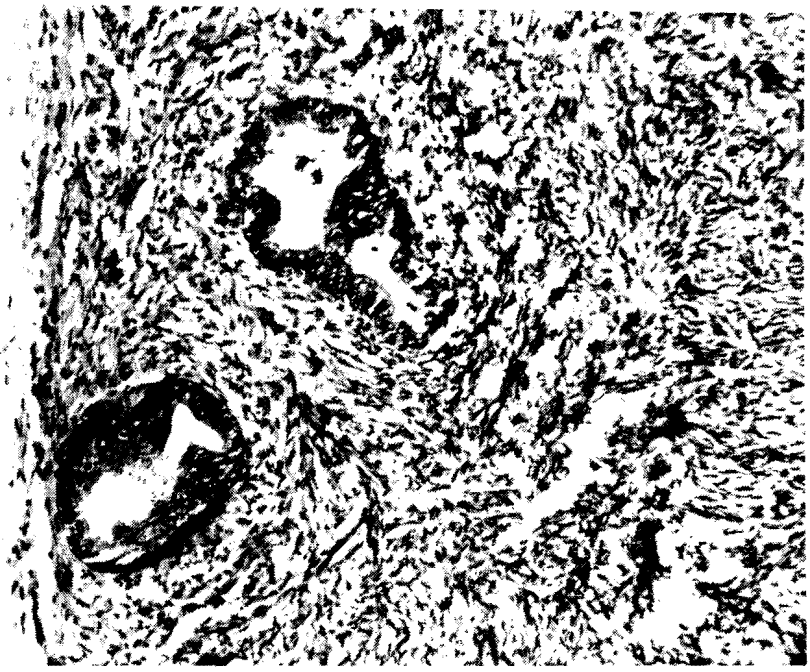


Fig. 3A.– Hematoxylin and eosin. Germinal inclusion cyst with metaplasia changes toward the endometrial glands. Magnification: 100 X.

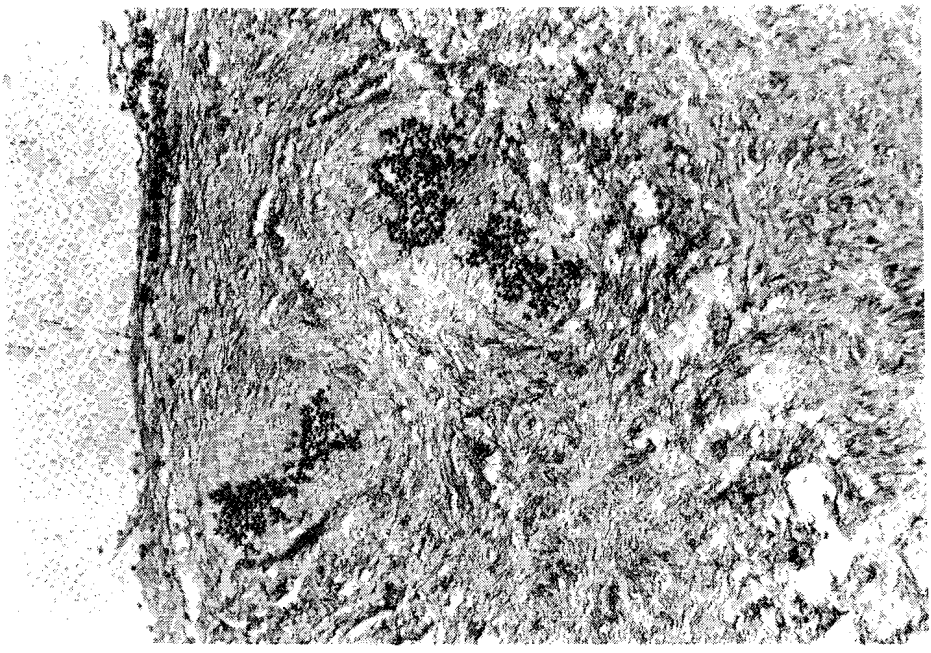


Fig. 3B.– RCA test. Metaplastic cyst giving a positive reaction in the lining and in its contents. Magnification: 100 X.

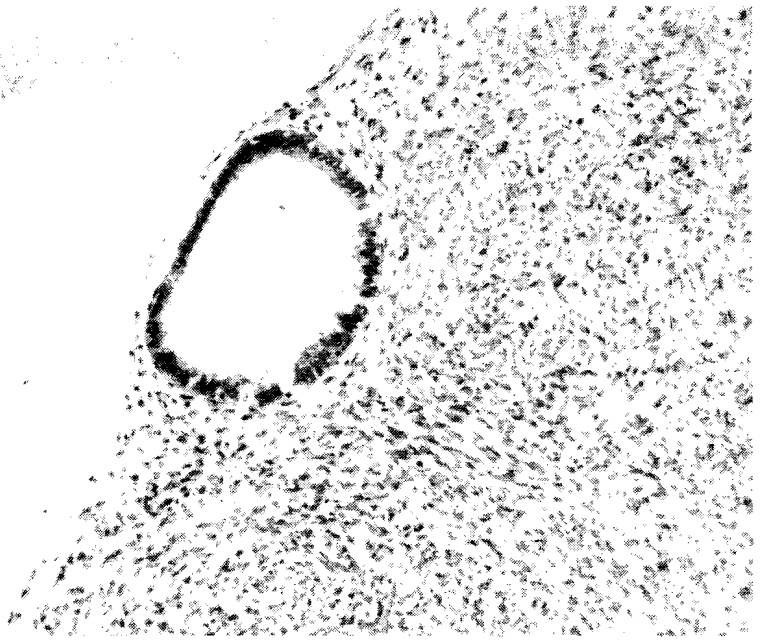


Fig. 4A.– Hematoxylin and eosin. Germinal inclusion cyst showing metaplastic changes to Fallopian tube epithelium. Magnification: 100 X.

