jection. Reduction of this delay—even investigation of the approach with another (injection) technique—should be attempted. The pulmonary arteries were injected according to the normally occurring blood flow, but the pulmonary veins could only be approached by an injection in a reverse flow manner (which did not seem to affect their complete filling, and hence can be considered unimportant).

Tissue perfusion may result from an equilibrated and extremely complex interplay of a wide spectrum of various morphological and functional factors, many of which are still unknown in health and disease. It may be an intricate "mosaic" which could possibly only be analyzed by a programmed computer tape (*16*).

Among the many mechanisms controlling tissue perfusion, an important morphological factor is the specific anatomical pattern of the organ's nutritive blood supply, which is peculiar to the lung and even changes with age. In this sense, the functional significance of the filling of the bronchial venous plexus, which we have again (*17*) observed in HMD lungs only after the injection of the pulmonary veins, should be further elucidated. Embryological (*18*), histological (*17, 19*), and pathological (*20*) studies have repeatedly revealed many widespread connections between the bronchial and the pulmonary circulation; they are even especially developed during embryogenesis and in the newborn infant may be important (even if technically difficult to assess) in collateral microcirculatory pathways during a hypoperfusion.

As important in the tissue perfusion of HMD lungs is the lymphatic microcirculation, which is an important factor in tissue-fluid balance and exchange, and to which, until now, only a casual interest has been paid. In HMD, the pulmonary lymphatics are frequently dilated and filled with an eosinophilic, presumably proteinaceous, possibly fibrinous fluid (*5*), as confirmed mathematically by histometrical measurements of the mean lymphatic diameter which is consistently greater in the idiopathic respiratory distress syndrome than in a control group of other newborns (*20*).

To summarize, although much information is still lacking, my observations support the argument for a disturbance of pulmonary perfusion localized largely at arterial level, the small muscular pulmonary arteries (50 to 30 μ) and especially the pulmonary arterioles ($< 30\ \mu$). The pulmonary venous vasculature does not seem to be the most important primary obstructive factor. These observations, as well as the filling of the bronchial venous plexus after injection of the pulmonary veins and the lymphatic angiectasis in such lungs, should be completed by urgently needed further data concerning the blood and lymphatic microcirculation in HMD.

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Auxin and Wall Extensibility: Reversibility of Auxin-Induced Wall-Loosening Process

Abstract. *The reversible nature of the auxin-mediated loosening of the cell wall is shown by the ability of respiratory inhibitors to cause the loss of the auxin-induced increase in wall extensibility without affecting the basal extensibility of the wall. This reversibility makes it unlikely that wall loosening is mediated by enzymes, such as cellulase and β-glucanase, which degrade polysaccharides, since their action is essentially irreversible.*

Auxin is believed to control the rate of cell elongation by regulating the extensibility of the cell wall (*1–3*). In stem and coleoptile tissues, auxin induces an increase in the wall extensibility. This potential for extension can, in turn, be converted by turgor pressure into a finite amount of wall extension (*2*). The process of cell elongation is the result of a continual series of these wall loosenings and extensions.

The biochemistry of the process of wall loosening is still unknown, but it may be mediated by enzymes, such as cellulase and β-glucanase, which degrade wall polysaccharides. Indeed, auxin can increase the endogenous activities of cellulase (*4*) and hemicellulases (*5*), and a crude β-glucanase preparation has been reported to replace auxin in inducing cell elongation (*6*). In addition, the extensibility of isolated cell walls can be increased by their treatment with cellulase (*7*).

Since the action of enzymes that degrade wall polysaccharides is essentially irreversible (*8*), any wall loosening that is induced in this way will persist until converted into extension or until the cleavage of the polysaccharide chains is repaired by separate, synthetic processes. One method of assessing the participation of these enzymes in wall loosening is to determine whether the wall-loosening process is irreversible.

