Reports

Factors Contributing to Production of "Virus-Free" Tumors in Turkeys by Rous Sarcoma Virus

Abstract. Virus-neutralizing factors in tumor extracts and sera, age of the tumor, and the infecting dose of virus are important factors contributing to the infective titer of tumor tissue. Tumors produced with small amounts of virus may contain no demonstrable infective virus, and detectable inhibitory factors may or may not be present in tumor extracts or sera.

Bryan, Calnan, and Moloney (*1*) observed that the amount of infective virus obtained from chicken sarcomas was related to the infecting dose (ID) of virus. Indeed, when less than 1 ID_{50} of virus was employed, about 24 percent of such tumors yielded no virus at all. Similarly, when chicks were inoculated intracerebrally with less than 1 LD_{50} of virus, brains removed from the relatively few chicks that died contained no infective virus in four of five instances (*2*). Recently it has been found that sarcomas produced in turkeys with as much as 10,000 ED_{50} of chicken tumor virus yielded little or no extractable virus despite the fact that the dilution end point for tumor production by chicken tumor virus was identical in chicks and turkeys (*3*).

In the studies described here Beltsville white turkey poults 3 to 5 days of age were infected subcutaneously by injection of 0.2 ml amounts of suitably diluted virus into the wing web. Standard chicken tumor virus prepared by differential centrifugation (*4*) was used as seed virus in all experiments. Infectivity titrations were carried out in embryonated chicken eggs, by a modified pock-counting technique (*5*). Serial decimal dilutions of the inocula were injected onto the chorioallantoic membrane of groups of five eggs each, and after 10 days of incubation at 38°C the membranes were examined. Tissue homogenates for assay in eggs were prepared by grinding the tissue in a mortar with Alundum and adding sufficient diluent (*2*) to make a 10 percent suspension by weight, which was then clarified by centrifugation at 2000 rev/min for 20 minutes. When such homogenates were used for serial passage in turkeys, each suspension was subjected to additional centrifugation (*1, 6*) to insure the absence of intact tumor cells in the inoculum.

It has recently been shown (*7*) that extracts of turkey sarcomas produced with chicken tumor virus contain a potent inhibitor and that this inhibitor was stable to 56°C for 30 minutes. This inhibitor rapidly neutralized large quantities of Rous sarcoma virus but did not affect fowl pox, Newcastle, bronchitis, or influenza viruses. The data in Table 1 show that a potent inhibitor may also be present in the serum and that both the presence of inhibitor and the infecting dose of virus exert a marked effect on the infective titer of the tumor. Individual birds were selected to illustrate the limits of variation observed to date. In all experiments, extracts of turkey tumor tissue and serum were heated to 56°C for 30 minutes before use. Neutralization tests were performed in the usual manner by mixing serial decimal dilutions of tumor extract or serum with 500 pock-forming units of virus and allowing the mixture to incubate for 30 minutes at room temperature before inoculation into groups of embryonated eggs. The data show that: (i) when large amounts of virus were employed there was an inverse relationship between the infective titer of the tumor tissue and the inhibitory titer of heated tumor extract and serum and (ii) when small amounts of virus were employed, little or no infective virus was present in tumor homogenates. However, the sera from such birds contained little or no inhibitor, although inhibitory factors were occasionally detected in low titer in tumor extracts. It is of interest to recall the observation of Carr (*8*) that slowly growing tumors induced in brown Leghorn chickens yielded noninfective filtrates, provided that the tumors selected had been growing for more than 40 days. Subsequently Carr reported (*9*) that such tumors contained an inhibitor which was associated with serum antibody, and he suggested that the amount of antibody contained in the tumor was sufficient to inactivate all of the virus that could be obtained from the tumor cells.

Previous studies (*3*) have shown that serial passage of virus in turkeys was associated with a progressive loss in infectivity with each passage until the fourth passage, when extracts of such tumors were noninfective. Our data (Table 1) suggest that the inhibitory factors pres-

Table 1. Selected examples of variations in infective titers of tumors produced in turkeys by Rous sarcoma virus.

Bird No.	Infecting dose of virus (PFU)*	Time after infection (day)	Size of tumor†	Infective titer (log)	Neutralization titer	
					Tumor extract‡ (log)	Serum‡ (log)
130	10^6	12	++	−7.8	< −1.0	< −1.0
4911	10^6	22	++	−5.3	−2.0	−2.0
4941	10^6	22	++	−2.1	−4.0	−4.0
254	10^6	33	++	< −1.0	−3.0	−4.0
229	10^8	21	++	−4.6	−2.0	−2.0
320	10^8	38	+++	< −1.0	−3.0	−4.0
5996	1	25	+	< −1.0	< −1.0	−1.0
169	1	42	+	< −1.0	−2.0	< −1.0
5993	1	42	++	< −1.0	< −1.0	< −1.0

* PFU, pock-forming units. † Size: +, 6 to 8 mm in diameter, confined to wing web; ++, 9 to 15 mm in diameter, with frequent invasion of adjacent muscle; +++, 6 to 30 mm in diameter, with extensive invasion of surrounding tissues. ‡ Tumor extracts and sera heated at 56°C for 30 minutes.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].