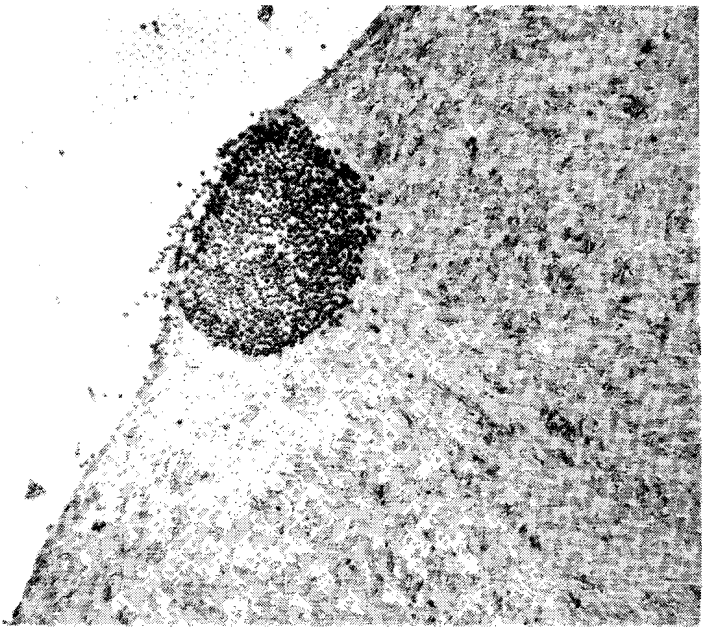


Fig. 4B.– RCA test. Positive reaction on the epithelium of the cyst and in its contents. Magnification: 100 X.

of Curtis (1) who found that the germinal epithelium overlies all the ovaries of the fetus in the last 2 to 3 months of fetal development except the hilus (Figs. 5, 6). The ovarian hilus and the mesoovarium of the adult patients also is lined by a columnar that gives a positive RCA test.

The tunica albuginea RCA was uniformly negative as was expected because the connective tissue is negative.

The cortex of the premature ovaries showed many isolated germ cells which are more abundant toward the surface of the organ where we see them mixed with the sex cords. The same was seen in the ovaries of the mature babies but in lesser numbers. The majority of the germ cells are surrounded by follicular epithelium or granulosa cells (Fig. 5). In some mature neonates and even in the premature babies, follicles deep in the cortex are lined by two or three layers of granulosa cells but we did not see there theca cells. In 7 and 8 month old babies and in the 3 year old child, theca cells were present. In all these structures the RCA test was uniformly negative demonstrating the absence of the isoantigens A, B and H.

The follicles of the adult ovaries (from 19 to 73 years of age) were present in all stages of development from primary follicles in young women to mature Graafian follicles with Call Exner bodies in several layers of granulosa cells and distinct theca interna with the associated blood vessels and theca externa, the antrum and the cumulus oophorus (Fig. 7). All these follicular structures and secretions do not contain blood group antigens. They gave negative RCA reactions, except the vascular endothelium of the theca interna which gave a positive RCA reaction like all endothelial cells of blood vessels of the bod·

The atretic follicles gave also a negative RCA test.

The Corpus Luteum.– The menstrual and the pregnant *corpus luteum*,, gave a negative RCA test in all stages of their development (Figs. 8, 9) and involution except for the blood vessels in the theca interna that showed a positive reaction which gradually became negative with the complete regression of the *corpus luteum* and the formation of *corpora albicantia* which are completely RCA negative.

The interfollicular stroma of the cortex consists of spindle cell conective tissue which also gave a negative RCA test but the vascular endothelium of the cortical stroma was RCA positive.

Ovarian Medulla.– The ovarian connective tissue matrix of the medulla gave a negative RCA reaction as does connective tissue elsewhere. The blood vessels so abundant in this area showed a distinct positive RCA test in the endothelial cells. The blood vessels engorged with the patient's blood besides the positive RCA test gave also a positive mixed cell agglutination reaction (MCAR) with the patien's own red cells.

The reaction of thick hyalinized blood vessel walls was negative. This finding is seen in the ovaries of the pre-menopausal and menopausal women.

Embryonal Rest.– The *rete ovarii* located partly in the medulla and partly in the broad ligament resembles anastomosing blind canaliculi in which the RCA test was positive (Fig. 10). Its structure corresponds to the testicular end of the seminiferous tubule (9). The mesonephric remnants are another set of vestigial tubules that remain in the broad ligaments between the ovary and the uterine tubes. These structures were seen in all tissues of infants and children as well as in some adults. The RCA test was positive proving the presence of the A, B and H antigens (Fig. 11). Hilus cells are present in the ovarian medulla and in the mesoovarium of infants and adults as an accumulation of polihedric clear (14) cells with round nuclei resembling regressing luteinized cells but without festooned appearance. The A, B and H antigens could not be demonstrated in the hilus cells (Fig. 12). Walthard rest was seen in two cases, one in the mesoovarium near the tube and the other in an ovarian cortex. It was represented by a nest of cells that resemble squamous epithelium One of them had a central cystic cavity. The RCA test in these embryonal structures were negative.

We examined also the Fallopian tubes of all infants and children included in this study. The ABH antigens were present in all the serial sections of the organ (4). On the contrary the delicate germinal epithelium of the ovary may be lost from one section to another. This can be explained by differences in thickness of the epithelium and/or differences in the amount of antigens secreted by these two varieties of epithelial cells that have the same embryonic origin, from the specialized mesodermal layer which forms the peritoneal mesothelium (14).

