A antigens is not the explanation of the B antigen adjuvant effect or (ii) the antigen processing machinery of macrophages from tolerant animals may be deficient not only with regard to the specific B antigens but also to the serologically distinct A antigens present on the same particle. From our study we do not know whether the birds tolerant of the B antigens responded less well to the A_2 antigens than one would expect of nontolerant birds immunized with A_2 alone, since so few of the latter responded (Table 2, group 1).

The possibility that macrophages which phagocytize two serologically distinct antigens pass on the necessary stimulus for the production of antibody to both antigens seems appealing since we have previous evidence that lymphoid cells which produce antibodies to *A* system antigens belong to the population of cells which produce antibodies to *B* system antigens (*8*). The ability to demonstrate that cells may produce two antibodies with different specificities may in part depend on the number of macrophages which phagocytize and suitably process both antigens. This may be a random phenomenon except in the situation where both antigens are present on the same carrier particle.

The adjuvant property of isoantigens may be valuable in the production of erythrocyte and leukocyte typing reagents which are ordinarily difficult to produce. By the same token it may complicate tissue typing studies in the human population since weak tissue antigens may acquire enhanced immunogenicity under the influence of a strong tissue antigen (*9*). Whether advantage can be taken of the isoantigen adjuvant phenomenon in inducing effective immunity to weak viral or tumor specific antigens has yet to be determined.

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1. The letter designation for the various chicken blood-group systems are specific and correspond to those used by other workers whereas the numerical superscripts, which designate specific alleles, apply only to the strain of chickens used in this study. Subscripts indicate antigen, and numbers on the line indicate antibody.
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Human Fibroblasts Infected with Rubella Virus Produce a Growth Inhibitor

Abstract. *A protein that inhibited mitosis of normal human diploid cells was demonstrated in extracts of WI-38 cells that were infected with rubella virus and that had gone into mitotic arrest subsequent to infection. A possible mechanism for the pathogenesis of the rubella syndrome is suggested.*

Human diploid fibroblasts infected with rubella virus in vitro are unchanged morphologically. When subcultured, however, these cells show effects ranging from mitotic inhibition to chromosomal breakage (*1, 2*). Infection of human fetal cells in vivo results in a complex of congenital anomalies referred to as the rubella syndrome. Naeye and Blanc (*3*) performed histopathologic analyses of the tissues of infants with rubella syndrome and found evidence of a general retardation in growth owing to mitotic inhibition. Cell strains carrying viruses derived from fetuses infected with rubella were studied in vitro by Rawls and Melnick (*4*), who found a slowed mitotic rate.

We have studied the mechanism by which rubella virus causes mitotic inhibition of WI-38 human diploid lung cells (*5*) and have found that infection induces the formation of a specific protein that slows mitosis. We call this substance rubella virus-induced mitotic inhibitor (RVIMI). Human diploid fibroblasts (WI-38) in the 18th to 28th passage in stoppered monolayer cultures containing about 4×10^6 cells were infected either with $10^{6.3}$ plaque-forming units (PFU) of rubella virus grown and titrated in BHK-21 cells (*6*) or with $10^{5.0}$ $TCID_{50}$ (tissue culture infectious dose, 50 percent effective) of rubella virus grown and titrated in WI-38. In both cases the growth and maintenance media were Eagle's basal medium and 10 percent fetal bovine serum. One week after infection the cells were suspended by treatment with trypsin. The suspension of cells was divided in half and subcultivated to two fresh bottles. A week later, or 2 weeks after they were inoculated with virus, infected and control cultures were treated with trypsin, washed, and resuspended in phosphate-buffered saline or in the maintenance medium. By the time of harvesting, the infected cultures showed visible evidence of arrested growth, while control cultures were confluent. After the cells were counted, the volumes were adjusted so that both suspensions contained 5×10^6 cells per milliliter. The suspensions were then subjected to three cycles of freezing and thawing, followed by 7 minutes (interrupted by shaking) of sonic oscillation in a Raytheon DF101 oscillator at 1 amp. The cell lysates were centrifuged for 2½ hours at 32,000*g* in a Sorvall centrifuge, a treatment which sediments infectious rubella virus (*7*). To remove additional live virus, we exposed the supernatants to 5 minutes of ultraviolet (UV) radiation delivered by a Westing-

Table 1. Effect of cell extracts on growth of WI-38 cells. To petri dishes were added 1×10^6 to 2×10^6 WI-38 cells. Extracts were added immediately. After 2 hours, about 30 to 40 percent of the cells were attached. On day 1, dishes to which nothing had been added showed a 25 percent increase in cell count above the number attached at 2 hours. Cells counts were performed in replicate and averaged. The error of the method equals ± 200,000. The dilutions were calculated by dividing the amount of undiluted cell extract added to 4 ml total volume by 4 ml.

