Table 3. Relation between antigen in the bloodstream and anticomplementary activity. Serum samples were from a patient who developed hepatitis after a transfusion. Samples were obtained at weekly intervals. Antigen was measured by agar-gel precipitation and by quantitative CF. The CF titer is the reciprocal maximum dilution of serum that fixed more than 2 units of C in the presence of optimum antibody. The patient's serum alone at 1:20 dilution fixed amounts of C listed as anticomplementary (AC) activity. The amount of antigen added to reverse AC activity was approximately 15 times the amount of optimum antigen for an amount of antibody capable of fixing 8 units of C at an optimum ratio of antigen to antibody (that is, sufficient antigen to cause less than 1 unit of CF as shown in Fig. 2). The source of antigen was highly antigenic serum (titer, 1 : > 10,000 by microtiter CF) from patients with serum or infectious hepatitis, or from nonhuman primates, as well as antigen purified on CsCl gradients. The amount of antibody added to reverse AC activity was 0.15 ml of the antibody with the highest titer (shown in Fig. 1), per 0.4 ml of mixture. Antigen or antibody was added to AC serum and incubated for 2 hours at 37°C or for 4 hours at 24°C before C was added.

Time after exposure (weeks)	Serum antigen + antibody		Serum AC activity (units)			
			Serum alone	Serum + excess antigen		Serum + excess antibody
	Agar gel	CF titer		Incubated before CF	No inc. before CF	Incubated before CF
3	0	0	0	0	0	0
4	0	0	6.0	0	4.5	1.5
6	0	0	7.5	0	6.5	2.8
8	0	40	2.1	0	1.8	
12	+	640	0	0	0	
16	+	320	1.5	0	0	
19	0	160	1.8	0	1.5	
21	0	0	7.5	0	6.5	2.5
28	0	0	7.4	0	7.0	3.2
32	0	0	4.9	0	3.0	2.0
36	0	0	1.5	0	1.0	0

that contains virus-like particles of antigenic serums. Three high-titered AC serums that were examined by electron microscopy after sedimentation on CsCl gradients, contained virus-like particles which were anticomplementary. These particles appeared to have an outer coat about 2 nm thick, which could be removed by extraction with dichlorodifluoromethane (*5*). This fluorocarbon removes antibody from neutralized virus, with the virus being in the aqueous phase and precipitated antibody in the organic phase (*13*). Extraction of AC serum for 2 hours at room temperature by Vortex mixing with an equal volume of fluorocarbon decreased or eliminated AC activity and often doubled the antigenic activity detectable with added antibody. Thus, hepatitis virus in complex with antibody appears to be responsible for AC activity.

Further evidence that antigen-antibody complexes account for AC activity is the ability of excess antigen to reverse it. Reversal was a time-dependent reaction, the rate being inversely proportional to the amount of antigen added. For example (Table 3), amounts of antigen that completely eliminated AC activity when incubated with AC serum for approximately 2 hours at 37°C or 4 hours at 24°C before addition of C, only slightly reversed AC activity if added immediately before C. Most antibodies used in Fig. 1 showed a "prozone" phenomenon of inhibiting CF at high ratios of antibody to antigen; and these antibodies in high concentration reversed AC activity as did excess antigen. Serums from normal individuals and from hepatitis patients without demonstrable antigen or antibodies in the blood did not reverse AC activity. Free antibody was detected much less frequently than AC activity. Complement fixing antibody could be demonstrated in only 3 of 22 hepatitis cases on whom weekly serum samples were obtained during the month after disappearance of antigen in the blood. None of the patients developed antibodies that precipitated antigen in agar gel (*2*). Thus it appears that CF antibody in most cases of hepatitis circulates while bound to antigen and manifests its presence as AC activity. Conversely, AC activity reversible with specific antigen or antibody appears to be as indicative of viremia as measurement of free antigen.

