ent in tumor extracts and serum probably are responsible for this failure to propagate virus serially in turkeys. Inasmuch as such inhibitory factors resemble antibody and are not demonstrable until two or more weeks after infection, a second attempt was made to establish the virus in young turkeys. In this experiment, tumors were initiated in turkeys with 10^6 pock-forming units of virus and the tumors were collected 8 to 14 days after inoculation. Tumor extracts were prepared as described above and were used as inocula in subsequent passage in turkeys. Under these conditions, a series of six passages of virus was successfully carried out, and the infective titer of tumor tissue from the sixth passage was $10^{-8.3}$. The latent periods for tumor production in each passage were less than 5 days.

The studies described in this report (*10*) indicate that inhibitory factors in tumor extracts and sera, time of collection of tumor tissue, and the infecting dose of virus employed are important factors contributing to the infective titer of tumor tissue. It seems clear that the presence of potent inhibitory factors in sera and in extracts of tumors produced in turkeys with large amounts of virus is responsible for the failure to demonstrate infective virus in such tumors. In addition, tumors produced with small amounts of virus also frequently yielded no infective virus demonstrable by the methods employed. In this instance it is of interest that potent inhibitory factors were not associated with the absence of infectivity. However, far less potent inhibitory factors were occasionally detected in tumor extracts but not in the homologous serum and vice versa. Inasmuch as tumors produced with small-to-moderate amounts of virus may reasonably be assumed to resemble naturally occurring viral neoplasia, a detailed study of their nature appears to be warranted.

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15 December 1958

Two Antigenically Different γ-Globulins in Domestic Rabbits Revealed by Isoprecipitins

Abstract. Isoprecipitins used in an agar-gel immunochemical analysis of 500 normal sera obtained from several breeds of rabbits show that the individual rabbits contain one or the other or both of two γ-globulin antigenic specificities in their sera but never lack both of them.

Recent investigations have shown that components of serum from individual rabbits are antigenic in other rabbits (*1, 2*). The isoantibodies were produced by subcutaneous injections of serum plus paraffin oil type adjuvants; they were detected by the precipitin reaction and by passive cutaneous anaphylaxis in the guinea pig (*1–3*). Isoprecipitins were found which reacted with serum antigens having electrophoretic mobilities corresponding to those of α-, β-, and γ-globulins (*2*). When the sera of 90 normal rabbits were tested with six isoimmune sera in agar-gel tubes, the 90 rabbits could be differentiated into 13 groups on the basis of the presence or absence of precipitin bands (*2*). These results and those of Oudin indicate that several components of serum may induce isoantibodies.

This report presents evidence concerning the number and incidence of the antigenically different γ-globulins in normal rabbit sera. The isoimmune sera were obtained from rabbits immunized with serum from individual normal rabbits as previously described (*2*). The agar-gel techniques employed were those of double diffusion in plates (*4*), microimmunoelectrophoresis (*5*), and a modification of Björklund's inhibition technique (*6*). For the purpose of inhibiting reactions of antiserum with a specific antigen in double diffusion experiments,

