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

(*3*, *4*). To elucidate the mechanism of polar auxin transport, it is important to know whether the functioning of the transit through the sections requires a supply of energy from aerobic metabolism. Naqvi *et al.* found, under both aerobic and anaerobic conditions, that the overall transport was proportional to uptake, and this led to the suggestion that anaerobic conditions do not directly inhibit transport in sections of corn coleoptiles but only inhibit uptake (*3*). On the other hand, Goldsmith (*4*) found that transport in sections of oat coleoptiles is inhibited anaerobically to a greater extent than uptake and concluded that transport is directly affected. A further and unambiguous test of whether transport is directly inhibited in anaerobic sections is provided by the new method. The inhibition of the movement of an auxin pulse in sections shifted from aerobic to anaerobic conditions supports the earlier conclusion that transport can be inhibited anaerobically.

The sections used were 20 mm long; they were cut starting 3 mm below the tip of 6-day-old etiolated coleoptiles of *Zea mays* L. var. Burpee Snowcross (*5*). During the 15-minute period of uptake, the apical cut surfaces of the sections were in contact with small blocks (16 μl) of 1.5 percent agar gel containing 2 percent sucrose and $10^{-5}M$ ^{14}C-carboxyl-labeled IAA (New England Nuclear Corporation, 13.5 mc/mmole). In the following 15-minute period, the radioactive donors were replaced by similar blocks containing unlabeled IAA. This allowed time for nearly all the ^{14}C-IAA that entered the tissue in the initial period to move some distance from the cut surface. Throughout both periods, the sections were supported vertically with their basal ends resting on agar blocks containing 2 percent sucrose.

