long way toward harmonizing many discoveries of the developmental geneticist (focusing to a greater extent on innate endowment) with those of the experimental zoologist (focusing more largely on environmental adversity) in respect to causes of congenital malformations.

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2 July 1963

Fixation of Complement to Fragments of Antibody

Abstract. *A specific precipitate of ovalbumin and its rabbit-serum antibody, after fixing human serum complement, was digested with papain. The digest was analyzed immunochemically for complexes of antigen, antibody fragments, and components of complement. The results indicated that complement is not bound to Porter fragment III, but very likely is bound to fragments I and II.*

The amount of complement (C′) fixed by antibody-antigen complexes at or near equivalence is proportional to the antibody present. Some antibodies, however, fix complement poorly or not at all despite their precipitation by homologous antigen. Precipitation of antibody by antigen is therefore not a sufficient condition for antibody to fix complement, and it has been assumed that fixation depends also upon other structural characteristics of antibody. It became possible to obtain direct evidence relating antibody structure to complement fixation when Porter (*1*) showed that antibody digested with papain-cysteine yields three distinct fragments, and Nisonoff *et al.* (*2*) obtained by pepsin digestion two pieces, one of which consisted of Porter fragments I and II.

Taranta and Franklin (*3*), Amiraian and Leikhim (*4*), and Ishizaka *et al.* (*5*) examined the Porter and Nisonoff fragments for their capacity to fix C′ in the presence of antigen or to inhibit immune lysis of sensitized sheep erythrocytes and concluded that fragment III is the portion of the antibody molecule associated with complement fixation.

Our approach to the problem of determining which of the antibody fragments is required for complement fixation differs from theirs in that complement was fixed to an antibody-antigen system under normal conditions before digestion of antibody by papain-cysteine. The specific precipitate, having fixed complement, was digested and examined for complexes consisting of antibody fragments, antigen, and components of complement which might have remained bound. Results of experiments of this type led to the conclusion that complement is not bound to fragment III but is probably fixed to fragments I and II of the antibody molecule.

In our experiments we used ovalbumin and its rabbit-serum antibody as the antigen-antibody system, fresh human serum as the source of complement, and heat-inactivated human serum (56°C, 30 min) as control. Components of complement from heat-inactivated serum are not taken up by immune-specific precipitates (*6*). For this reason the rabbit antiserum was also heated at 56°C for 30 minutes. The order in which the reactants were mixed was antiserum, human serum (C′) or saline, and antigen. Each mixture contained 12.5 mg of antibody nitrogen, enough antigen to precipitate the antibody at equivalence, and 100 ml of fresh human serum, heat-inactivated serum, or saline. After 4 hours at 37°C and about 16 hours at 0°C, the specific precipitates were separated, washed thoroughly until the supernatants were free from inert constituents of rabbit antiserum and human serum, and digested with mercuripapain in the presence of cysteine and EDTA. The soluble papain digest was dialyzed against 0.01*M* acetate buffer (*p*H 5) and centrifuged. The water-soluble fraction, upon chromatography on carboxymethyl (CM) cellulose, yielded three fractions.

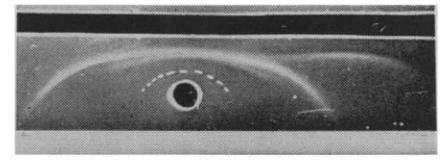

Fig. 1. Immunoelectrophoretic analysis of a saline-soluble extract for antibody fragments. The extract was from a specific precipitate containing C′. Sheep antirabbit serum was added to the trough. Electrophoretic migration was to the right toward the anode.

The insoluble fraction was taken up in 0.15*M* saline-veronal buffer (*p*H 7.4) giving the saline-soluble fraction. The original digest and its fractions were analyzed by gel-diffusion methods for antigens derived from rabbit anti-ovalbumin, ovalbumin, and human serum. Rabbit antisera to human serum were used for the detection of constituents of human serum. One was a polyvalent antiserum and the other was prepared against the fraction of human serum which does not contain γ-globulin. Both inactivated specifically the hemolytic activity of human serum and therefore contained antibodies against hemolytic components of complement. Sheep antirabbit serum was used to detect fragments of rabbit anti-ovalbumin. The digest was also tested for precipitating antibody against ovalbumin; none was found, indicating that the digestion of rabbit antibody had been complete.

The chromatographic patterns of the water-soluble fractions were identical whether or not the water-soluble fractions were derived from specific precipitates containing C′; three fractions were recovered from each. Fractions

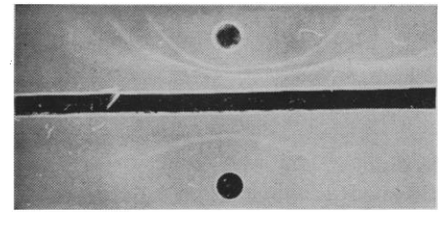

Fig. 2. Immunoelectrophoretic analysis for components of C′. The top well contained the same saline-soluble fraction used in the analysis in Fig. 1. The bottom well contained the water-soluble fraction of the digested specific precipitate formed in the presence of C′ from fresh human serum. Rabbit antihuman serum was added to the trough. Electrophoretic migration was to the right toward the anode.