Complement-fixation techniques provide a valuable diagnostic test for hepatitis and lend themselves to screening large numbers of blood donors to exclude carriers of hepatitis virus. Screening tests must include procedures for detecting not only free antigen, but also the AC activity of antigen-antibody complexes that can be reversed with specific antigen or antibody. Antigen in the blood may be confirmed by electron microscopy (*5*).

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Radiation Leukemia Virus: Quantitative Tissue Culture Assay

Abstract. *Radiation leukemia virus does not propagate in tissue cultures from either Swiss or C57BL mouse embryos, but it does augment focus formation by the defective Moloney leukemia pseudotype of murine sarcoma virus in Swiss mouse cells and thus can be quantitatively assayed.*

X-irradiation of C57BL mice, which have a low spontaneous incidence of leukemia, induces lymphomas from which a leukemogenic virus (Rad LV) and viral-specific antigen can be consistently recovered (*1*). The host range, the ensuing pathology, and the thymic

dependency of Rad LV have been studied (*2*). A complex procedure for preparing a murine sarcoma virus (MSV) of the Rad LV pseudotype (Rad LV-MSV), involving Rad LV infection of cocultivated C57BL mouse cells and MSV-induced but nonvirus shedding hamster tumor cells, has been reported (*3*). Rad LV-MSV can form foci of morphologically altered cells in cultures of C57BL cells but not in cultures of Swiss mouse cells; it appears to have only a limited capacity for further propagation of C57BL cells. In contrast, Rad LV alone, unlike other murine leukemia viruses, cannot propagate in either C57BL or Swiss mouse tissue culture cells, and quantitative assays in vivo of Rad LV require a minimum of several months (*3*, *4*).

We have reported a quantitative 6-day tissue culture assay method for a variety of murine leukemia viruses; this method depends on their capacity to promote formation of foci in mouse cell cultures infected with defective MSV (*5*). We now report that Rad LV can promote focus formation in Swiss mouse embryo fibroblast (MEF) cells infected with defective MSV of the Moloney leukemia virus pseudotype (MLV-MSV), and can thus be quantitatively determined by the helper assay.

Swiss or C57BL/6 mouse embryo fibroblast secondary cultures were prepared according to the protocol of the helper assay (*5*) and were then inoculated with 0.2 ml of a dilution of defective MLV-MSV and simultaneously inoculated with 0.1 ml of serial dilutions of MLV or Rad LV. The MLV-MSV was obtained as a second-day viral harvest from infected 3T3 mouse cells; its focus titration pattern indicated that it contained MSV only in its defective state (*6*). This indicator MSV stock also contained an excess, approximately 100-fold, of endogenous MLV as determined by the induction of helper activity in mouse embryo fibroblast cells inoculated with virus dilutions beyond the sarcoma focus-inducing endpoint. The MLV used as helper virus in our experiments consisted of a 10 percent extract of a Swiss mouse lymphoma induced by MLV originally isolated from 3T3 cells infected with the Moloney sarcoma-leukemia virus complex (*7*). The Rad LV stock was prepared as an extract of C57BL/Ka mouse lymphoma tissues in 20 percent phosphate-buffered saline (*8*).

The dilution of defective MLV-MSV was adjusted so as to give an average of 2.7 foci per dish when plated on Swiss MEF cells. Coinfection of the cultures with the optimum dilutions of MLV yielded between 60 to 80 foci per dish. A curve of the increase in foci registering as a function of dilution of the MLV virus stock (Fig. 1) followed a pattern characteristic of the helper assay and consisted initially of a region of less than maximum expression of foci, then a plateau region of maximum expression of foci, and finally a region of linear decline of focus expression to the background of the indicator MLV-MSV alone. Identical infection with serial dilutions of the Rad LV on Swiss mouse embryo fibroblast cells gave a generally similar pattern except that the increase in numbers of foci given by the plateau dilutions of Rad LV consisted of 15 to 25 foci per dish. The value of the increase in the number of foci at zero dilution obtained from this curve indicated the presence of 6.7×10^7 leukemia virus helper units per milliliter in the Rad LV stock according to the standard formulation of helper activity (*5*).