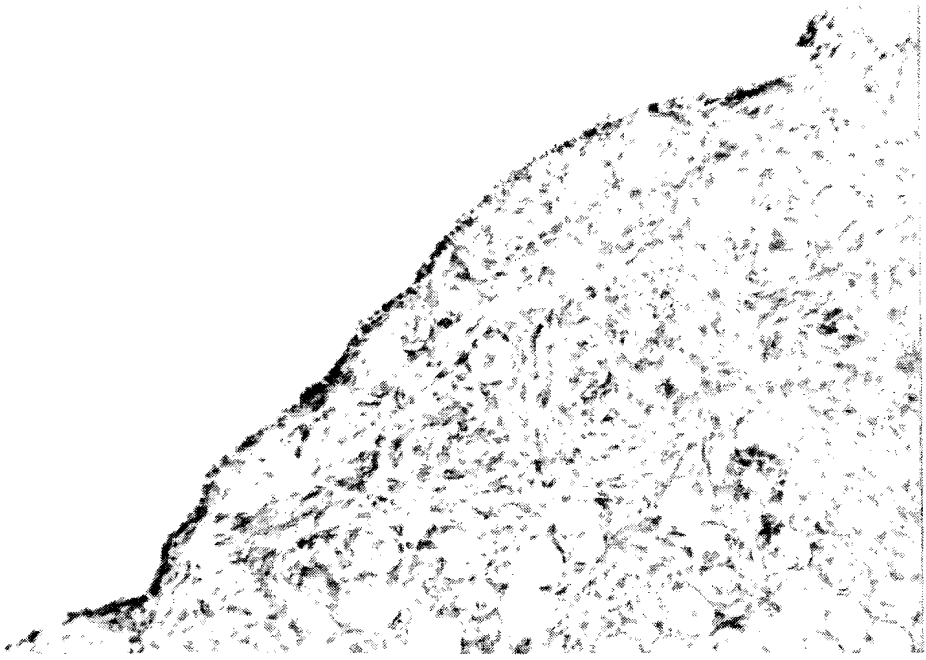
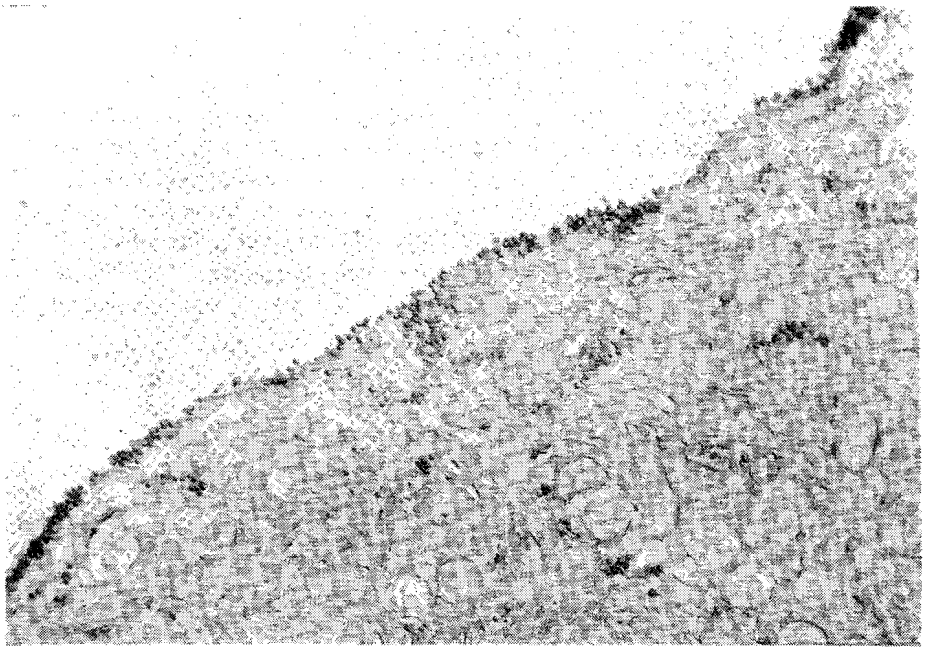


Fig. 5A.– Hematoxylin and eosin. Germinal epithelium, ovarian stroma with numerous primordial follicles in a three year-old child. Magnification: 100 X.



Fiq. 5B. RCA test. Positive along the germinal epithelium. Negative in the primordial follicles and intrafollicular stroma. Magnification: 100 X.

