

A STUDY OF THE FIRST GENERATION OF RABBITS
FROM MOTHERS IMMUNIZED WITH HOMOLOGOUS
THYROID GLAND PLUS ADJUVANT

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A study of the first generation of rabbits from mothers immunized with homologous thyroid gland plus adjuvant showed that maternal antithyroid antibodies pass through the placenta, appear in the fetal blood, and are still present in the neonatal blood until the 40th day of life. The highest titers of hemagglutinating antibodies reached 1:128 and of complement-fixing antibodies 4+. Histological examination of the thyroid glands of the experimental rabbits showed marked changes resembling spontaneous thyroiditis. The thyroid gland tissue was more highly differentiated, intensive vacuolation of the colloid was observed, and the severity of the lymphoid changes was assessed as 2+ and 3+. These lesions of the thyroid gland are the result of the action of "early" antithyroid antibodies transferred from mother to fetus.

After the demonstration that antithyroid antibodies can pass through the human and animal placenta [1, 2] and the high frequency of their appearance in mothers of atrophic cretins [3, 4] it was postulated that some causes of atrophic cretinism in man may be associated with the autoimmune process in the maternal thyroid gland. However, attempts to induce experimental cretinism in rabbits and guinea pigs by the placental transfer of antithyroid antibodies did not result in the development of histological thyroiditis by the newborn animals despite high titers of circulating antibodies [3, 4, 7]. The experiments of Nakamura et al., [5, 6] showed that "early" antibodies against the thyroid gland, formed 2 weeks after the end of immunization in thyroidectomized donors, have a cytotoxic action.

The object of this investigation was to study the times of appearance of lesions in the thyroid gland of mother rabbits immunized with thyroid gland antigen together with adjuvant, and to attempt to produce a disease of the thyroid gland in newborn rabbits by the action of "early" antithyroid antibodies transferred from mother to fetus during the period of organogenesis and fetal development.

EXPERIMENTAL METHOD

Female chinchilla rabbits weighing 3-4 kg were used; eight litters each of 6-12 young rabbits were obtained from each female. Altogether 62 newborn rabbits were studied. Five healthy females and 20 of their young were investigated. Tests were carried out on the 1st, 7th, 10th, 14th, 21st, 30th, and 40th days of life.

The experimental rabbits were immunized by subcutaneous (once a week) and intravenous (three times a week) injections of antigen from the homologous thyroid gland (with adjuvant). Each animal received one cycle of immunization consisting of three subcutaneous and five intravenous injections. To increase the permeability of the placenta, hyaluronidase was injected into the immunized mothers, starting on the 11th day of pregnancy, in doses of 300-600 turbidity units every 3 days.

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TABLE 1. Serological and Histological Changes in Rabbits Born from Immunized Mothers

| Serological and histological parameters | Age of newborn rabbits (days) | | | | | | |
|---|----------------------------------|-------------------------------|------------------|-------------------------|---------------------|---------------------------|------------------|
| | 1 | 7 | 10 | 14 | 21 | 30 | 40 |
| Hemagglutinating antibodies | 0 1:128 1:128 1:64 — | 0 1:8 1:32 0 1:16 | 1:2 0 1:32 | 0 1:2 1:8 1:16 | 1:32 0 — — | 0 1:128 1:32 1:2 | 0 0 0 0 |
| Complement-fixing antibodies | — | 0 ++ — | ++ ++ | ++ ++ — | 0 +++ | +++ 0 | 0 0 |
| Degree of thyroiditis | 1+ or 2+ | 2+ | 2+ or 3+ | 3+ | 2+ | 1+ or 2+ | 0 |

