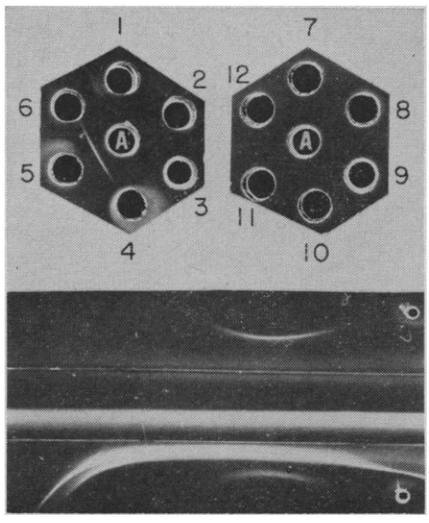


Fig. 1. (Top) Agar plate analysis showing the specific reaction of isolated Th anti-A antibody with antiserum to Th antibody (central wells A). Outer wells: 1, normal serum; 2, Fr II; 3, 4, high γ-globulin sera; 5, isolated Th anti-A antibody; 6–12, other isolated anti-A antibodies. (Bottom) Immunoelectrophoresis pattern of serum Th developed against antiserum to Th antibody. With the antiserum absorbed with normal serum, a single sharp line is visible, representing Th anti-A antibody. With the unabsorbed antiserum the long γ-globulin line is seen with the unique short band below it.

or γ-globulins described above. Only one anti-levan antibody was available for study. As in the case of Th anti-A this antibody could be detected in whole serum by immunoelectrophoresis. Individual specific antibodies were also obtained with one of two anti-dextran antibodies, as well as with one cold agglutinin and two anti-γ-globulins. The latter were 19*S* proteins. Four other anti-γ-globulin factors failed to show individual specificity. Three antinuclear antibodies failed to show specificity.

Starch-gel electrophoresis of a number of the antibodies described above, after reduction with mercaptoethanol in the presence of urea, indicated a possible association between the specific antigenicity and sharp banding of the L chains. Anti-A Th and anti-levan Ka showed particularly sharp lines.

The present studies demonstrate that, contrary to accepted opinion, individual antibodies will elicit secondary antibodies in the rabbit which are specific for the antibody. This was shown with four antibodies of the 7*S* class, including anti-A, anti-dextran, and anti-levan antibodies; and with three proteins of the 19*S* class. There is some possible question about the antibody nature of the latter three despite their specific reactivity, because in contrast to the first group of antibodies they were not produced by intentional immunization. It is of interest that two of the antibodies showing antigenic specificity came from the same serum.

Thus far, the specificity obtained was found directed only against the individual antibody used for immunization. No cross-specificity between a large group of anti-A antibodies from different individuals was obtained. However, it must be stressed that further work is necessary to settle this point, particularly since many of the anti-A antibodies studied appeared to be extremely heterogeneous. Evidence for this heterogeneity was obtained from a number of directions in addition to the gel electrophoresis experiments described; the ratio of group-specific antigens and the content of genetic factors approached that of whole γ-globulin and contrasted with the findings for more homogeneous antibodies such as anti-A Th and anti-levan Ka. The accumulated evidence suggests that all antibodies might well serve as specific antigens, provided that those directed against individual antigenic sites are selected out.

The localization of the individual antigenic specificity on the γ-globulin molecule is currently under study. The *S* fragment, obtained from papain- as well as pepsin-splitting of Th anti-A, contained all the specificity. This fragment is known to contain combining sites. It seems probable that previous data on the antigenic specificities of various myeloma proteins (*2*), Waldenström's macroglobulins (*3*), cold agglutinins (*8*), and certain other "monoclonal" γ-globulins (*4*) are directly applicable to the antibody problem. These antigenic differences are marked and readily demonstrable. Their exact relationship to the antibody sites and to the L chains (see *5*, *9*) remains to be determined (*10*).

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8 May 1963

## Antibody Formation in Embryos

Abstract. *The production of agglutinins to* Salmonella typhi *was studied in the opossum embryo in the period immediately before and after the development of thymic and lymph node lymphoid tissue. Antibody was found only in embryos older than 8 days which corresponds to an 8- to 10-week human embryo in terms of organogenesis and is the earliest stage at which antibody production has been recorded.*

Embryos and neonatal animals are commonly considered to be immunologically unresponsive (*1*, *2*). In fact, injection of antigen into newborn animals or embryos leads to immunological tolerance in adult life (*3*, *4*). Exceptions to this concept are formation of antibodies to a leptospira in bovine embryos (*5*) and to phage antigen in the neonatal opossum (*6*). The opossum has a gestation period of 12½ days and the newborn corresponds in development to an 8- to 10-week human embryo and to a 10-day rat or mouse embryo. These animals live in the maternal pouch until about the 60th day. Essentially then, they are embryos at and after birth, and organ development continues while they are in the pouch. Lymphoid tissue begins to appear in the thymus on the 2nd day, in the lymph nodes on the 5th or 6th day, and in the spleen on the 17th day (*7*).