There is some evidence (*9*) that wall

loosening may, in fact, be reversible. When sections of *Avena* coleoptiles were treated with auxin under conditions where elongation could not occur, a potential for extension was built up which could be used whenever the sections were allowed to extend. But if the sections were treated just before the extension with a respiratory inhibitor such as cyanide, the potential for extra extension was nullified. This finding suggested that wall loosening is reversed whenever respiration is inhibited.

Because wall extensibility can now be measured with the Instron technique (*2, 7*), one can test directly whether an inhibition of respiration leads to a reversal of the wall loosening which is induced by auxin. *Avena* seedlings were grown and sections were obtained as described earlier (*10*). Wall loosening was induced by incubation for 120 minutes in potassium maleate buffer (5 m*M*, *p*H 4.7) containing indoleacetic acid (IAA, 5 μg/ml). To prevent continued conversion of the wall loosening into wall extension, the sections were then transferred to solutions which also contained 0.15 or 0.2*M* mannitol. Control sections were incubated in a similar manner in solutions without IAA. After an additional 90 to 120 minutes, a respiratory inhibitor was added to certain groups of sections. Sections were harvested after the desired incubation period, killed in boiling methanol, and deproteinized with Pronase. The walls were then subjected to force-extension analysis with an Instron linear extensometer, and the resulting data were analyzed so as to obtain compliance values (*DP*) which characterize the plastic extensibility of the walls. This technique has been described in detail in an earlier paper (*2*).

Auxin causes a doubling of wall extensibility, and, once induced by auxin, the wall loosening persists as long as elongation is prevented osmotically (*2, 3*). When 0.3 m*M* KCN is added to auxin-treated *Avena* coleoptiles under these same conditions, there is a rapid decrease in the wall extensibility until it nearly reaches the control value (Fig. 1). In contrast, cyanide has no effect on the extensibility of control sections. It is apparent that cyanide causes a reversal of the auxin-induced wall loosening.

Other respiratory inhibitors can cause a similar reversal of wall loosening. This is illustrated for dinitrophenol (DNP) and the sulfhydral inhibitor *N*-ethylmaleimide (NEM) by the data of Table 1. An inhibitor of protein synthesis, cycloheximide (CHA), has also been tested in this system. Since respiratory inhibitors also block protein synthesis, and since protein synthesis is needed for the induction of wall loosening (*11, 12*), it is possible that the respiratory inhibitors act simply by blocking protein synthesis. However, incubation of auxin-treated coleoptiles with cycloheximide (4 μg/ml) for 2 hours had no effect on wall extensibility (Table 2), even though protein synthesis was inhibited by over 90 percent (*11*). The effect of respiratory inhibitors, then, cannot be due to their inhibition of protein synthesis but must be ascribed to their inhibition of respiration.

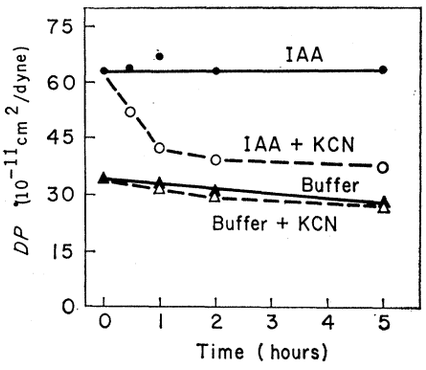

Fig. 1. Reversal of auxin-induced wall loosening by cyanide. Sections were first incubated for 2 hours in potassium maleate buffer (5 m*M*, *p*H 4.7), with or without IAA, then for 2 hours in the same solutions plus 0.15*M* mannitol; KCN (0.3m*M*) was added at time zero, and sections were harvested after 0 to 5 hours. Plastic extensibility of walls (*DP*) was determined as described earlier (*2*).

Table 1. Reversal of auxin-induced wall loosening by respiratory inhibitors. All sections were first incubated with (+) or without (−) IAA, then for 2 hours in the same solutions with 0.2*M* mannitol. Dinitrophenol (DNP), *N*-ethylmaleimide (NEM), or water was added, and incubation continued for 4 hours. The plastic extensibility of walls (DP) was then determined.