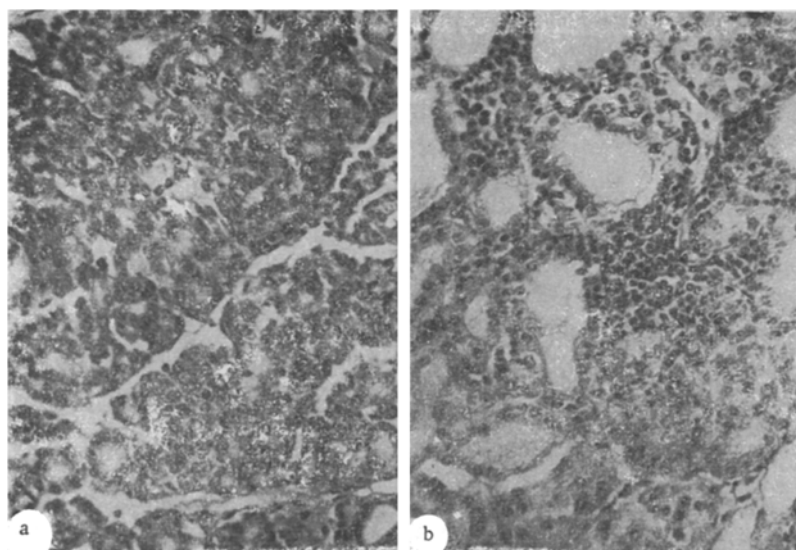


Fig. 1. Thyroid gland of a day-old rabbit: A (from a healthy mother; B) from an immunized mother: follicles enlarged, focus of lymphoid infiltration (2+). Here and in Figs. 2 and 3: hematoxylin-eosin, 200 ×.

Antithyroid antibodies in the blood of the newborn rabbits and their mothers were determined by the passive hamagglutination and complement fixation tests. For the histological investigations the thyroid gland was fixed in Carnoy's fluid and stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

Hemagglutinating antibodies appeared on the third day after the end of the cycle in the immunized rabbits in fairly high titers (1:128). Complement-fixing antibodies appeared later and persisted much longer; their level did not correlate with the titers of hemagglutinating antibodies. Lesions of the thyroid gland in these rabbits was investigated at various times after immunization: 30, 40, 60, 90, and 120 days. No changes were found in the thyroid gland of animals killed on the 30th and 40th days; after 2 months the structure of the gland remained normal in one mother rabbit, while in the other lymphocytes appeared and the lesions were assessed as minimal (1+). In the subsequent months the rabbits showed a picture of thyroiditis of varying severity. It was concluded that during the first month of pregnancy no lesions developed in the gland of the mother rabbits after immunization; for that reason circulating antibodies of all types, including aggressive, evidently can pass through the maternal placenta and act on the thyroid gland of the fetus at various periods of prenatal development.

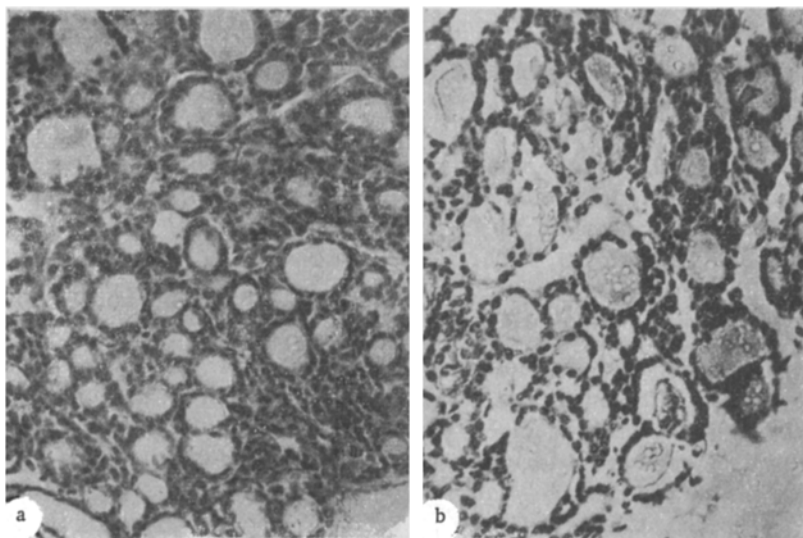


Fig. 2. Thyroid gland of a 7-day rabbit: A) from a healthy mother; B) from an immunized mother: vacuolation of colloid, lymphoid infiltration (1+).