Dilution	Cell counts 2 days after seeding ($\times 10^5$) in experiment			
	1	2	3	4
Rubella-infected cell extract				
1/20	4.5	12.7	8.0	8.5
1/40	6.2	11.9	8.5	8.0
1/80	7.9	13.4	10.0	9.9
1/160	8.6	17.5	11.9	11.4
Control-cell extract				
1/20	9.0	18.3	10.1	9.6
1/40	9.2	19.0	12.5	12.7
1/80	8.4	18.7	11.8	11.5
1/160	8.5	18.0	12.0	12.2
No extract				
	8.5	19.3	12.2	12.4

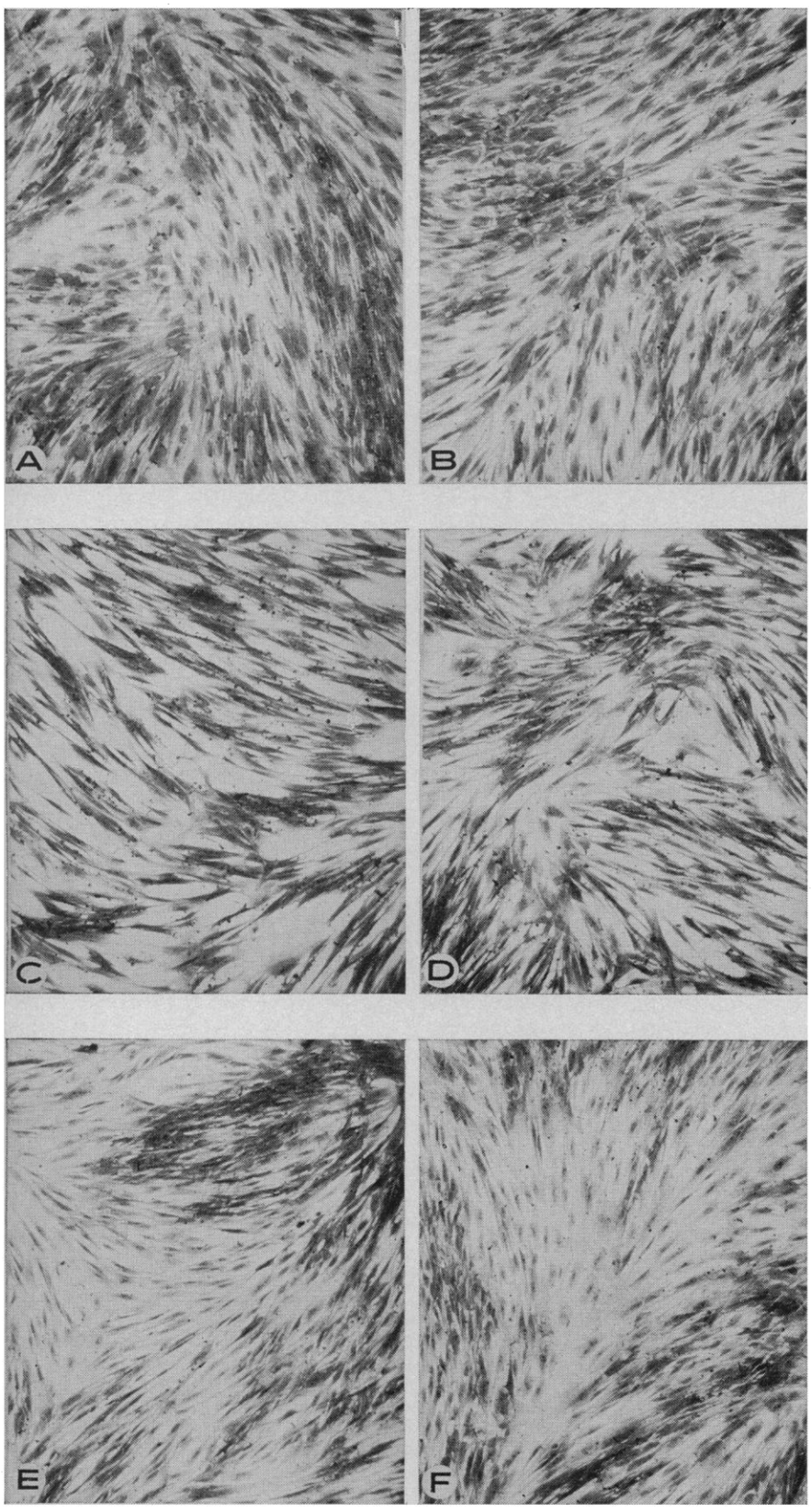

Fig. 1. (A) Control. WI-38 cells in monolayer 2 days after petri dish was seeded (× 60). (B) WI-38 cells to which control cell extract diluted to one part in 20 had been added; no effect. (C) WI-38 cells to which virus-infected cell extract diluted to one part in 20 had been added; growth inhibition. (D) Virus-infected cell extract diluted to one part in 40; growth inhibition. (E) Virus-infected cell extract diluted to one part in 80; slight growth inhibition. (F) Virus-infected cell extract diluted to one part in 160; no effect.

house Steri-Lamp 782-L 20 at 30 cm to a liquid layer 1 mm deep. After these procedures, extracts from infected cells contained less than $10^{0.5}$ PFU of rubella virus per milliliter. All extracts were negative for mycoplasma on culture. Irradiated cell extracts were tested for growth inhibitors with uninfected WI-38 cells used as indicators. When activity was tested, 5×10^5 to 1×10^6 indicator cells were seeded in 50-mm petri dishes containing 4 ml of double-strength Eagle's medium in single-strength Earle's salts. The cell extracts were added immediately, and the plates were placed in an incubator (35°C) perfused with 3 percent CO_2 in air.

In Table 1 are shown the results of four experiments in which extracts prepared from control cells or from those infected with rubella were added to freshly suspended WI-38 cells. As determined by cell counts, the extract of virus-infected cells diluted up to 80 times inhibited multiplication of uninfected WI-38. In other experiments, extracts of virus-infected cells showed activity at dilutions ranging from one part in 80 to one part in 320. While in the experiment illustrated control extracts showed no activity, in other experiments control extracts showed slight activity at a dilution of one part in 20 and moderate activity at one part in 10. The microscopic appearance of the cultures in a typical experiment 2 days after the addition of extracts is shown in Fig. 1.