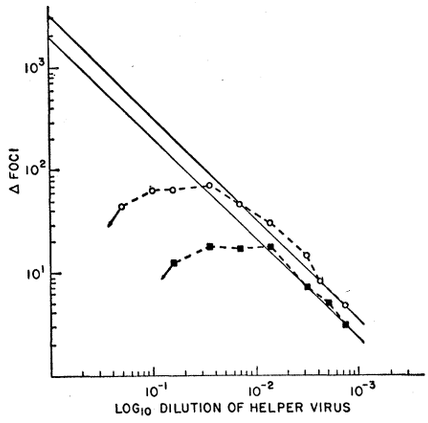

Fig. 1. Helper assay of MLV (○——○) and Rad LV (■——■) in Swiss mouse embryo fibroblasts cells. Both leukemia viruses were in the form of extracts of lymphomatous organs. The indicator virus was 3T3 cell-derived defective MLV-MSV. At the single dilution of the indicator virus employed, 2.7 foci per dish were present if no helper was added.

Because the difference in the numbers of foci registering on Swiss mouse embryo fibroblasts with Rad LV was not as pronounced as with MLV, we explored the use of several other dilutions of the indicator MLV-MSV. Figure 2 illustrates the infection of Swiss mouse embryo fibroblast cells with Rad LV and two different twofold dilutions of the indicator MLV-MSV. Use of different indicator stocks does yield differences in the increases in the number of foci for zero dilution. However, these differences are compensated for by the values of the maximum number of foci obtained with each indicator so that the final calculation of helper activity (5.8×10^7 leukemia virus helper units) yields an essentially unaltered value.

The difference in the capacity of Rad LV and MLV to promote focus formation in Swiss cells infected with defective MLV-MSV could arise from a difference in the relative absorption of both leukemia viruses to the cells. Accordingly, we exposed similar Swiss mouse embryo fibroblast cultures to 1×10^6 leukemia virus helper units of MLV and 1×10^6 of Rad LV. After the standard absorption we assayed the unabsorbed virus on Swiss mouse embryo fibroblast cells; in each case it amounted to 40 percent of the input virus.

Igel and colleagues reported that MLV-MSV preparations showed equal infectivity in either Swiss mouse embryo fibroblast or C57BL cultures (*3*), but the nature of the MSV employed

Table 1. Titration of defective and partially competent MSV-MLV in mouse embryo secondary cultures. When added, the quantity of MLV was 2×10^5 helper units.

Dilutions of tumor-derived MSV-MLV	Effect on mouse embryo fibroblasts			
	Swiss		C57BL	
	By itself	With MLV added	By itself	With MLV added
		Defective		
1 : 500	1.7×10^5	Confluent	3.1×10^4	1.2×10^5
1 : 2000	8.1×10^4	1.14×10^6	1.7×10^4	1.3×10^5
1 : 8000	2.0×10^4	1.28×10^6	4.1×10^3	1.4×10^5
		Part competent		
1 : 400	4.8×10^4	2.5×10^5	2.3×10^4	4.8×10^4
1 : 1600	6.3×10^4	3.5×10^5	1.5×10^4	4.0×10^4
1 : 6400	5.5×10^4	2.9×10^5	3.2×10^4	3.2×10^4

was not defined in terms of defectiveness, competence (*6*), and focus potentiation by optimum amounts of exogenous murine leukemia virus. We have examined the infection of both Swiss MEF and C57BL MEF cultures with completely defective and partly competent stocks of MLV-MSV, either alone or with concomitant infection with optimum helper levels of exogenous MLV. Our data (Table 1) would suggest that the capacity of C57BL cells for expression of foci after infection with MLV-MSV is only a fraction of that of Swiss MEF cells under similar physiological conditions. Focus assays of the unabsorbed virus indicated that both types of cell cultures had similar capacities for viral absorption.