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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References and Notes

1. W. R. Bryan, D. Calnan, J. B. Moloney, *J. Natl. Cancer Inst.* 16, 317 (1955).
2. V. Groupé and F. J. Rauscher, *ibid.* 18, 507 (1957).
3. ———, *Science* 125, 694 (1957).
4. W. R. Bryan, J. B. Moloney, D. Calnan, *J. Natl. Cancer Inst.* 15, 315 (1954).
5. V. Groupé, V. C. Dunkel, R. A. Manaker, *J. Bacteriol.* 74, 409 (1957).
6. V. Groupé, F. J. Rauscher, W. R. Bryan, *J. Natl. Cancer Inst.* 19, 37 (1957).
7. F. J. Rauscher and V. Groupé, *Federation Proc.* 17, 532 (1958).
8. J. C. Carr, *Brit. J. Exptl. Pathol.* 24, 133 (1943).
9. ———, *ibid.* 25, 56 (1944).
10. These studies were aided by a grant from the American Cancer Society.

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,

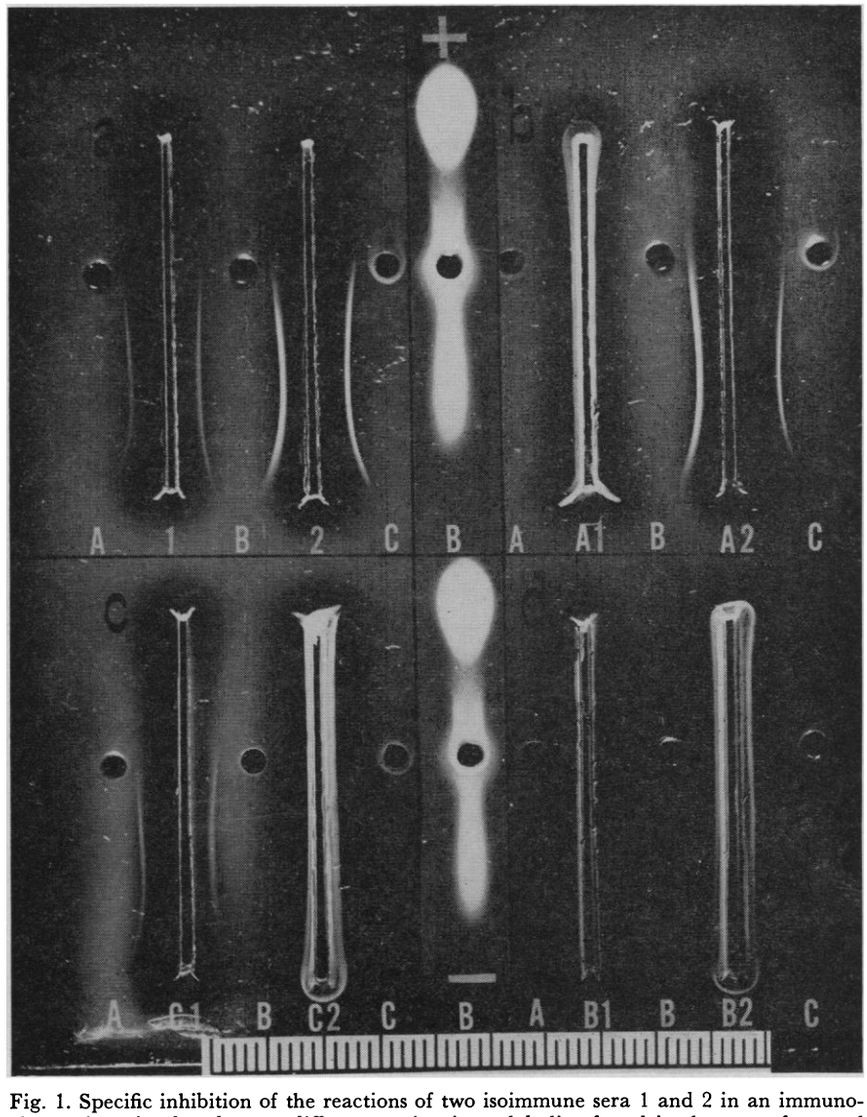

Fig. 1. Specific inhibition of the reactions of two isoimmune sera 1 and 2 in an immunoelectrophoretic plate by two different antigenic γ-globulins found in the sera of normal rabbits, A, B, and C.