ic implantation by use of a perivascular transducer, it has since been modified for transcutaneous detection of flow in peripheral vessels (*5*). The first trials, in which air emboli were injected into animals, indicated that this technique was as sensitive to the presence of emboli as the perivascular technique was.

To provide variation in skin characteristics and circulation, three animal species—the dog, swine, and goat—were selected for air-decompression experiments. In each case, a transcutaneous flow transducer was taped to the skin overlying the chosen superficial vessel, and the anesthetized animal was placed in the hyperbaric chamber. Flowmeter output was telemetered (*6*) to a receiver outside the chamber and was recorded on magnetic tape throughout each run.

The dog (2 years, female beagle, 10 kg) was linearly decompressed over a 10-minute period after a 1-hour exposure to 5.3 atm (gauge reading; 53 m equivalent depth of sea water). A transducer over the right saphenous vein detected the first embolus at 4.3 atm (43 m) with several more occurring at 3.3 atm (33 m), both well before the first "stop" recommended for man by U.S. Navy tables (15 m) (*4*). Immediately after 0 atm was reached, the transducer was relocated over the right cephalic vein where embolic artifacts were so loud and numerous that the blood-flow background noise was obliterated. The swine (1 year, castrated male miniature, 48 kg) was linearly decompressed over a 12-minute period after a 30-minute exposure to 5.3 atm (53 m); emboli were detected in the left superficial epigastric vein at 0.6 atm (6 m) and became more numerous as decompression continued. The goat (2 years, male pygmy, 28 kg) was linearly decompressed over a 7-minute period after a 40-minute exposure to the same pressure. During decompression the animal struggled, displacing the transducer so that the time of the first embolic event could not be recorded; however, immediately after arrival at 0 atm, the jugular and saphenous veins contained emboli which produced embolic signals of great number and magnitude, nearly obliterating the background noise of blood flow.

These experiments establish that circulating gas emboli associated with decompression are detectable in major veins with the transcutaneous Doppler flowmeter. Although surgically implanted perivascular transducers will continue to be useful in applied research on animals, transcutaneous application is possibly of greater practical importance to man, on whom this technique has been applied in other studies (*7*). In particular, it has been long suspected that gas emboli form and circulate in the venous system, during marginal decompression procedures, before the onset of symptoms; some believe these "silent bubbles" may even occur during procedures which are now considered safe.

With no method to detect these emboli (if they exist) in man, decompression tables are now based on empirical procedures in which the announced discomfort of the subject is the indicator. Such methods are time-consuming and fraught with subjective error, and must be considered hazardous. If "silent bubbles" exist, and if the transcutaneous Doppler flowmeter can detect them before onset of symptoms, the situation could be improved greatly. Furthermore, there is no method available to correlate severity of symptoms with the degree of embolism or to assess the embolic status of unconscious patients undergoing therapeutic decompression. Current efforts to attain greater depths and longer exposure periods by the use of new gas mixtures can be expected to increase the hazards beyond those now endured, and may create an even greater need for objective methods of monitoring subjects. Based on our animal trials, it is evident that the transcutaneous Doppler technique warrants further evaluation.

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Cytoplasmic Activity in Type I Pulmonary Epithelial Cells Induced by Macroaggregated Albumin

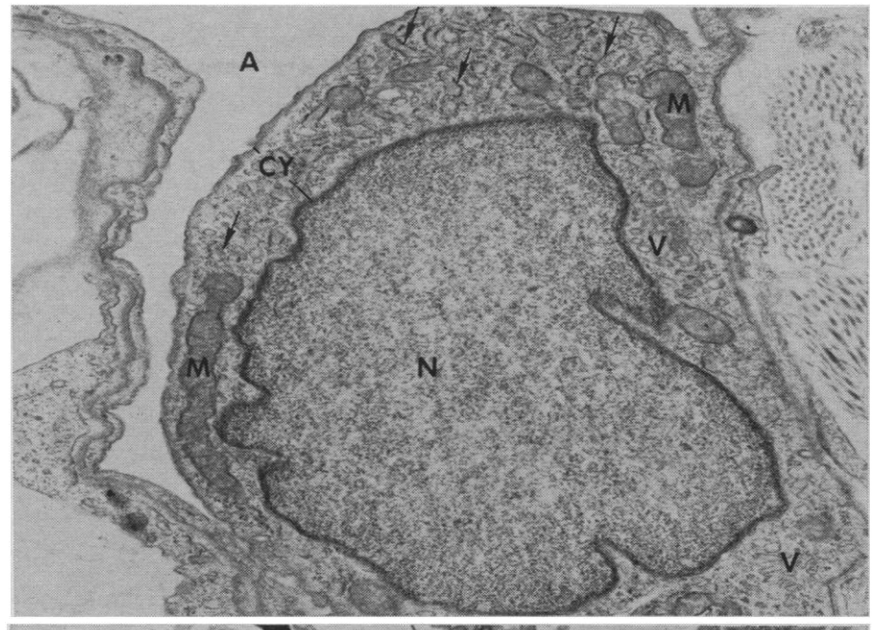
Abstract. *After the intravenous injection of radioalbumin macroaggregate, large numbers of cytoplasmic inclusion bodies were observed in the lung tissue of rats. The inclusions were located mainly in the cytoplasm of type I alveolar lining cells, appeared 40 minutes after the injection, and lasted up to 2 days. These observations suggest that the type I alveolar lining cells participate in the clearing mechanism of the lung tissue, a function that thus far has not been attributed to this type of cell.*