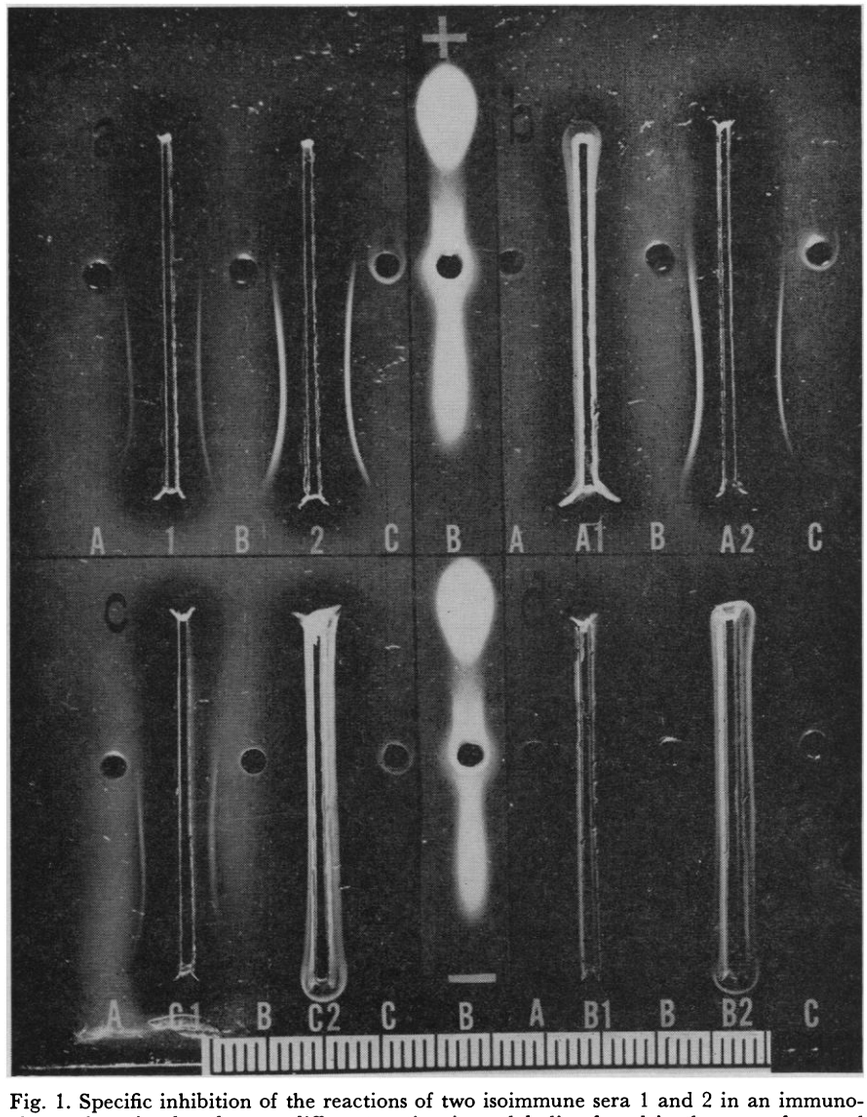

Fig. 1. Specific inhibition of the reactions of two isoimmune sera 1 and 2 in an immunoelectrophoretic plate by two different antigenic γ-globulins found in the sera of normal rabbits, A, B, and C.

the antigen solution was placed into the well or trough ½ to 1 hour prior to filling with the antiserum. This procedure permits the antigen to permeate the surrounding agar and provide an excess antigen zone of inhibition similar to that in Björklund's procedure, in which the antigen is incorporated into all of the agar (*6*). The procedure used is simpler and avoids heating the antigen, and less antigen needs to be employed.

Figure 1 is a photograph of the results of a typical microimmunoelectrophoretic experiment (*2, 5*). First, three undiluted normal rabbit sera, A, B, and C, were subjected to electrophoresis for 2½ hours under a field strength of approximately 3 v/cm in 1.5 percent (Noble) agar containing Veronal buffer at ionic strength 0.05 and *p*H 8.6. The troughs were then filled with 1:4 dilutions of sera in 0.15*N* NaCl as follows: no antigen in Fig. 1*a*, serum A in Fig. 1*b*, serum C in Fig. 1*c* and serum B in Fig. 1*d*. One hour later, when the troughs were practically empty, they were filled with isoimmune sera 1 and 2 (as indicated) which had been lyophylized and redissolved in distilled water to one-half the original volume. Twelve hours later the precipitin lines involving the γ-globulins were fully developed and photographed. The electrophoretic diagram of normal serum B obtained under the same conditions is superimposed on the results of double diffusion.