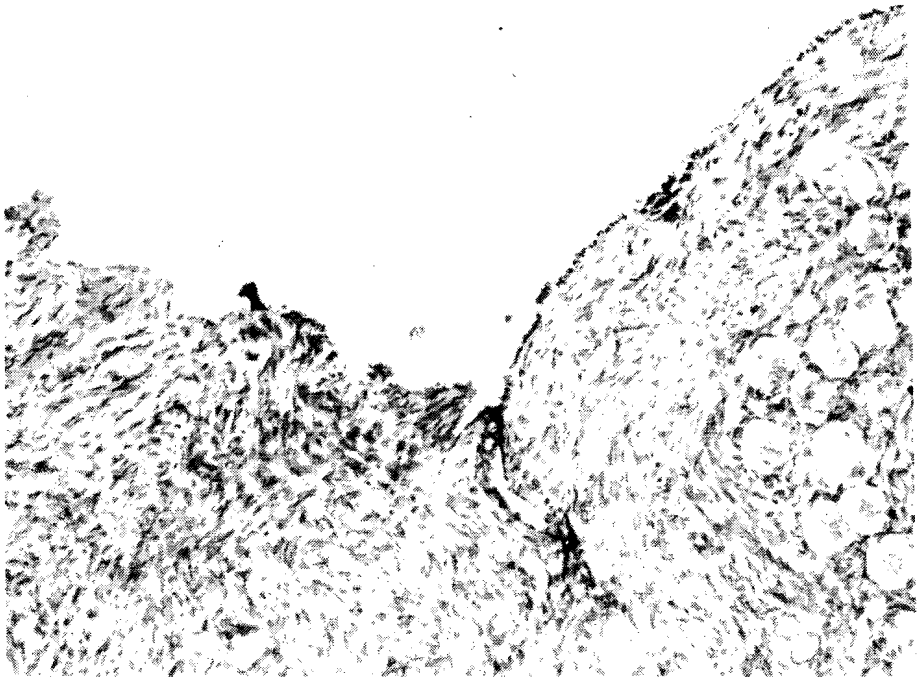


Fig. 6A.– Hematoxylin and eosin. Germinal epithelium along the ovarian cortex, extending along hilus of the organ. Magnification: 100 X.

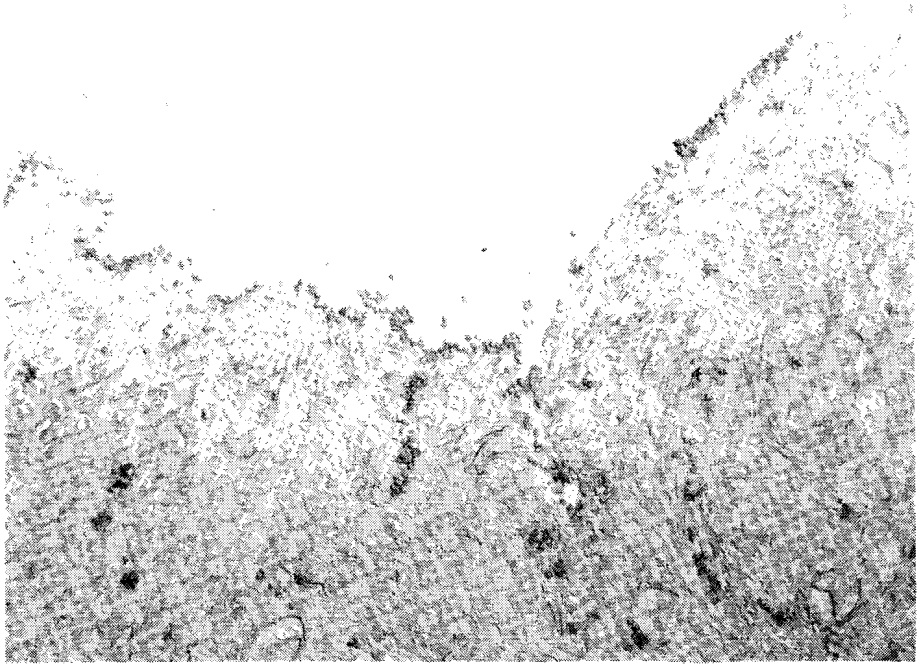


Fig. 6B.– RCA test. Positive on the coelomic epithelium of the ovarian cortex and hilus. Magnification: 100 X.

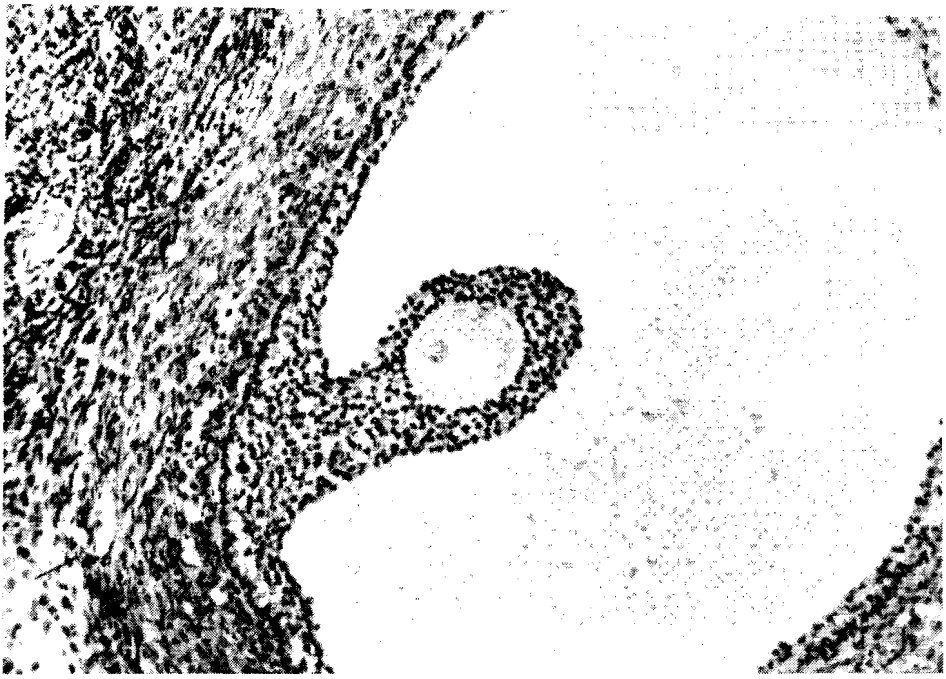


Fig. 7A.– Hematoxylin and eosin. Graaphian follicles with small blood vessels on the theca. Magnification: 100 X.