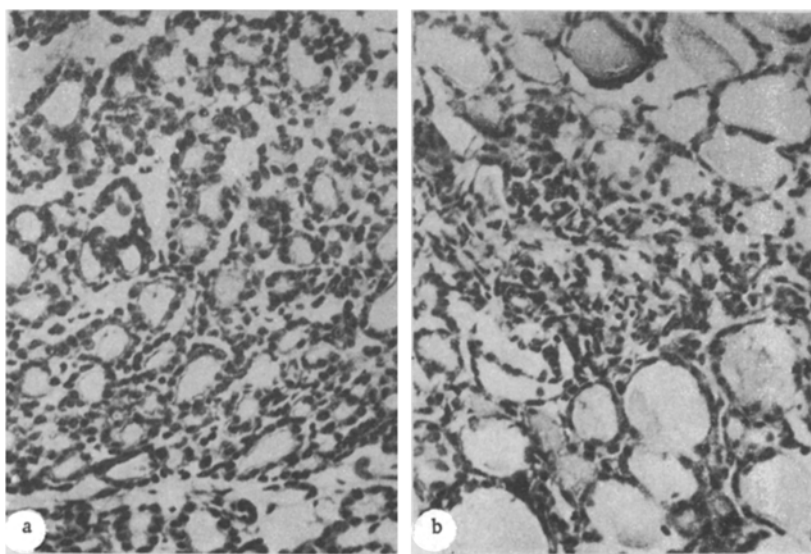


Fig. 3. Thyroid gland of a 14-day rabbit: A) from a healthy mother; B) from an immunized mother: follicles enlarged, lymphoid infiltration (3+).

Antibodies against thyroid gland were found on the first day of life in newborn rabbits from immunized mothers in the highest titers: later the titers fell and by the 40th day the antibodies had disappeared completely. Both hemagglutinating and complement-fixing antibodies passed through the placenta (Table 1).

On histological examination of the thyroid glands of the experimental rabbits, marked inflammatory changes were found in the thyroid gland (by contrast with the findings of Western observers); these changes could be classified descriptively as follows:

- 1) The presence of scattered lymphoid infiltration without destruction of the follicles (1+);
- 2) The appearance of foci of lymphocytes in the interfollicular tissue with slight destruction of the adjacent follicles (2+);
- 3) The presence of an inflammatory exudate occupying one-third of the field of vision, with preservation of the normal structure of the gland elsewhere (3+).

The thyroid glands of the day-old experimental rabbits differed from those of healthy animals of the same age in their more highly differentiated structure, the more intensive vacuolation of the colloid, and the presence of lymphoid lesions assessed as 2+. The thyroid epithelium was high in both experiment and control (Fig. 1).

By the 7th day of life the difference in the level of differentiation of the glands was more clearly apparent still, as reflected in the truly follicular structure of the glands in the experimental animals compared with the very small follicles of the healthy animals. Inflammatory changes in the experimental animals of this age were assessed at 1+ or 2+. The height of the thyroid epithelium was slightly reduced both in the experiment and in the control; resorption of the colloid was much more complete in the experimental animals, however, and it did not completely line the cavity of the follicles (Fig. 2).

By the 10th day the inflammatory changes were rather more severe (2+ or 3+) and the resorption vacuoles were very large. The difference in the level of differentiation of the thyroid gland tissue of the experimental and healthy rabbits persisted until the age of 14 days (Fig. 3). Starting from the age of 2 weeks, the lesions began to diminish and they disappeared completely on the 40th day of life, parallel with disappearance of antibodies from the blood of the young experimental rabbits.

It is considered that the lesions thus produced in the thyroid gland are the result of the action of early antithyroid antibodies transferred from mother to fetus.

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