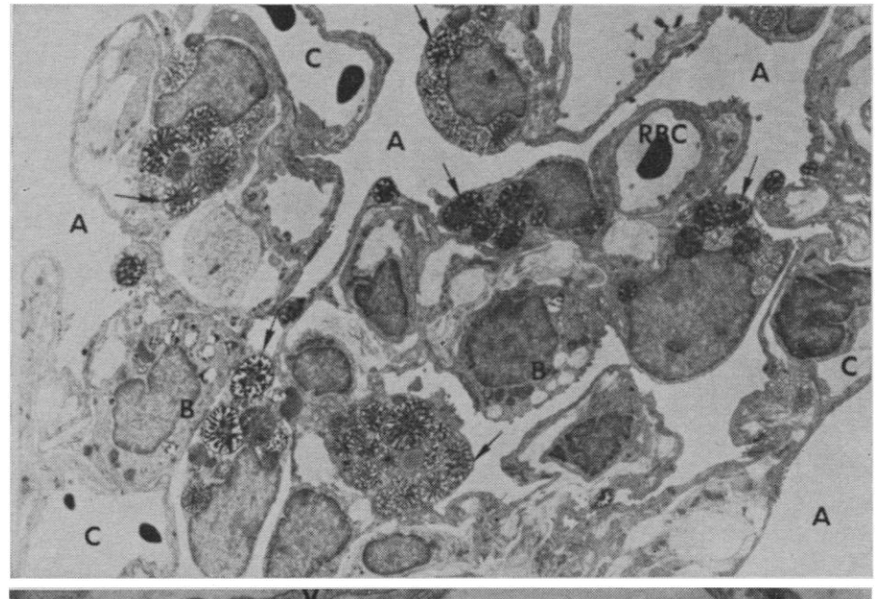
The alveolar surface of the lung is continuously lined by a single layer of two types of epithelial cells: the type I and type II cells. At least 90 percent of the alveolar surface is covered by type I cells (*1*) that have attenuated cytoplasmic processes containing few mitochondria and sparse endoplasmic reticulum. Type II cells, wedged between type I cells, are cuboidal and their cytoplasm is rich in mitochondria, endoplasmic reticulum, and also electron-dense lamellar inclusion bodies. While type II cells have been of constant interest to investigators concerned with lung tissue, especially to those studying alveolar surfactant, type I cells have thus far received little attention. Except for their being part of the "air-blood membrane," no specific metabolic activity has been suggested for these cells. This report provides, for the first time, evidence that type I alveolar lining cells are capable of participating in physiologic processes occurring in lung tissue which apparently require high cellular activity.

Macroaggregated albumin labeled with I^{131} (15 μc/kg; specific activity 2 to 300 μc/mg) (*2*), a pharmaceutical used in radioisotope scanning of human lungs for diagnostic evaluation of certain disease conditions (*3*), was injected into the tail vein of Sprague-Dawley rats. Rectolinear scintiscans were carried out immediately thereafter to verify that the radioisotope had arrived in the lung. The animals were then killed at intervals varying from immediately after the injection to 6 days later. The lung tissue was prepared for electron-microscopic examination by fixation in phosphate-buffered osmium tetroxide, embedding in Epon, and staining with uranyl acetate and

lead citrate. The tissue was examined with an RCA EMU 3G electron microscope at 100 kv.