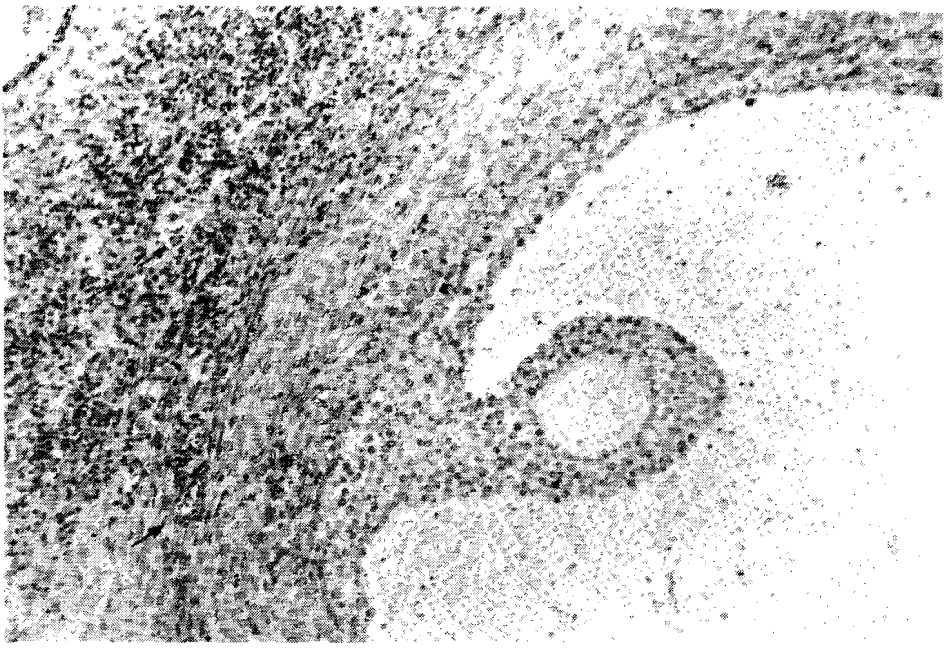


Fig. 7B.– RCA test. Negative in all the structures of the Graaphian follicles except in the blood vessels of the theca. Magnification: 100 X.

Fig. 8A.– Hematoxylin and eosin. Early corpus luteum. Magnification: 100 X.

Fig. 8B.– RCA test. Negative except for the blood vessels of the theca. Magnification: 100 X.

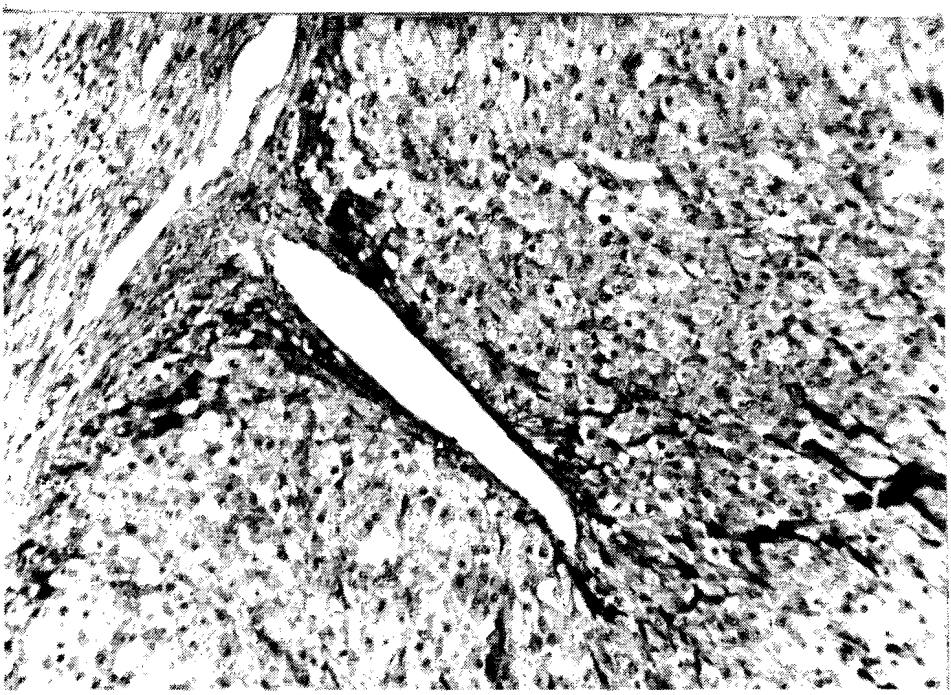


Fig. 9A.— Hematoxylin and eosin. Corpus luteum. Magnification: 100 X.

