fading seems to have been correct.

To determine quantitatively the production of ethylene, 12 freshly harvested, normal *Vanda* blossoms (approximately 24 g) were placed in a 500-ml wide-mouth Erlenmeyer flask maintained in a water bath at 25°C. A stream of humidified air was passed through the flask at the rate of 200 ml/min and bubbled into a solution of mercuric perchlorate for the manometric determination of ethylene (*4*). After an initial 1-hour collection period, the pollinia of the blooms were removed to cause premature fading, and sampling was continued for consecutive 60-minute intervals. The results of a typical test are shown in Table 1. Normal blossoms did not produce ethylene; however, about 15 hours after the pollinia were removed, ethylene was produced in a measurable quantity. The production then rapidly increased up to 32 hours, the time of the peak rate. In general, ethylene production was correlated with the degree of fading; the peak production occurred when 97 percent of the flowers had faded. The unusually high production of ethylene in these blooms is noteworthy, for the observed maximum value of over 3400 μl/kg hr is approximately 8.5 times greater than the peak production rate in the purple passion fruit, heretofore the highest reported for any plant material (*5; 6*).

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Individual Antigenic Specificity of Isolated Antibodies

Abstract. *Antisera produced against certain isolated human antibodies showed clear antigenic differences between these antibodies. Individual antigenic specificity was demonstrated for two anti-A antibodies, one anti-dextran, and one anti-levan antibody. Failure to produce specific antisera in other instances appeared to be correlated with a greater heterogeneity of the antibody population used as antigen.*

Numerous studies in the past have failed to show that isolated antibodies possess individual specificity as antigens. This work has been summarized in several reviews (*1*). However, observations from a number of laboratories have indicated that myeloma proteins and macroglobulins, with many of the characteristics of individual antibodies, do show antigenic specificity (*2, 3*). Recently, this work has been extended to include "monoclonal" γ-globulins appearing in smaller amounts in certain presumed normal individuals, and to certain antibodies (*4*). In view of these findings a reinvestigation of the antigenicity of isolated antibodies was undertaken, particularly since new evidence for a close similarity between myeloma proteins and isolated antibodies has arisen from a variety of studies including starch-gel electrophoresis after reduction in the presence of urea (5) and localization of genetic characters (*6*).

Anti-A antibodies were isolated from the serum of seven individuals who developed high titers after immunization with hog gastric A substance. Complement and natural anti-γ-globulin factors were removed by heating at 56°C for 30 minutes and absorption with aggregated γ-globulin. Specific precipitates formed at equivalence, with highly purified A substance, were washed and eluted with acetate buffer *p*H 3.8. After removal of residual precipitate, the supernatant was dialyzed rapidly against phosphate buffer, *p*H 7.5. These eluates were employed as antigens and were injected into rabbits primarily by the intraperitoneal route with complete Freund's adjuvants. Dextran and levan antibodies were prepared in a similar fashion from specific precipitates formed with the respective antigens. Subject Ka had produced antibodies to A substance, dextran and levan following immunization. Data concerning the antibody levels have been published (*7*).

Two of the seven anti-A antibodies produced individual, specific antibodies after injection into rabbits. The results with the antibodies from serum Th are representative. Three of six rabbits injected with Th antibody formed antibodies which still reacted strongly with the antigen after absorption with normal serum or normal γ-globulin. Figure 1 (top) illustrates this result. Antiserum against Th antibody, after absorption with normal serum, failed to react with Fr II γ-globulin, normal and high γ-globulin sera, or with any of seven other isolated anti-A antibodies. Many additional γ-globulins and other isolated antibodies were tested but only the Th antibody showed the specific reaction with the three different antisera to Th antibody.

These antisera to Th antibody also showed the antibody in whole Th serum. Figure 1 (bottom) illustrates an immunoelectrophoresis experiment demonstrating the sharp antibody line with both the absorbed and unabsorbed antiserum. The unabsorbed antiserum showed a strong reaction with 7*S* γ-globulin of the serum which is seen in the long bifurcated line. Clear identification of the sharp line as the anti-A antibody was obtained in two ways. First, absorption of Th serum with A substance completely removed the line. Second, immunoelectrophoresis experiments of Th serum with the antiserum in one trough and A substance in the other showed that the line with the rabbit antiserum corresponded exactly in position with the line obtained with A substance. Detailed studies of the antigenic character of isolated Th antibody demonstrated that it had the antigenic characteristics of ordinary 7*S* γ-globulin. Of particular significance was the finding that all the specificity could be precipitated by ordinary antisera to 7*S* γ-globulin and antisera to the F portion of 7*S* γ-globulin.

Antisera to five other isolated anti-A antibodies were also studied in detail. Only one of these showed similar individual specificity. This absorbed antiserum to Ka anti-A failed to react with Th anti-A or any other anti-A studied and reacted only with Ka antibody. Two antisera against other anti-A antibodies failed to show specificity despite the formation of extremely high titers of γ-globulin antibodies.

Two antisera to levan antibodies of Ka serum also showed strong individual specificity. These antisera, after absorption with normal serum, reacted only with the levan antibodies from this serum and not with the other antibodies

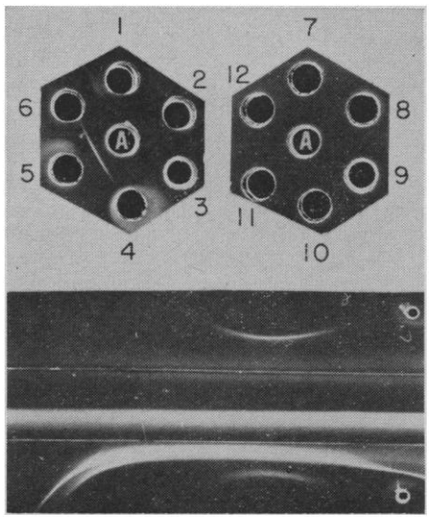

Fig. 1. (Top) Agar plate analysis showing the specific reaction of isolated Th anti-A antibody with antiserum to Th antibody (central wells A). Outer wells: 1, normal serum; 2, Fr II; 3, 4, high γ-globulin sera; 5, isolated Th anti-A antibody; 6–12, other isolated anti-A antibodies. (Bottom) Immunoelectrophoresis 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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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