Figure 1*a* shows that isoimmune serum 1 has an antibody to a γ-globulin present in normal sera A and B; isoimmune serum 2, an antibody to a γ-globulin in normal sera B and C. Figure 1*b* shows that the previous filling of both troughs with normal serum A blocks the reactions of immune serum 1 but not that of immune serum 2. Also, the blocking reaction between normal serum A and immune serum 1 results in a precipitin line which encircles the trough. Similarly, Fig. 1*c* shows that the initial filling of both troughs with normal serum C blocks the reactions of immune serum 2 but not that of immune serum 1. Finally, Fig. 1*d* shows that all reactions are blocked by the previous filling of both troughs with normal serum B. Thus, the γ-globulin isoantigen in rabbit A has one type of specificity, hereafter designated as RGG-I, and the γ-globulin isoantigen in rabbit C has another type of specificity, designated RGG-II. Both types of γ-globulin specificities occur in rabbit B.

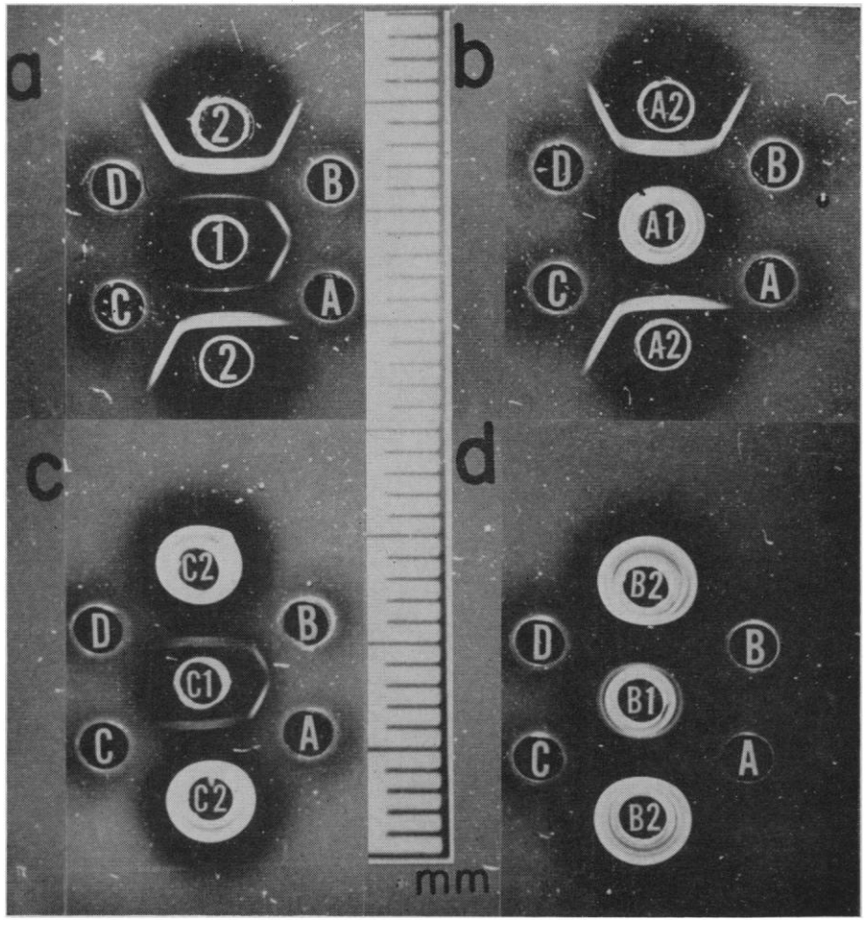

Fig. 2. Coalescence and specific inhibition of the precipitin lines resulting from the reactions between the sera of normal rabbits A, B, C, and D with the isoimmune sera 1 and 2 are used as criteria for identity or nonidentity of γ-globulin antigens in the various sera. Rabbit B was the donor to rabbit 1; rabbit D was the donor to rabbit 2.

Figure 2 is a photograph of the results of a typical double-diffusion experiment in agar-gel plates (*4*), including the inhibition technique described above. The antigens and antibodies were used in the same concentrations as in Fig. 1. The notations A1, A2, C1, C2, B1, and B2 indicate that the wells were initially filled with the normal rabbit sera A, B, or C and 30 minutes later were filled with isoimmune sera 1 or 2.