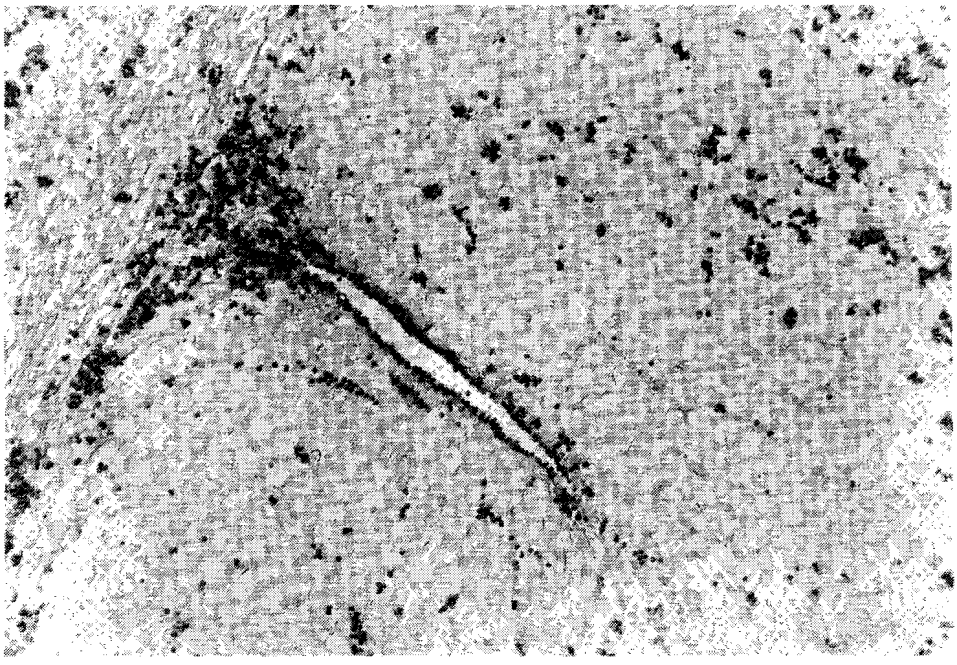


Fig. 9B.— RCA test. Negative structure except for the blood vessels of the theca. Magnification: 100 X.

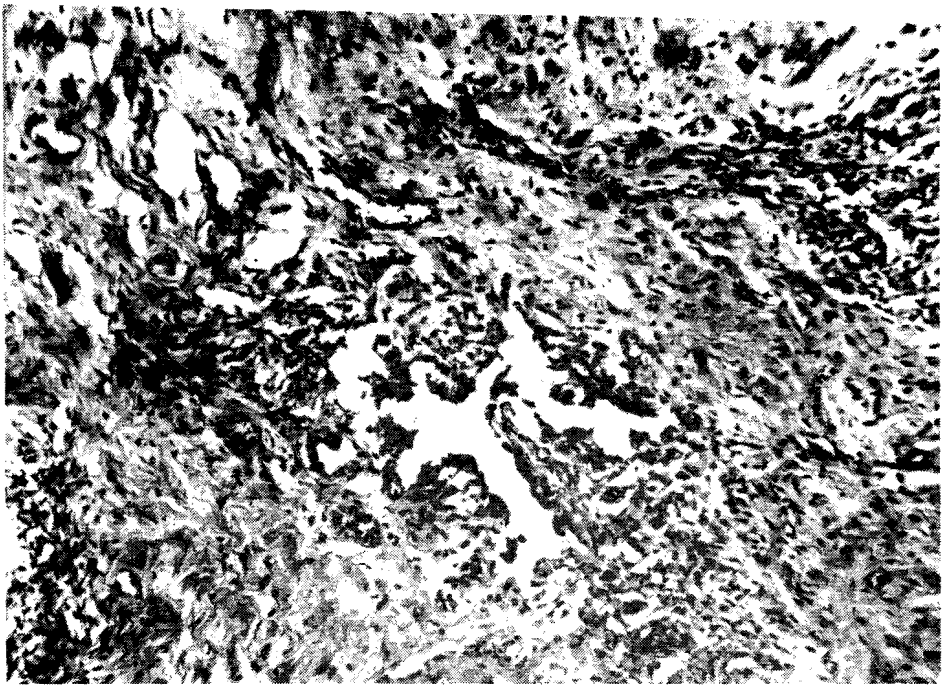


Fig. 10A.— Hematoxylin and eosin. *Rete ovarii*, represented by anastomosing canaliculi. Magnification: 100 X.

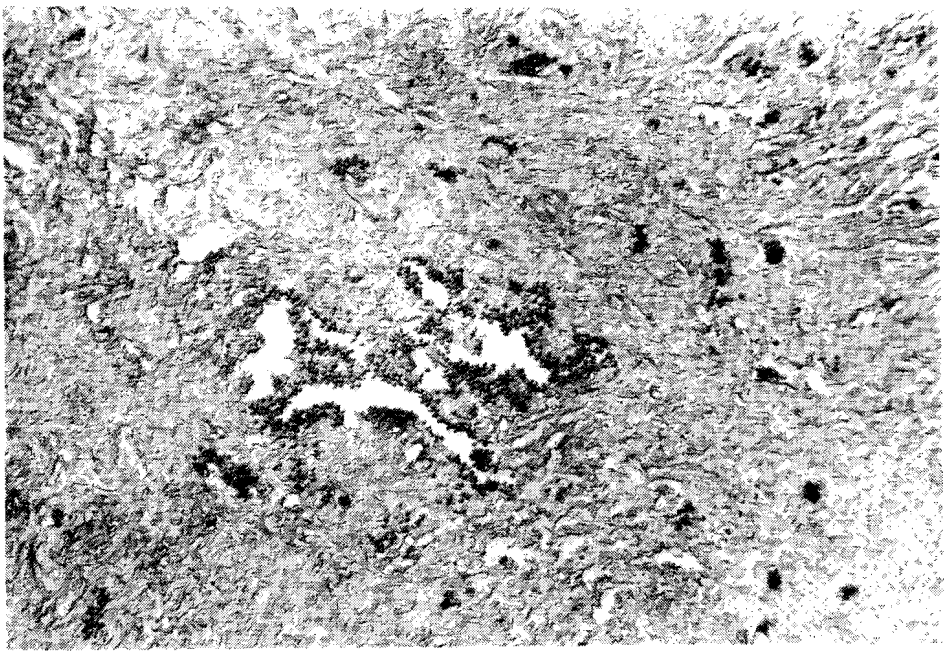


Fig. 10B.— RCA test. Showing a positive reaction on the epithelium of the embryonal rest. Magnification: 100 X.