We explored the possibility that C57BL cells could better register Rad LV helper activity despite their lower efficiency for focus formation by MLV-MSV. We titrated MLV and Rad LV in C57BL cells with defective MLV-MSV but at a concentration of the indicator virus four times higher to compensate for the lower focus expression. The C57BL cells permitted a meaningful helper assay of MLV, but the plateau of maximally expressed foci was only about 20 percent of that observed with the same indicator virus in the Swiss MEF cells (Fig. 3). However, calculation of the helper activity with the experimentally obtained values for the maximum and the increases in the number of foci at zero dilution, yielded a value (4.3×10^7) not significantly different from that obtained on mouse embryo fibroblast cells. Surprisingly, Rad LV was essentially nonfunctional as a helper in the C57BL cells with MLV-MSV. Use of other concentrations of the indicator MLV-MSV did not affect this result.

We have used the helper assay to demonstrate propagation of the Rauscher (RLV) and Moloney leukemia viruses in various murine cells (*7*, *9*). We now examine possible propagation of Rad LV alone in either Swiss or C57BL/6 embryo cells. Secondary cultures were infected with a multiplicity of input of 1 or 10 leukemia virus helper units per cell. After an incubation for 1.5 hours the cells were washed twice, maintenance medium was replaced, and the cultures were then incubated at 37°C in a moist atmosphere of 5 percent CO_2 and air. On subsequent days, up to 18 days after infection, plates of cells were collected. No detectable helper

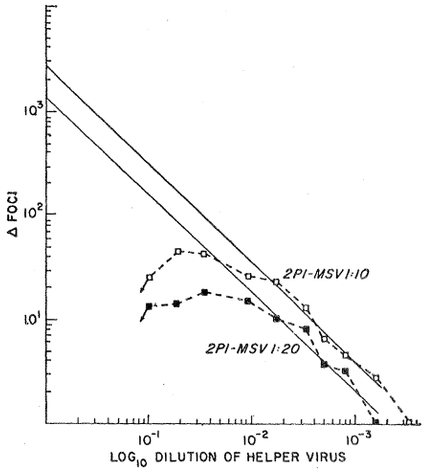

Fig. 2. Helper assay of Rad LV in Swiss mouse embryo fibroblasts cells with two dilutions of defective MLV-MSV—designated (2P1-MSV). Without helper virus a 1:20 dilution of 2P1-MSV showed an average of 3.2 foci per dish. The plateaus of expressed foci subsequent to sufficient quantities of Rad LV additions were 23 and 49 foci per dish for a 1:20 and 1:10 dilution of 2P1-MSV.

activity developed in Rad LV infected cells, whereas MLV and RLV infected cells developed high helper activity.

The existence of a murine leukemia virus which could not replicate alone but which could function as a biological helper was puzzling since helper activity had always been associated

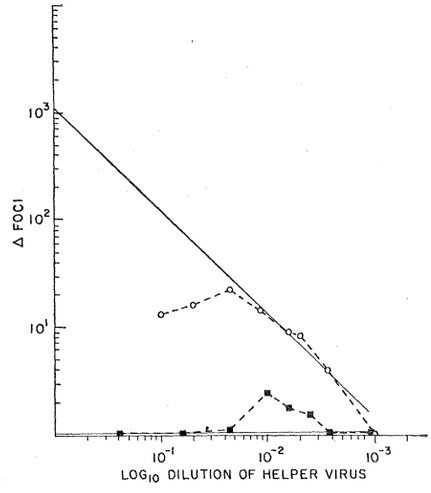

Fig. 3. Helper assay of MLV (○——○) and Rad LV (■——■) in C57BL/6 MEF cells. The source of defective MSV is that used for previous assays which in C57BL cells gave an average of 1.5 foci per dish and in Swiss cells 10 foci. An optimal dilution of MLV which gave a plateau value of about 22 foci in the C57BL/6 cells gave, under identical assay conditions in the Swiss mouse embryo fibroblasts system, foci too numerous to count.