Treatment	DP (10^{-11} cm²/dyne)	
	+IAA	−IAA
None (initial value)	50	22
Buffer, 4 hours	49	21
DNP ($3 \times 10^{-5}M$)	33	19
NEM ($4 \times 10^{-4}M$)	24	19

Table 2. Difference in ability of inhibitors of protein synthesis and respiration to cause reversal of auxin-induced wall loosening. Sections were first incubated 120 minutes in buffer with or without IAA, then for 90 minutes in the same solutions with 0.2*M* mannitol. Cycloheximide (CHA), KCN, or water was then added, and incubation continued for 2 hours. The plastic extensibility of walls (DP) was then determined.

Initial incubation	Treatment	DP (10^{-11} cm²/dyne)
IAA	None	68
IAA	IAA	70
IAA	IAA + CHA (4 μg/ml)	69
IAA	IAA + KCN (0.3 m*M*)	43
No IAA	Buffer	31

There are at least two ways in which an inhibition of respiration could lead to a reversal of wall loosening. Wall loosening may involve an enzymatic reaction in which one of the wall polysaccharides is cleaved whenever the enzyme, whose presence is presumedly controlled by auxin, and a product of respiration are both present. If the change in free energy in this reaction is nearly zero, the reaction will be driven toward polymer cleavage whenever there is an adequate supply of the respiratory product; but if the supply of this product is depressed by an inhibition of respiration, the direction of the reaction will reverse and the polymers will reform. Because some movement of the chain ends may occur, complete reformation of the polymers is unlikely to occur and the wall loosening is unlikely ever to be completely reversible. No such enzyme is known at present—cellulase and β-glucanase are not capable of carrying out such a reversible reaction—but this may simply be due to the fact that no attempt has been made to find an enzyme with these properties.

A second possibility is that the loosening and stiffening processes are mediated by different enzymes. In this case the nature of the loosening process is more difficult to predict, although it is apparent that it cannot be mediated by cellulase or β-glucanase since the process requires respiration. The possibility that the loosening involves the synthesis and intussusception of new wall polysaccharides has been suggested (*13*); if so, the reversal could be due to removal of these polysaccharides by cellulase or β-glucanase. When respiration is active, the synthetic processes would predominate and wall loosening would occur, whereas an inhibition of respiration would lead to an excess of the degradative, stiffening processes.

Evidence of whether the loosening and stiffening processes are mediated by the same or different enzymes is now needed before the enzymes involved in auxin-induced wall loosening can be identified.

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Antibody Formation: Initiation in "Nonresponder" Mice by Macrophage Synthetic Polypeptide RNA

Abstract. *The RNA extracted from normal peritoneal macrophages exposed to a linear, random synthetic polypeptide, $Glu^{60}Ala^{30}Tyr^{10}$, initiated an immune response in C57B1/6J mice, although this strain responds very poorly to the antigen itself. From 10 to 150 micrograms of RNA obtained from mouse, rat, or rabbit macrophages was injected intraperitoneally into recipient mice, and specific antibody was detectable by passive hemagglutination 3 to 4 weeks later. Treatment of the RNA with ribonuclease destroyed its ability to initiate a specific immune response. The RNA contained by weight 0.02 percent of the (specific) antigen. The RNA obtained from cells incubated with a second polypeptide, $Glu^{36}Lys^{24}Ala^{40}$, initiated a response specific for this polymer. This RNA even when incubated in vitro with $Glu^{60}Ala^{30}Tyr^{10}$ failed to initiate antibody formation specific for $Glu^{60}Ala^{30}Tyr^{10}$.*

The role of the macrophage in antibody formation has received much attention. Ribonucleic acid (RNA) extracted from macrophages that had been exposed in vitro to antigen can elicit antibody formation in lymph node cells from nonimmune animals in vitro (*1*) when antigen alone failed to do so. Such RNA is also a potent immunogen in vivo (*2*). We report now on a study of the role of macrophage RNA in a better-defined system, namely, inbred animals and synthetic antigens. The response of inbred mice to structurally simple synthetic polypeptides is genetically determined, both with regard to whether or not the animal can respond ("responder" as opposed to "nonresponder" strains) (*3*), the specificity of the response, and the type and amounts of immunoglobulins produced (*4*). In addition, the animals are free of "background" antibody activity against the antigen.