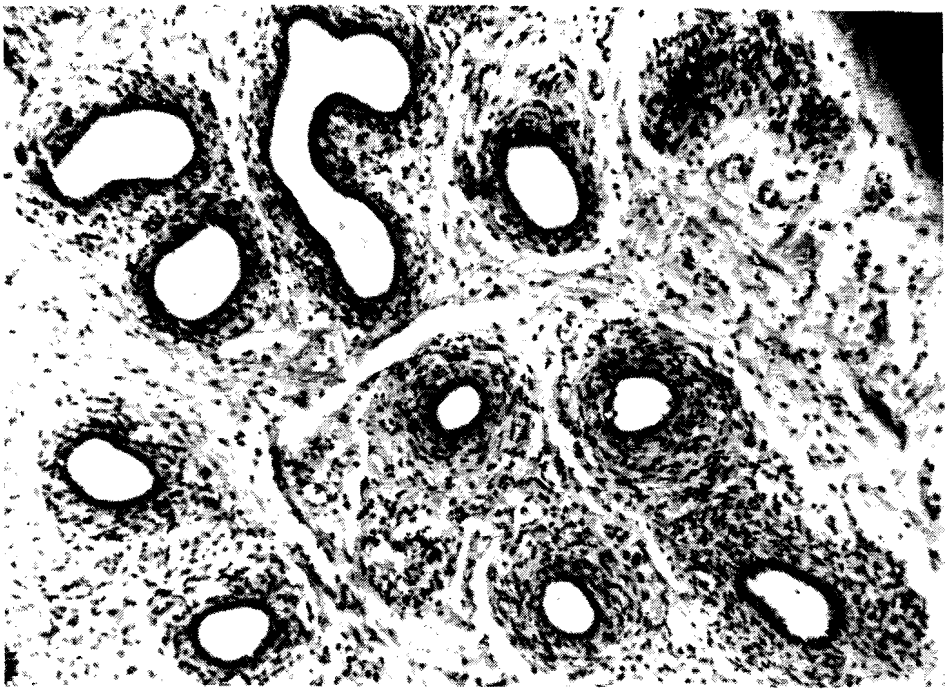


Fig. 11A.– Hematoxylin and eosin. Mesonephric vestigial tubules in a five-months-old baby. Magnification: 100 X.

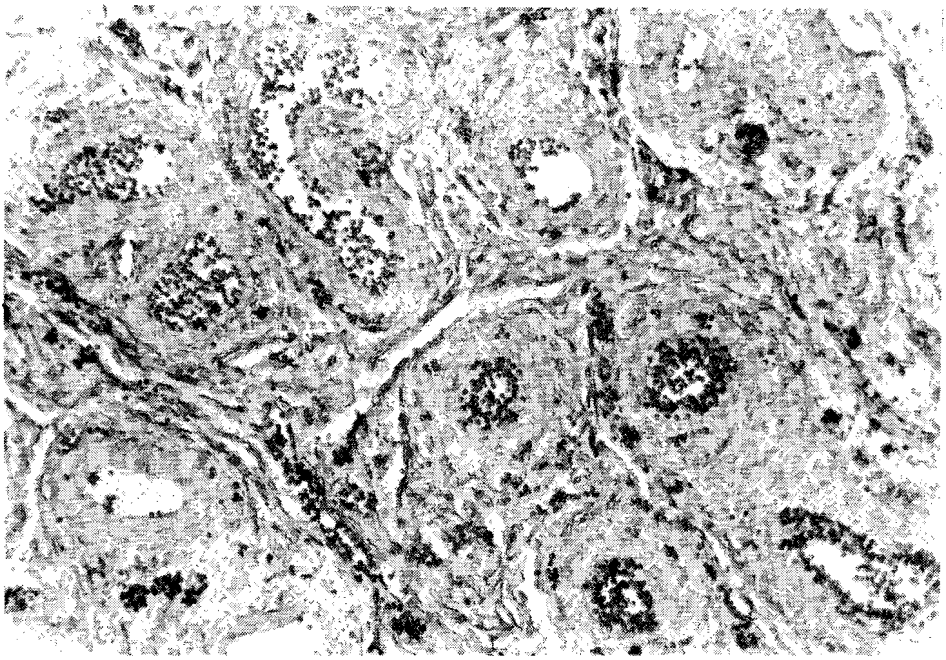


Fig. 11B.– RCA test. Showing a positive reaction on the lining and contents of the mesonephric rest. Magnification: 100 X.

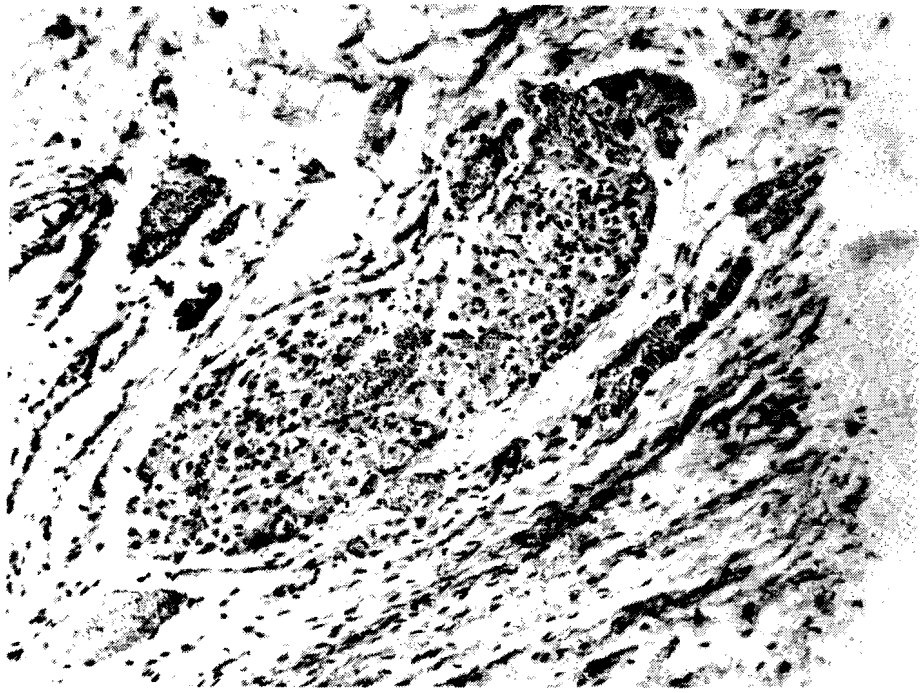


Fig. 12A.– Hematoxylin and eosin. Hilus cells. An accumulation of polihedric clear cells resembling luteinized cells. Magnification: 100 X.