with multiplying helper virus. To clarify the physical nature of Rad LV helper entity we subjected MLV and Rad LV of equal helper-unit content to similar purification procedures. In the case of both viruses, low-speed centrifugation (5000*g*, 15 minutes) left essentially all helper activity in the supernatant, whereas a subsequent high-speed centrifugation (51,000*g*, 45 minutes) deposited more than 90 percent of the helper activities as a pellet. We resuspended the pellets, layered them on identical phosphate-buffered saline, sucrose density gradients (1.004 to 1.250 g/cm^3), and centrifuged them at 105,000*g* for 3.5 hours. We collected fractions by side puncture at various densities for the determination of density and biological activity. In both tubes containing MLV or Rad LV, the peak of helper activity corresponded to the buoyant density region of 1.15 to 1.18 g/cm^3. Thus in each case the helper activity was a function of the respective virions. In the course of the helper assay of the supernatant from low speed centrifugation, the resuspended pellet and the buoyant density region at 1.16 g/cm^3 of Rad LV, the same plateau of maximum foci expression was observed. Thus, it appears unlikely that this lower focus expression on Swiss embryo cells as compared to other murine leukemia viruses arises from the action of a nonvirion inhibitor. Some stocks of RLV also showed a lower plateau value in promoting maximum focus expression as compared to the MLV used in these experiments, but the difference was small compared to the depression in the plateau value observed in experiments with Rad LV. The factors underlying the intrinsic capacity of a given murine leukemia virus for maximum focus expression in a given cell type are undefined.

The appearance of foci formed by cooperative infection of Swiss embryo cells by MLV-MSV and Rad LV was indistinguishable from that of foci formed with MLV-MSV and other murine leukemia viruses. Although focus formation by MSV is generally concomitant with release of infectious progeny virus we directly tested for progeny virus. Plates containing Swiss embryo secondary cultures were inoculated with a dilution (1 : 20) of completely defective MLV-MSV to yield an average of 1 focus alone and 7.5 foci on coinfection with a fixed optimum dilution of Rad LV. A 1 : 100 dilution

of the indicator MLV-MSV on plating alone yielded no foci but on plating together with the fixed dilution of Rad LV yielded 2, 1, 1, 0, and 2 foci, respectively, on five separate plates. The probability was high that these foci arose solely from cooperative infection of the MLV-MSV and Rad LV. On day 6 after infection, the cells were frozen and thawed and the cell-free fluids from individual dishes were tested for focus-forming capacity. Each of the five dishes which contained foci yielded enough virus to ensure confluent focus transformation on plating on either Swiss or C57BL mouse embryo cells.

The finding of nonreplicating, virion-associated helper activity in Rad LV stocks emphasized the potential existence of defective murine leukemia viruses whose biological activity could become apparent only after coinfection of a leukemia virus–infected cell with sarcoma virus (*3*). We have observed a degree of synergism between MLV-MSV and other leukemia virus strains also. An infection of 3T3 cell cultures with MLV alone gave less than 10^4 detectable leukemia virus helper units after 2 to 3 days of infection regardless of the number of infecting viral particles; whereas a cooperative infection of 1 leukemia virus helper unit per cell and 0.1 competent MSV per cell yielded, together with 10^4 focus forming units of MSV, more than 2×10^5 helper units of MLV on day 2. Leukemia virus titer was determined by the induction of helper activity in Swiss mouse embryo fibroblasts after 14 days by terminal dilutions of this MSV-LV yield. Our findings are compatible with the hypothesis that Rad LV is defective and that synergistic interactions occur between the sarcoma and leukemia viruses within a cell. If leukemia virus potentiation by sarcoma viruses is a general phenomenon, then a sarcoma virus infection of cells which do not overtly exhibit viral particles might elicit cryptic leukemia viruses. The utilization of this rapid assay of Rad LV could extend explorations of Rad LV tissue culture defectiveness, its manner of replication in vivo, and possibly its mode of neoplastic transformation.