We used a synthetic, linear, random polymer of the α-amino acids L-glutamic acid, L-alanine, and L-tyrosine, $Glu^{60}Ala^{30}Tyr^{10}$, as antigen (superscripts refer to moles per 100 moles of the amino acid; the molecular weight of the preparation used was 25,000); C57B1/6J mice were the recipients of the RNA. The antibody response of this strain to $Glu^{60}Ala^{30}Tyr^{10}$ is normally very poor. The animals fail to respond to 0.01 μg to 1 mg of the polymer in saline, and respond poorly even to polymer in Freund's complete adjuvant; mean passive hemagglutination titers on day 14 were 1:16, and they declined rapidly thereafter. This is in contrast to the excellent antibody response (100 to 800 μg of antibody nitrogen per milliliter of serum) of other strains (*3*). Upon restimulation, the C57B1/6J mice fail to show an increased response, and in fact become completely unresponsive. With pertussis vaccine as adjuvant an antibody response—first detectable 10 days after immunization—is produced; the biological properties of the antibody produced, which is also transient and cannot be restimulated, indicate that it is the murine equivalent of immunoglobulin E (IgE) (*5*).

The techniques used for obtaining peritoneal exudates that contain large amounts of macrophages, incubation with antigen, and extraction of RNA with phenol have been described (*6*). Antibody was determined by passive hemagglutination (*7*). Macrophages of other inbred mouse strains and of heterologous species were examined as sources of active RNA (Table 1). Antibody was detected in the serums of a significant number of mice 2 or 3 weeks after the intraperitoneal injection of 25 to 100 μg of macrophage RNA. Although the titers were low, rarely exceeding 1:16, they were reproducible and specific. The titrations [micro-Takatsy method (*3*)] yield values which are consistently three to five doubling dilutions lower than those obtained with a tube method.

The macrophage donors for groups 1 and 2 were nonimmunized mice of strains that produce antibodies to the

Table 1. Antibody to $Glu^{60}Ala^{30}Tyr^{10}$ (GAT) in primed and unprimed C57B1/6J mice subsequently treated with RNA extracted from homologous and heterologous normal macrophages that had been incubated with GAT. Priming consisted of injecting (intraperitoneally) the recipient mice with (A) 1 mg of GAT in 0.15*M* NaCl plus 500 μg of pertussis vaccine; (B) 1 mg of GAT in 0.15*M* NaCl; or (C) 0.15*M* NaCl. Three months later RNA in sterile tris buffer, *p*H 7.2, was injected intraperitoneally. Antibody titers were determined by the passive hemagglutination of sheep red cells coupled with polymer by bis-diazobenzidine, by serums which had been absorbed with sheep red cells. Results are the ratio of the number responding to the number tested.

Group	Donor macrophages		Treatment of recipient mice		Antibody response on day:				
	Source	Polymer added per 10^9 cells (mg)	Priming	RNA (μg)	8	14	21	28	35
1	C3H/HeJ mice	10	A	35, 50*	5/6	5/6	5/6	0/2	
2	DBA/2 mice	50	A	25		1/5	1/5		1/4
			B	25		1/6	1/5		1/5
			C	25		2/9	4/7		1/7
3	Rat	50	A	56		3/5	2/4		0/4
			B	56		2/5	3/5		2/5
4	Rabbit	45	A	100		1/3	1/2		2/2
			B	100		2/5	4/4		2/4
			C	100		1/4	3/4		3/3

* Two separate preparations of RNA were used.