As in Fig. 1, Fig. 2*a* shows the precipitin lines between isoimmune serum 1 and normal sera A and B and shows those between isoimmune serum 2 and normal sera B and C. The precipitin rings around the wells in Figs. 2*b*, 2*c*, and 2*d* show the selective blocking of these reactions by prefilling the antisera wells with sera A, C, and B, respectively. Moreover, as is shown in Fig. 2*a*, the reaction between immune serum 1 and immune serum 2 results in two precipitin lines. The line closest to the well containing immune serum 1 represents the reaction between the antibody from this well and the RGG-I present in immune serum 2. Similarly, the line closest to the well containing immune serum 2 represents the reaction between the antibody from this well and the RGG-II present in immune serum 1. This follows from the fact that these two lines coalesce with the corresponding lines resulting from the reactions of the immune sera with normal sera A, B, C, and D. The results of the inhibition procedure in Figs. 2*b*, 2*c*, and 2*d* also indicate that the coalescing lines represent identical antigen-antibody systems.

In order to pursue the question as to the number of such γ-globulin antigens in rabbits, the sera of 500 rabbits of several breeds (New Zealand white, Chinchilla, Dutch, Flemish giant, Race III, ACCR line, New Zealand red, and mixed breeds) and from three sources (NIH, Roscoe B. Jackson Memorial Laboratory, and local commercial breeders) were tested for the presence of RGG-I, and RGG-II; 24 rabbits were found with only RGG-I, 379 rabbits with only RGG-II, and 97 rabbits with both RGG-I and RGG-II in their sera. No rabbits were found to lack both γ-globulin specificities. All the possible "cross immunizations" among the above three known groups of rabbits have as yet (in 35 rabbits) failed to reveal a third γ-globulin specificity.

Quantitative studies are in progress to determine the proportion of the γ-globulin molecules in rabbit sera which have the specificity of RGG-I or of RGG-II

with respect to the isoimmune sera and what proportion of the molecules may have neither specificity. Also, it will be interesting to determine whether in such rabbits as B, the specificities of RGG-I and RGG-II are on the same or different molecules (*7*).

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22 October 1958

Development of a Chick Embryo Heart Cell for the Cultivation of Poliovirus

Abstract. An epithelial-like cell has been developed in line culture that apparently is stable. Although initially isolated cells were incapable of supporting the growth of poliovirus, the cells of the sixth and later passages allowed virus to propagate. The early, nonsusceptible cells were fibroblastic in appearance, in contrast to the epithelial type, poliovirus-susceptible, derived cell of later passages.

The use of cells in tissue culture for the propagation of poliovirus has been limited until recently to cells of primate origin. In 1957, Westwood, Macpherson, and Titmuss (*1*) developed a cell in line culture from embryo rabbit kidney. This cell was shown, by Sheffield and Churcher (*2*), to be capable of supporting the growth of the three types of poliovirus. Subsequently, Drew (*3*) and McCarthy and Tytell (*4*) reported the growth of poliovirus in other line cultures derived from rabbit kidney. Dunham and Ewing (*5*) demonstrated that cells derived from the chorioallantoic membrane of chick embryos were susceptible to the three types of poliovirus after five serial passages. Although it seems clear that cultures other than those of primate origin are capable of acquiring susceptibility to poliovirus when carried in passage, there may be an inclination by some to explain the susceptibility to poliovirus on the basis of contamination of the nonprimate cultures with cells of other susceptible lines. The work described in this report was conducted under conditions designed to minimize the possibility of such contamination.

This study concerns the development of a cell line from trypsinized chick embryo hearts which is capable of supporting the growth of poliovirus. Particular care was taken to insure against the possibility of contamination by other cell lines at all steps of the procedure. Trypsinized preparations were made in an area not used for other line cell work. Particular care was taken to limit the possibility of bacterial contamination of the various cell passages. The cultures were examined for contamination by pleuropneumonia-like organisms, with negative results.