I and II contained antibody fragments I and II, respectively; antibody fragment III was found in fraction III. Fractions I and II also contained ovalbumin that reacted with rabbit antibody to ovalbumin, most of it being in I; in fraction III none was evident by the precipitin method, although a trace was detected with the more sensitive complement fixation method. The ovalbumin was unquestionably bound to antibody fragments I and II because it migrated electrophoretically with these. An antigenic constituent of human serum was present only in fraction II obtained from the specific precipitate formed in the presence of fresh human serum, an indication that the antigen was a component of C′. Its electrophoretic mobility was the same as that of antibody fragment II, but it could not be concluded on the basis of this finding alone that C′ was bound to fragment II in the water-soluble fraction because the known antigenic components of C′ and fragment II normally have similar β-mobilities.

Analysis of the saline-soluble fraction from the precipitate formed in the presence of fresh human serum showed only antibody fragments I and II and constituents of human serum (Figs. 1 and 2). The presence of fragment III was excluded because no component with its characteristic slow electrophoretic mobility was ever found in it.

No constituents of human serum were present in the control preparations in which heat-inactivated human serum or saline had been used, an indication that the antigens of fresh human serum precipitated with antigen-antibody were components of C′. The concentrations of antibody fragments I and II were greater in the water-insoluble fraction from the preparation containing human C′ than in those with heat-inactivated C′ or saline. This result could have been and very likely was due to the fixation of water-insoluble components of C′ to antibody fragments; antigenic components of C′ were found predominantly in the water-soluble fraction (Fig. 2).

These results show that C′ is not bound to fragment III because fragment III was never found in any fraction containing antigens of fresh human serum fixed by the specific precipitate. Rather, the results suggest that components of C′ are complexed to fragments I and II. This conclusion is consistent with the very recent findings of Schur and Becker (*7*) that 5*S* antibody fragments of rabbit and sheep antibody, which apparently lack fragment III, do fix C′ significantly when washed specific precipitates are used.

Our conclusion that C′ is not fixed to antibody fragment III in the normal course of complement fixation may be reconciled with the observations that fragment III inhibits immune hemolysis and that heat-aggregated fragment III fixes complement by assuming that fragment III is primarily involved in a possible activation step which must take place before actual fixation of complement to antibody-antigen aggregates can occur. Alternatively, it may be that the action of papain-cysteine differs, depending upon whether the antibody is in its native state or is part of a specific precipitate, in which case it is possible that the portion of fragment III that fixes complement goes with fragment II or fragment I when the specific precipitate is digested (*8*).

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Dissociation of Homograft Response to Allogeneic versus Xenogeneic Skin Grafts in Irradiated Mice

Abstract. *Lethally x-irradiated (C3H × DBA/2)F₁ mice were protected with syngeneic (isogenic) bone marrow and/or prior treatment with urethan and skin grafted at various times after irradiation. The ability to reject a first-set xenogeneic (heterologous) skin graft with normal vigor returned within 92 days; the first-set response to allogeneic (homologous) grafts was still impaired 350 days after irradiation.*

Previous reports (*1*) have presented data demonstrating in lethally x-irradiated (C57L × A)F₁ mice, protected with syngeneic bone marrow, that the ability to reject a first-set allogeneic skin graft recovers from the effects of x-irradiation prior to the ability to reject a first-set xenogeneic skin graft. The converse has been found to be true with respect to (C3H × DBA/2)F₁ mice (designated C3D/2 F₁), and the data supporting this are herein presented.

These data are interpreted as representing positive evidence for the existence within the mammalian "immune system" of two physiologically distinct "lines" of immunologically competent cells which possess dissimilar functional capabilities. That is, the xenogeneic skin grafts were rejected by a cell system not capable of rejecting allogeneic grafts and vice versa. These two cell systems manifest widely disparate rates of recovery from the effects of radiation.

Twelve- to 16-week-old male C3D/2 F₁ mice were used as skin graft recipients. Skin graft donors were adult male C3D/2 F₁ ($H2^k$-$H2^d$), A/HeJ ($H2^a$), BALB/c ($H2^d$), and C57L ($H2^b$) mice, and 3- to 4-week-old male and female Sprague-Dawley rats. The orthotopic tail skin grafting method of Bailey and Usoma was used (*2*). Mean survival time of the grafts and standard deviation are reported. The mice were protected against the lethal effects of the radiation by means of the intravenous injection of 4 to 6 × 10^6 nucleated syngeneic bone marrow cells and/or by means of urethan (1 mg/g, intraperitoneally), given daily for the 2 days prior to irradiation (*3*). The mice received 870 rad whole body x-radiation [250 kv (peak), 15 ma; HVL 1.5 mm Cu; 30 rad/min] and were grafted with C3D/2 F₁, A/HeJ, BALB/c, and rat skin 0.2, 62, 92, 240, or 350 days after irradiation. Several groups were regrafted after the first-set of grafts had been rejected; in addition, a skin graft