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Hyperpolarizing and Depolarizing Receptor Potentials in the Scallop Eye

Abstract. *Depolarizing and hyperpolarizing responses to light were recorded intracellularly from different cells in the scallop retina. Both types of potentials appear to be primary effects of light on photoreceptor cells.*

The eye of the scallop *Aequipecten irradians* (*1*) contains two retinal layers of cells whose axons give rise to separate branches of the optic nerve (Fig. 1A). The visual cells in the distal retinal layers have a ciliated photoreceptor structure while the cells of the proximal layer possess microvilli (*2*). Although early microscopic work reported interconnections between the two retinal layers (*3*), more recent light and electron microscopic studies reveal no evidence for synaptic connections (*2, 3*). In examining the electrical responses of the two branches of the optic nerve, Hartline (*4*) found that the fibers from the proximal retinal cells discharged only upon illumina-

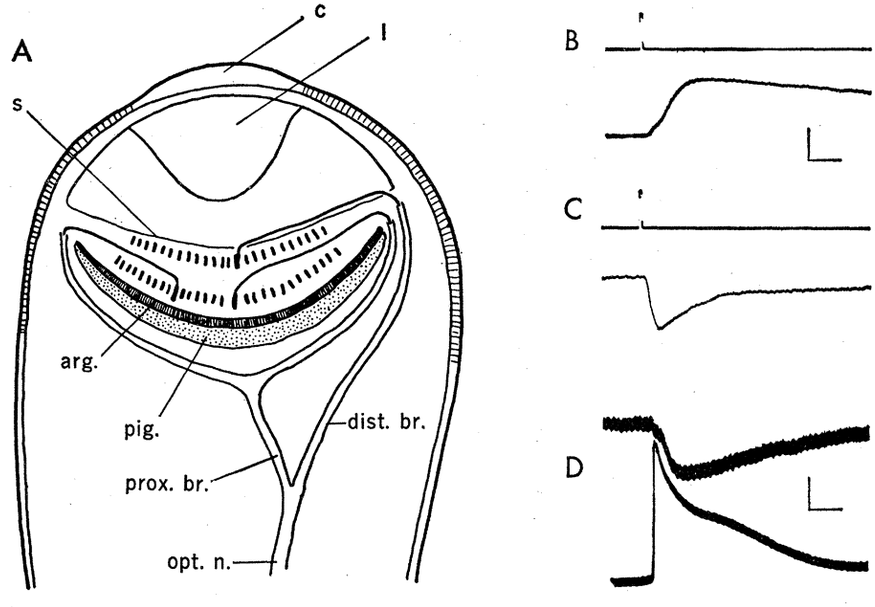

Fig. 1. Responses of visual cells in the scallop eye. (A) Schematic diagram of eye (after Dakin, 1928). In the retina one cell from the distal layer and two from the proximal layer are shown enlarged with their axons running to the respective branches of the optic nerve. Behind the retina are the argentea (*arg.*) and pigment layer (*pig.*). The proximal (*prox. br.*) and distal (*dist. br.*) branches join behind the eye to form the main optic nerve (*opt. n.*); (*c*), cornea; (*l*), lens; and (*s*), septum. (B and C) Intracellular recordings of depolarizing (B) and hyperpolarizing (C) responses to flashes of light. Photocell output monitoring light flash shown above each response. Calibration: 10 mv, 100 msec. (D) Simultaneous recording from extracellular electrodes located in proximal (upper trace) and distal (lower trace) regions of the retina. Light flash (not shown) at same time as in (B) and (C). Calibration: 0.5 mv, 100 msec for upper trace; 1 mv, 100 msec for lower trace.