To confirm that the visually observed inhibition of growth and the cell count were due to true antimitotic activity, we studied DNA synthesis by autoradiography. Cells (WI-38) were seeded on cover slips in media with or without cell extracts diluted one part in ten. At intervals, 1 μc of tritiated thymidine per milliliter was added to the plates and allowed to remain for 60 minutes, after which the cells on cover slips were fixed and processed for autoradiography.

The addition of extract from rubella-infected cells resulted in a decrease in the percentage of cells taking up thymidine during the first 48 hours. At this low dilution, extract of uninfected cells also showed activity, but, in terms of cumulative reduction of cells taking up thymidine, the rubella-infected cell extract was 59 percent more active.

The antimitotic activity is unrelated to the procedure used in making the extracts: in particular, treatment with UV does not induce activity in the extracts. Activity is also unrelated to the

age of the cells, since extracts of slowly multiplying phase III (40th to 50th passage) cells do not give more inhibition. The inhibition of cultures treated with cell extracts can be readily reversed by treatment of the cells with trypsin and transfer to fresh medium or simply by washing the affected monolayers.

The development of RVIMI (growth inhibitor) during infection was studied in two experiments in which several bottles containing monolayers of WI-38 were exposed to 2 PFU of rubella virus per cell. Antimitotic activity appeared first in cell extracts made 5 days after inoculation.

The substance RVIMI was not dialyzable and was resistant to the actions of ribonuclease, deoxyribonuclease, and ether. Treatment with 0.2 percent trypsin or chymotrypsin for 2 hours at 37°C followed by the addition of an equal amount of soybean inhibitor of trypsin lowered the RVIMI titer to one-fourth of the original value, while incubation alone for the same time had no effect.