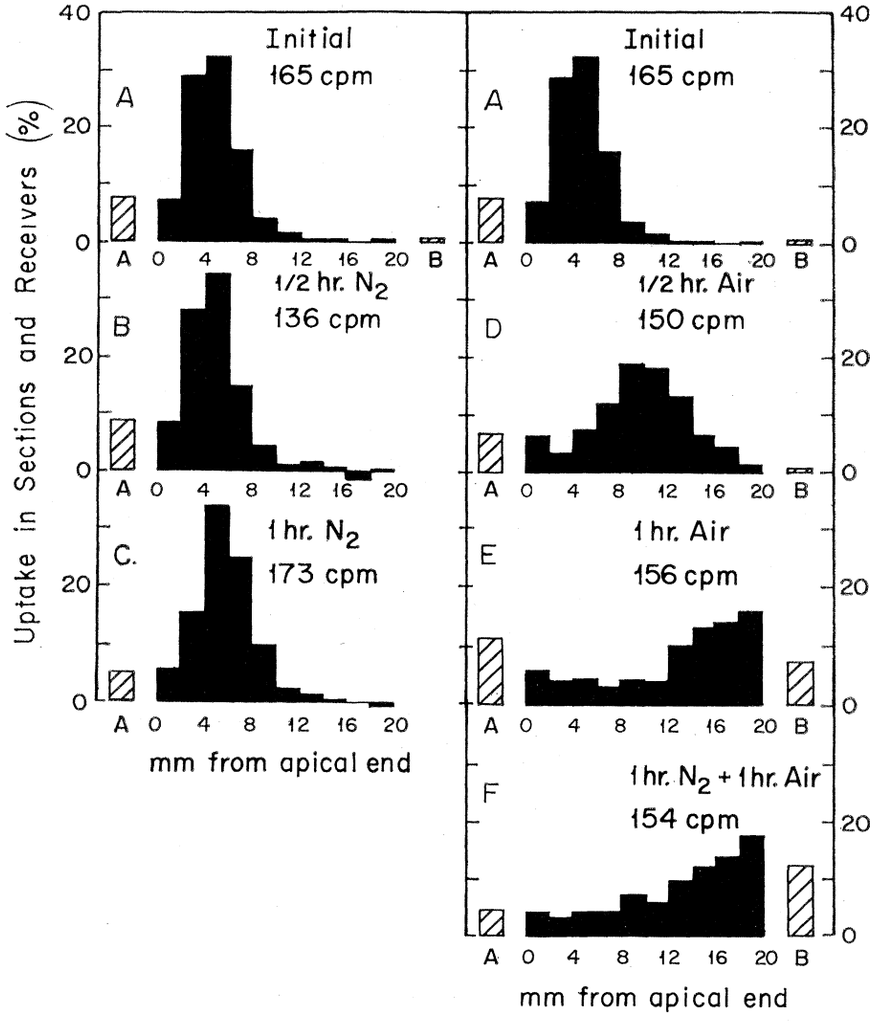

Fig. 1. (A to F) Movement of ^{14}C-IAA down aerobic and anaerobic coleoptiles. The sections in part A (shown twice for comparison with other parts) were subdivided 15 minutes after the donor block containing ^{14}C-IAA was removed. The sections in parts B to F were treated similarly but were subdivided after the indicated additional periods in air, nitrogen, or nitrogen followed by return to air. The percentage of the total uptake recovered in the apical (*A*, hatched lines) and basal (*B*, hatched lines) receivers and each 2-mm piece of section are shown, as well as the average total uptake of each group of sections. Apical receivers contained unlabeled IAA and sucrose and were present only during the first 15 minutes after the radioactive donor was removed. Basal receivers were present only during aerobic periods. Part A, average of data from four sections; other parts, average of data from duplicate sections.

At the end of the second period, some of the coleoptiles were subdivided in order to determine the distribution of IAA. Other coleoptiles were either kept in air or transferred to nitrogen for additional periods before they were subdivided. These sections were removed from their blocks and transferred to 125-ml gas-tight flasks. In order to speed the equilibration of the tissue with the anaerobic atmosphere, the flasks were evacuated five times to 0.1 atm and released to prepurified nitrogen. Flasks with control aerobic sections were also evacuated but released to room air. After evacuation, the test sections remained in a flow (250 ml/min) of moist nitrogen, and the control sections were left in room air. Only the aerobic sections were returned to their receiving blocks. The sections were subdivided after ½ or 1 hour.

Sections received white light during the short periods needed to perform the experimental manipulations but were shielded with black cloths during the actual uptake and movement of auxin. To determine the distribution of radioactivity, each section was cut into ten successive 2-mm pieces with a multirazor-blade cutter. Each piece of section and all blocks were counted separately in Bray's scintillation solvent (*4*). Counting efficiency was approximately 80 percent. Results consistent with those recorded in Fig. 1 have been obtained repeatedly. After the external source of ^{14}C was removed, the total radioactivity of the section plus its receiver did not vary significantly with time (Fig. 1) and equaled the uptake by the sections during the initial period.

Within 15 minutes after the ^{14}C-IAA donor was removed, the peak of radioactive IAA had moved down the section 2 to 6 mm from the apical end, and the ^{14}C in the first 2 mm of the section had dropped to less than 10 percent of the total uptake (Fig. 1, part A). After the first 15-minute period, the ^{14}C in this

piece did not decline further. The rapid appearance of a peak of ^{14}C at some distance from the apical end, coupled with the decrease of radioactivity in the first 2 mm, is a polar movement of IAA and indicates that within 15 minutes after removal of the donor the bulk of ^{14}C-IAA is being transported. During the first hour, ^{14}C moved down the aerobic section (Fig. 1, parts D and E) and began to appear in the basal receiving block. As the peak of ^{14}C moved down the aerobic coleoptile, it became flatter and more diffuse (for example, Fig. 1, parts A, D, and E). Nevertheless the position of maximum radioactivity can be estimated with reasonable accuracy for at least 1 hour, and an average velocity of the bulk of the auxin molecules can be obtained. The location of the peak shifted from the vicinity of 4 mm (Fig. 1, part A) to approximately 10 mm (Fig. 1, part D) in the first ½ interval and to about 19 mm (Fig. 1, part E) in the next; therefore, it moved at an average rate of about 15 mm/hr.

Routinely, the velocity of auxin transport in various tissues has been determined by the method introduced by van der Weij (*2*). Although the present method lacks precision, it does have the advantage over van der Weij's procedure that the time required for uptake from the donor and exit to the receiver is not lumped with the time of movement through the section. The present average rate of 15 mm/hr agrees with the velocity of IAA movements through coleoptiles of corn as determined by the classical method (*6*), and this supports the suggestion of McCready (*7*) that loading and unloading are not rate-limiting in the method of van der Weij.