The lung tissue of animals killed immediately and 10 minutes after the injection revealed no structural changes when compared with the tissue of control animals. Albumin aggregates were not observed in the capillary bed of these or any other specimens examined. In specimens obtained 20 and 30 minutes after the injection, a marked increase in the rough endoplasmic reticulum, free ribosomes, and mitochondria was seen in the cytoplasm of type I alveolar lining cells, together with clusters of small vacuoles, comparable in size to pinocytotic vesicles (Fig. 1). Similar changes, especially the appearance of small vacuoles, were observed in the endothelium of capillaries. At 40 minutes after the injection, large numbers of clearly visible, round and oval cytoplasmic inclusion bodies were seen throughout the lung tissue. Most of these were located in the cytoplasm of type I cells; only a few were seen in cells within the alveolar septum and none were observed in type II or endothelial cells (Fig. 2). The majority of these cytoplasmic inclusion bodies revealed a distinctive structural arrangement: electron-dense rays radiated from the center of the body in all directions, the remaining background was light, but a concentrically arranged, fine lamellar system was noted especially in those bodies that appeared to be sectioned close to their center. There was no limiting membrane toward the cytoplasm, but an electron-dense outer rim that seemed to be part of the concentric lamellar system (Fig. 3). A number of the inclusion bodies were homogeneous





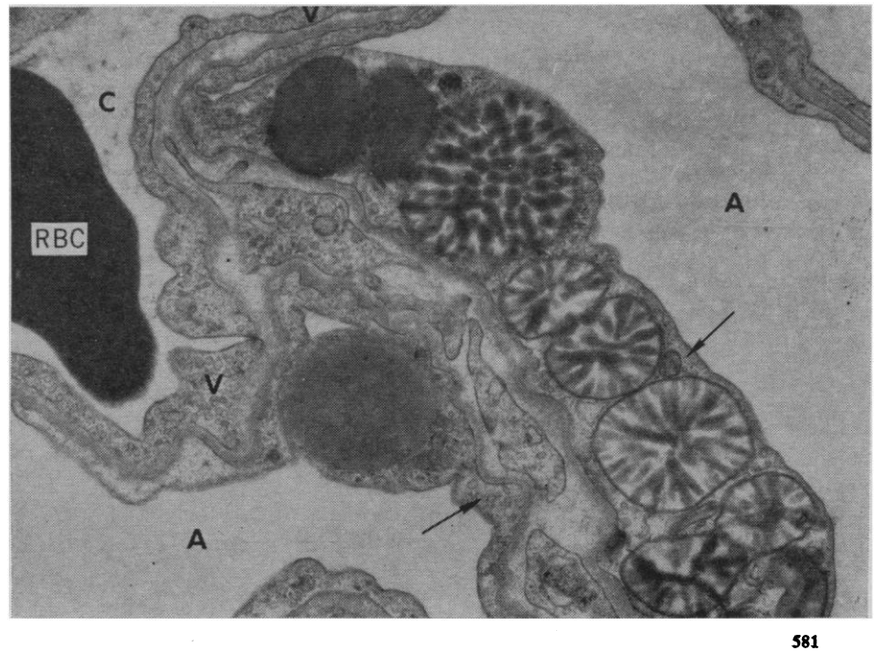


Fig. 1 (top). Type I alveolar lining cells in tissue of animal killed 30 minutes after injection. Note the abundance of rough endoplasmic reticulum (arrows), mitochondria (*M*), and small vesicles (*V*) in the cytoplasm (*Cy*). *A*, Alveolar space; *N*, nucleus. (× 20,900) Fig. 2. (middle). Lung tissue of animal killed 2 days after injection. Note the cytoplasmic inclusion bodies in the type I alveolar lining cells (arrows). Type II cells (*B*) are free of such inclusion bodies. *A*, Alveolar space; *C*, capillary; and *RBC*, red blood cell. (× 1800) Fig. 3 (bottom). Higher magnification of type I alveolar lining cells (arrows) 50 minutes after injection, showing multiple inclusion bodies; some reveal an internal structure, and others are homogeneous. Note the multiple small vesicles (*V*) in the endothelial cytoplasm. *A*, Alveolar space; *C*, capillary; and *RBC*, red blood cell. (× 15,300)

and more or less electron dense, while others exhibited various stages from a homogeneous appearance to the striking wheel-like arrangement (Fig. 3). Quite frequently type I cells were so laden with inclusions that their cytoplasm bulged out locally or their nuclei were indented. An increased number of free cells in the alveolar space was also observed. These cells were often arranged in groups of three or more and their cytoplasm revealed numerous inclusion bodies of various electron densities, closely resembling the homogeneous inclusions in type I cells. Up to 2 days after the injection there was no apparent change in the number and appearance of the cytoplasmic bodies in type I cells. At 3 days and thereafter, very few of these bodies were seen and the cytoplasm of type I cells appeared indistinguishable from that of control animals.