The activity of RVIMI in cell extracts kept 1 hour at 56°C was partially lost; that of extracts kept 1 hour at 65°C was completely lost. Centrifugation for 150 minutes either at 3000 rev/min or 32,000*g* did not affect the titer of RVIMI. This fact, along with the fact that RVIMI is resistant to ether and is not inactivated by antiserum to rubella virus, suggested that RVIMI was not the virus particle. In addition, a concentrate of rubella virus containing 10^9 PFU/ml was irradiated with UV for 5 minutes to destroy its infectivity; it was then tested for antimitotic activity. None was found.

The cell specificity of RVIMI derived from WI-38 cells was tested with additions of extracts to growing cultures of a human amnion cell line (WISH), a human diploid lung strain transformed by SV40 virus (WI-26-VA4), baby hamster kidney-21, and a human diploid skin-cell strain cultivated in this laboratory. Only the skin strain was inhibited, while the continuous and nonhuman cell lines were unaffected.

That WI-38 cells treated with RVIMI for 24 hours were not protected against the cytopathic effect of vesicular stomatitis virus suggests that RVIMI is not an interferon. This fact was ascertained from results of endpoint titration of vesicular stomatitis virus in petri dishes containing cells treated with RVIMI, control cell extract, or nothing. The titer of this virus was unaffected by previous treatment with the extracts. Furthermore, RVIMI was not resistant when kept in solution at *p*H 2.5 for 18 hours at 4°C.

The presence of RVIMI in extracellular fluid was investigated with variable results. Infected lung cells (whose multiplication is inhibited by rubella virus) were mixed with infected skin cells (whose multiplication is not inhibited by rubella virus) (*2*), and all infected cells were inhibited. Infected and control WI-38 cells were placed in U-tubes and were separated by a 50 mμ millipore filter to prevent passage of virus; there was inconstant inhibition of control cells. Direct addition of tissue culture fluids free of virus to indicator WI-38 cells also resulted in variable inhibition. We conclude that, if extracellular RVIMI occurs, it is present in low titer.

The exact characterization of RVIMI and its relationship to the phenomenon of contact inhibition (*8*) would be of interest. Because some activity was found in uninfected cells, it is possible that RVIMI is a normal cell component, such as an enzyme. The presence of RVIMI in high titer in cells infected with rubella may account for the frequent retardation of growth in infants with rubella syndrome (*9*) as well as for the mitotic inhibition of human cells produced by infection in vitro (*1, 2*).

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Separation of Transit of Auxin from Uptake: Average Velocity and Reversible Inhibition by Anaerobic Conditions

Abstract. *An applied pulse of ^{14}C-indole-3-acetic acid moved down aerobic corn coleoptiles at about 15 millimeters per hour. When sections with a moving pulse were transferred to nitrogen, the rate fell below 2 millimeters per hour. This inhibition was completely reversible; sections returned to air moved the same amount of auxin as untreated aerobic controls.*

The auxin, indole-3-acetic acid (IAA), is produced in the tips of growing coleoptiles and moves from there basipetally to the cells whose elongation growth it controls (*1*). Under appropriate conditions, movement of auxin in coleoptile sections is both highly polar and independent of the orientation of the concentration gradient (*2*). The study of auxin transport is complicated by the complexity of the usual experimental system; auxin is supplied in a donor block of agar gel to one cut surface of an isolated section, and the auxin that is taken up and moved through the section is collected at the opposite end in a receiver block. Traditionally, any auxin collected in the receiver is said to have been transported there (*1, 2*). In this experimental system (i) uptake from the donor and (ii) exit to the receiver may be unique to the transport of auxin in isolated sections, but (iii) transit through the section should be comparable to the transport of endogenous auxin in the intact plant. In the work reported here the interpretation has been simplified by measuring transport uncomplicated by uptake and exit. Sections of corn coleoptiles were briefly exposed to ^{14}C-IAA, and then the movement of this labeled pulse was followed down the coleoptile.

Two examples illustrate the usefulness of the new approach. (i) It provides the first direct estimate of the velocity of auxin movement within the tissue. With this technique, evidence emerges that auxin molecules move within the section at different rates, but that the average velocity of the bulk of the auxin is similar to the rates reported for overall transport (*2*). (ii) It helps to resolve a recent discrepancy