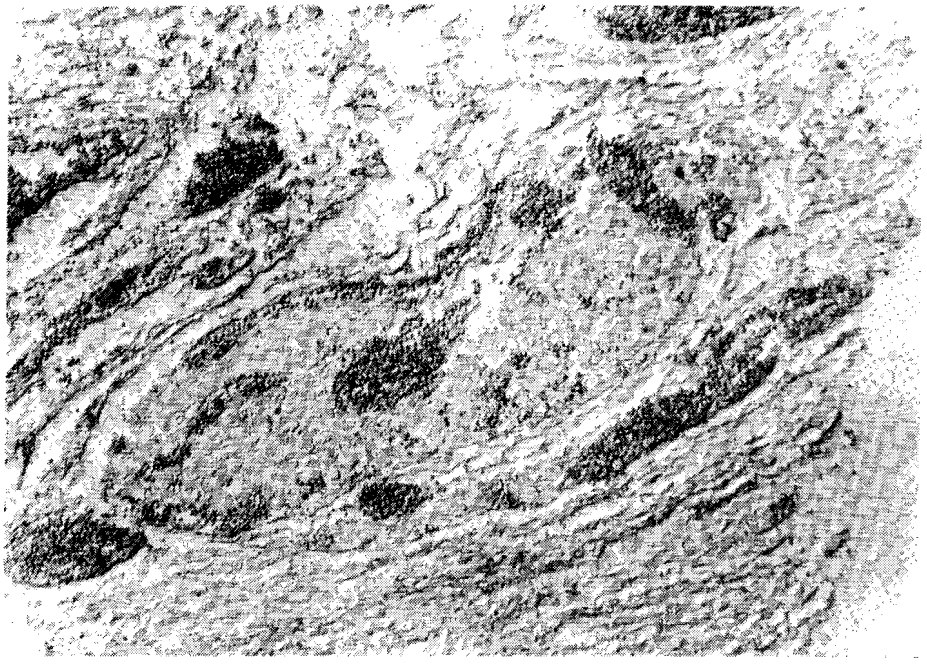


Fig. 12B.– RCA test. Showing a negative reaction on the hilus cells and a positive one in the blood vessels. Magnification: 100 X.

DISCUSSION

The coelomic epithelium has a mesodermal embryonal origin (9). The finding that it contains the blood group antigens is probably important because 75 to 80% of ovarian tumors arise from this specialized peritoneal mesothelium.

If our hypothesis is correct the benign neoplasm originating in this tissue should contain the blood group antigens occasionally in large quantities depending on the degree of cellular proliferation. On the other hand, the antigens cannot be demonstrated in malignant neoplasm because they are lost; the RCA test may help in differentiating the two. The germinal epithelium does not originate in the germ cells as was claimed previously. The negative RCA test at all stages of development of germ cells supports the concept of the extra germinal epithelial origin of the cells (9).

It seems likely that the presence of blood group substances of the germinal epithelium is not related to the histochemical composition or to hormonal influence.

McKay, Pinkerton, Hertig and Danziger (12) demonstrated with histochemical methods, in the cystoplasmic glycogen granules and ribonucleic acid (germinal epithelium of adult ovaries), but no enzymatic activity.

The mature Graafian follicles contain glycogen and ribonucleic acid in the three layers of their walls. These structures do not contain the glycolipid complexes of the blood group substances as shown by us above.

The steroid producing tissue of the human ovary (12) (some cells of corpus luteum, theca interna of Graafian follicles, hilus cells that correspond in morphology to the interstitial cells of the testes) does not normally contain the blood group antigen. The germinal epithelium does not produce steroids. Neither is it influenced by them.

The *rete ovarii* which is an embryonic rest corresponding to the male seminiferous tubules, and the mesonephric tubules which correspond to the Wolfian duct of the male which contain the blood group antigen. We assume that the male corresponding structure will also contain the antigen. The benign neoplasms of these structures in the female is expected to contain the antigens, the malignant neoplasms do not.

RESUMEN

Distribución de los antígenos A, B y H en el tejido ovárico normal. *Gaskin-Urdaneta A. (Cátedra de Anatomía Patológica, Facultad de Medicina, Universidad del Zulia, Maracaibo, Venezuela) y Davidsohn I.* **Invest Clín.** *18(1): 12-32, 1977.*– Se estudió la distribución de los antígenos A, B y H, en el tejido ovárico normal de 92 pacientes. La edad de los pacientes varió desde prematuros (1000 g de peso) a adultos de 73 años. El estudio fue realizado utilizando la prueba de adherencia de las células rojas (RCA) descrita por Kovarik, Davidsohn y Stejskal en 1968. Encontramos que solamente el epitelio coelómico, la *rate ovarii*, y los restos embrionarios mesonéfricos, contienen el correspondiente isoantígeno. Consideramos importante este hallazgo, porque esperamos que las neoplasias benignas que se originan de estas estructuras, contengan los correspondientes isoantígenos; mientras que las neoplasias malignas que se originan de las mismas, las pierdan. Este hallazgo podría tener valor diagnóstico.

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