The hearts were removed from 15- to 19-day-old chick embryos and placed in 100 ml of Hanks' balanced salt solution in a 250-ml flask. Approximately 45 hearts were used in each batch. A magnetic bar was placed in the flask and agitated in a magnetic field for 20 minutes. The fluid was poured off and replaced with an equal amount of 0.25-percent trypsin (Difco 1–250). Agitation was resumed for 2 to 3 hours, at which time the fluid was poured off and centrifuged at 1000 rev/min for 5 minutes; the pellet was then resuspended in medium 199 (*6*) containing 10 percent calf serum and adjusted to *p*H 6.8. Cells were planted in roller tubes at a concentration of 600,000 per tube, as counted in a hemocytometer.

Figure 1 shows fibroblastic-like cells isolated in the first passage of culture, and Fig. 2 shows epithelial-like cells which appeared in the fifth passage of culture. As transfers progressed, the line cells throughout the entire sheet were consistently of this type. Usually each passage was incubated for 7 to 10 days. The first four passages had as their typical and predominant cell an elongated fibroblast-like structure. In each passage, however, a few epithelial-like cells were present, and these increased in number with each transfer until at the fifth passage the culture consisted of approximately half fibroblastic and half epithelial cells. At the sixth passage most of the cells were epithelial, and at the seventh, a complete epithelial sheet was seen.

The three antigenic types of poliovirus were placed on chick heart cells of the first and sixth passages. No replication of virus was obtained on the first transfer, but on the sixth transfer all three virus types were propagated, yielding titers of 5.6, 5.5, and 5.2 for types I, II, and III, respectively, as determined by a metabolic inhibition test (*7*) with monkey kidney tissue. Virus produced by these cells was identified specifically by the complement-fixation procedure described by Mayer *et al.* (*8*).

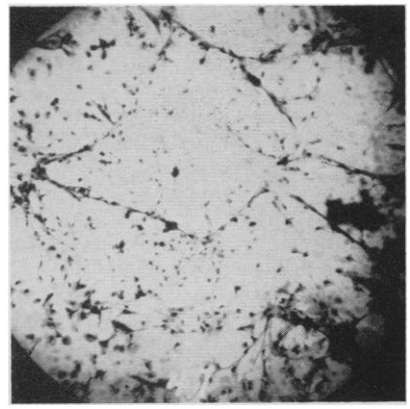

Fig. 1. Chick embryo heart cell, first passage (Giemsa stain). (About × 94)

It has been shown that several line cell cultures from various mammalian sources have a common antigen, as determined by complement-fixation techniques (*9, 10*). A. A. Tytell of the Merck Sharp and Dohme research laboratories (*11*) has examined the chick embryo heart cells and has found that those permitting poliovirus replication contained the common antigen, whereas the early passage strains resistant to poliovirus did not have the common antigen. Several explanations have been offered regarding this observation (*10*), and projects under consideration for the use of cells containing the common antigen for the production of virus vaccines must be deferred until the significance of the common antigen is understood.

It is of interest that a tissue culture series, originating with heart tissue, in which the cell type appears almost entirely fibroblastic has yielded an epithelial culture. The cell type has been obtained on two occasions from four attempts. One line now has been main-

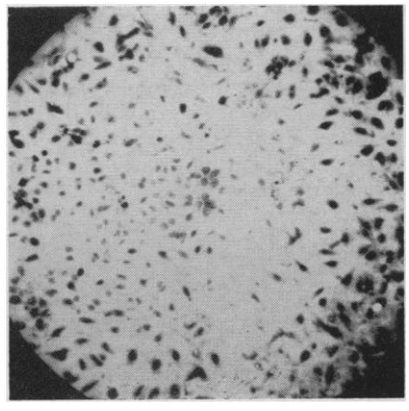

Fig. 2. Chick embryo heart cell, fifth passage (Giemsa stain). (About × 94)