Litters of opossums (*Didelphys virginiana*) were injected subcutaneously with single doses of antigen at varying times from the 6th to the 16th day. The antigen was a flagellar preparation from *Salmonella typhi* which is known to be a powerful antibody inducer (*8*). Each animal received a standard dose of 1.0 μg of antigen protein in 0.01 ml of saline. The animals were decapitated on the 7th, 9th, or 16th day after

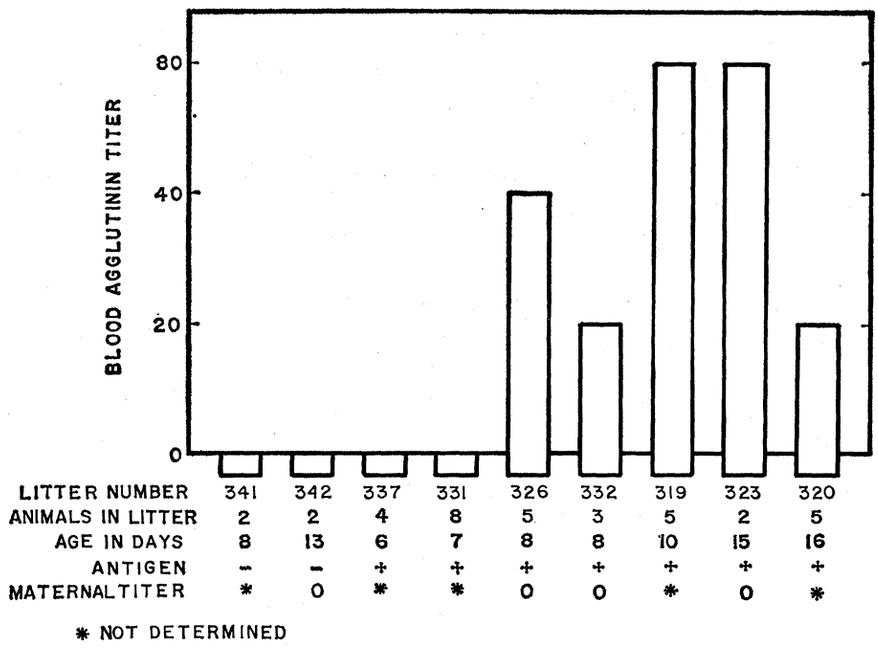


Fig. 1. Agglutinin titers of immunized and nonimmunized opossum embryos and of randomly selected mothers. Each bar represents the titer of a pool of heparinized blood from the number of embryos indicated. Titers of maternal serum are recorded in the bottom line of the figure. Each titer is expressed as the reciprocal of the highest dilution of serum in saline which gave a weak agglutination as judged by visual examination after resuspension. Serums giving no agglutination at the lowest dilution were considered negative.

injection, and heparinized blood from one litter was pooled to obtain a volume sufficient for agglutinin titration. Antibody titration was carried out by the method of Cannon *et al.* (*9*).

An antibody response was obtained when the antigen was injected on the 8th day or thereafter. No measurable titer was present in animals injected before the 8th day. To exclude the possibility that the antibody measured was of maternal origin, serums from mothers selected at random from both immunized and nonimmunized litters were taken for titration. Serums from noninjected opossum embryos were also titrated. All these controls gave negative results (Fig. 1).

These observations suggest that thymic or lymph node lymphoid tissue, or both, are required for an immune response to a flagellar antigen of *Salmonella typhi*. This phenomenon is particularly important in view of the role of the thymus in immunogenesis recently proposed (*10*). In the time covered by this experiment, namely between the 6th and 16th day, splenic lymphoid tissue, plasma cells, and secondary lymphoid nodules have not yet appeared in normal opossums (*7*).

Our work then extends previous work (*5*, *6*) to an earlier stage in embryonic development and indicates that antibody can be formed by embryos immediately after the appearance of lymphoid tissue in the thymus and in the lymph nodes. The observation that embryos can respond to an antigenic stimulus by forming antibody is thus emphasized and suggests these questions: (i) is this response accompanied by a proliferation of cells which normally develop only in more mature animals, (ii) if so, what cell types are involved (*11*)?

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## Endotrophic Mycorrhizae Influence Yellow Poplar Seedling Growth

Abstract. *Yellow poplar seedlings infected with endotrophic mycorrhizal fungi grow much faster than seedlings grown without mycorrhizae. A method of pot culture that uses natural soil structure provides an excellent means of studying growth differences due to microorganisms.*

Studies begun in 1959, designed to show effects of various soil factors on tree seedling growth, clearly indicate that endotrophic mycorrhizal fungi are important for the vigorous growth of seedlings of the yellow poplar, or tulip tree (*Liriodendron tulipifera* L.). Uninfected plants were small and chlorotic, but plants infected with the mycorrhizal fungi were large and vigorous.

The well-known ectotrophic mycorrhizae are comprised of a fungal sheath of plant roots and hyphal penetration between cortical cells. Endotrophic mycorrhizae do not form a fungal sheath and hyphae grow inside the cortical cells. Past research has shown that ectotrophic mycorrhizae are important in plant nutrition. However, work on the functions of the more common endotrophic mycorrhizae has been very limited. Mosse (*1*) in England found that inoculation of apple cuttings with an *Endogone* species from strawberry roots produced endotrophic mycorrhizal infection and generally greater growth. Baylis (*2*) used the soil under *Griselinia littoralis* (Cornaceae) to inoculate seedlings grown in sterile soil to show that trees infected with fungus producing vesicular-arbuscular mycorrhiza were bigger after 1 to 2 years' growth.

In the yellow poplar studies samples of undisturbed soil were taken in 1-gal tin cans driven into forest soil (*3*). The large plugs or cores in the tin containers served as excellent media for growing