Although it is usually assumed that all the IAA moves with the same velocity, as if, for example, it were being carried in a stream (*2, 8*), the rapidly changing shape of the peak indicates that individual auxin molecules move at different rates. This could be the result of (i) auxin molecules moving largely independently of each other, (ii) several independent streams moving at different velocities, or (iii) a decreasing capacity of transport toward the base of the section, so that auxin molecules depart from the stream and either diffuse in the tissue or equilibrate with the immobile phase. In view of the experimental results, it seems appropriate to reevaluate the confining concept of a single constant velocity of auxin transport.

The movement of the pulse of radioactivity down the section was sharply inhibited by anaerobic conditions. In coleoptiles that were rapidly equilibrated with an anaerobic atmosphere as soon as the ^{14}C-IAA had been transported as far as shown in Fig. 1, part A, the peak shifted less than 2 mm during the following hour (Fig. 1, part C). In this particular experiment, no significant change in the distribution of ^{14}C was detected during the first half hour in nitrogen (Fig. 1, part B), but with cuts at only 2-mm intervals a movement of as much as 1 mm could have been just below the level of detection. Although the inhibition appeared nearly immediately in this particular experiment, the time of onset of inhibition varied somewhat in different experiments. This variation may reflect differences in the rate at which the sections equilibrated with the anaerobic atmosphere. The inhibition established under anaerobic conditions appears to be completely reversible. If after 1 hour under nitrogen, coleoptiles were rapidly reequilibrated with air by evacuation and release to air, the amount of movement in them was not significantly different from control aerobic sections that had never been inhibited (compare parts E and F, Fig. 1).

Obviously anaerobic conditions, at least for 1 hour, had no permanent adverse effect on the transport. Furthermore, since the amount of movement was the same after an hour's inhibition as in control sections, transport must have either (i) resumed within 10 minutes of return to air, or (ii) if it resumed somewhat later it must have been at a greater than normal rate. The second alternative is suggested by the report that the growth rate of sections is stimulated following an anaerobic period (*9*).

Naqvi *et al.* reported that anaerobic conditions inhibit the overall transport in corn coleoptiles by virtue of inhibiting uptake alone (*3*). On the other hand, the present results show that the effect of anaerobic conditions is not limited to uptake; the rate of movement down the section can be reversibly inhibited by an anaerobic atmosphere. The explanation for the discrepancy between these results and those of Naqvi *et al.* is that the present procedures must render the tissues more nearly anaerobic.

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Dechlorination of DDT in Frozen Blood

Abstract. *Dechlorination of a commercial mixture of* o,p′-*DDT and* p,p′-*DDT to* p,p′-*DDD and* p,p′-*DDE occurs in avian blood stored at −20°C, indicating nonenzymatic degradation. The subsequent disappearance of these two metabolites suggests conversion to other metabolites which cannot be detected by gas chromatography with an electron-capture detector.*

During the routine analysis of DDT (*1*) in avian blood samples stored at −20°C, one sample was accidentally repeated at an interval of 3 weeks after the initial analysis. We were surprised to observe that the quantities (in parts per million) of DDT and the metabolites DDD and DDE were different from those of the first analysis, the amount of the metabolites being higher and that of DDT being much lower. Because this presented a possible source of error in the accurate analysis of insecticide residues, we investigated this apparent degradation of DDT in frozen (−20°C) whole blood.

DDT can be dechlorinated under anaerobic conditions by yeast (*2*), by the bacteria *Proteus vulgaris* (*3*), *Serratia marcescens* and *Escherichia coli* (*4, 5*), and *Aerobacter aerogenes* (*5, 6*), DDD being the major product with a small amount of DDE being formed. Castro (*7*) and Miskus *et al.* (*8*) observed a conversion of DDT to DDD by dilute solutions of Fe (II) porphyrin complexes, and Wedemeyer (*6*) observed a similar conversion by reduced