These observations strongly suggest that the type I alveolar lining cells participate in the clearing mechanism of the lung tissue. Such function, thus far, has never been attributed to these cells but rather to a variety of other cells in the lung tissue, including the type II cells. Although much additional study is necessary to elucidate the nature of these cytoplasmic bodies, as well as the molecular events and control mechanisms responsible for the activity exhibited in the type I pneumocytes, the observations reported here may well initiate a revision of our present concept of the still poorly understood clearing mechanism in lung tissue.

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Pesticide Transformations: Production of Chloroazobenzenes from Chloroanilines

Abstract. *Aniline and 11 different chloroanilines were added to soil. No azo compound was formed from aniline, but all monochloro- and some dichloroanilines were transformed to their corresponding dichloro- and tetrachloroazobenzenes. Other dichloroanilines and the trichloroanilines were stable in soil. Peroxidase catalyzed the formation of azo compounds by some chloroanilines. Correspondence in the range of substrates used and products formed in the two systems suggests a peroxidatic mechanism for the synthesis of azo compounds from anilines in soil.*

It was recently demonstrated that an aniline intermediate in the decomposition of a pesticide was condensed to form an azo compound; specifically the herbicide propanil (3′,4′-dichloropropionanilide) was degraded microbiologically in soil to 3,4-dichloroaniline, two molecules of which were oxidatively linked as 3,3′,4,4′-tetrachloroazobenzene (*1*). Since anilines are known to be produced during decomposition of various other substances, including phenylcarbamate, phenylurea, and acylanilide herbicides (*2*), formation of azo compounds may be a common rather than a rare phenomenon, and studies of the requirements and mechanism for aniline-condensation reactions appeared warranted. We now describe the influence of molecular configuration on the ability of anilines to be transformed to azo compounds in soil, and present evidence that the reaction is catalyzed by the enzyme peroxidase. The work relates directly to questions of environmental pollution, and is of concern to public health and welfare since some azo compounds are carcinogenic (*3*).

Formation of azo compounds in soil was investigated by mixing 50 mg of each of several different anilines separately with 50 g (dry weight) of Nixon sandy loam (*p*H 5.5). The soil samples were moistened to 60 percent of capacity, incubated in covered beakers at 27°C for 14 days, and then extracted with acetone. The extracts were concentrated and subjected to gas-chromatographic analysis with a flame ionization detector; the stainless steel column was 1.8 m long by 3 mm in outside diameter and packed with 5 percent UC-W98 on Chromosorb W. A portion of each extract was evaporated to dryness, and the residue was dissolved in methanol and examined spectrophotometrically with a Beckman-DB instrument.

Peroxidatic formation of azo compounds was measured in a system containing 50 mg of an aniline substrate in 50 ml of 0.2*M* acetate buffer at *p*H 5, 0.1 ml of a 30-percent solution of H_2O_2, and 13.5 units of peroxidase (Sigma Chemical Co.; horseradish peroxidase type II, 135 purpurogallin units per milligram). Additional increments of H_2O_2 and peroxidase were supplied at each of three consecutive 30-minute intervals, and the reaction mixture was stirred magnetically for a total of 2 hours. The system was then extracted with three 20-ml quantities of hexane, which were combined, concentrated, and analyzed for azo compounds by the gas-chromatographic procedure used for examination of the soil extracts.

All commercial chemicals were examined for homogeneity by gas chromatography. Chlorinated anilines were purified by repeated recrystallization from ligroin. Chlorinated azobenzenes were synthesized by oxidation of appropriate chloroanilines or by reduction of their corresponding nitrobenzenes (*4*).

Our concern was with the detection and identification of azo compounds as a means of understanding the requirements for and mechanism of their formation in soil. All analyses were qualitative, with no attempt to measure either the rate or extent of a transformation. Products other than azo compounds were not isolated or identified. No azo compounds were detected in soil that was untreated or in soil that was sterilized and treated with filter-sterilized aniline solutions (*1*). Moreover, there was no transformation of anilines to azo compounds in buffered H_2O_2 solutions unless the reaction was catalyzed by peroxidase.

The 12 anilines employed by us are illustrated in Fig. 1 along with the azo compounds that were formed from the anilines in soil and by peroxidase. In every instance the identification of an azo compound was based on two independent characteristics: its retention time and absorption spectrum.

Aniline itself was transformed in soil and by peroxidase, but no azo compound was among the products. The expected dichloroazobenzene was produced enzymically and in soil from each of the three monochloroanilines.